Bronchial Responsiveness to Ultrasonic Fog in Occupational Asthma Due to Toluene Diisocyanate*

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To determine the validity of ultrasonic nebulization of distilled water (UNDW, "fog") in comparison with methacholine challenge, in the assessment of toluene diisocyanate (TDI) asthma, we evaluated 75 subjects exposed to TDI with work-related respiratory symptoms. Subjects were submitted to bronchial challenge with methacholine at first, thereafter to UNDW inhalation and to specific challenge with TDI. The diagnosis of TDI-asthma was made in 30 of 75 patients (40 percent) who developed a bronchoconstrictive response to the specific challenge (reactors). Sensitivity and specificity of UNDW alone, methacholine alone, and of the combination of the two tests were determined with the results of the specific challenge with TDI as the "gold standard." Both frequency and severity of bronchoconstrictive response to UNDW (FEV₁ decrease ≥15 percent) and the degree (PD15 FEV₁) and frequency of bronchial hyperresponsiveness to methacholine were significantly higher in TDI reactors than in nonreactors. The UNDW had higher specificity (82.2 percent vs 51.1 percent) but lower sensitivity (40 percent vs 76.7 percent) than methacholine. The combination in parallel (positivity of any of the two challenges) of methacholine and UNDW challenge did not change sensitivity to a great extent (80 percent vs 76.7 percent), whereas combination in series (positivity of both challenges) had considerably greater specificity (86.7 percent vs 51.1 percent) than methacholine alone. We conclude that in the assessment of TDI-asthma, the validity of UNDW challenge alone is limited since it is insufficiently sensitive. Instead, combining UNDW and methacholine challenge when methacholine is positive improves our ability in identifying subjects with TDI-asthma diagnosed with the specific challenge. This procedure constitutes a first objective confirmation of a suggestive history of TDI-asthma that is useful for clinical purposes. However, especially for medicolegal purposes, the definitive diagnosis requires the specific challenge. (Chest 1993; 104:1127-32)

In the diagnosis of occupational asthma, the objective confirmation of a suggestive history has been proved necessary both for clinical and medicolegal purposes.¹ In diagnosing toluene diisocyanate (TDI)-asthma, the specific challenge is considered to be the "gold standard,"² but it is time-consuming and can be carried out only in specialized centers. The value of basal (before challenge) nonspecific bronchial responsiveness in assessing TDI-induced asthma and in predicting TDI sensitization in exposed subjects has been widely investigated by means of methacholine challenge.³-¹⁰ The bulk of studies indicates that this value is limited. Bronchial hyperresponsiveness to methacholine is not always present in TDI-induced asthma and TDI-asthma may exist also without bronchial hyperresponsiveness to methacholine.¹⁰,¹¹ Moreover, exposed individuals with bronchial hyperresponsiveness to methacholine may not have occupational asthma due to TDI,⁴-⁷ and in particular, the test lacks specificity. Bronchial responsiveness can be detected also by so-called "indirect" stimuli,¹² such as ultrasonic nebulization of distilled water (UNDW, "fog") or cold air, which provoke bronchoconstriction through mechanisms different from methacholine, by contrast called "direct." These mechanisms are presently thought to involve the release of inflammatory mediators,¹³-¹⁷ and this different pathway of response might more clearly reflect clinical disease.¹² A recent investigation by Brooks et al¹⁸ in platinum refinery workers has suggested that cold air challenge testing is useful in evaluating asymptomatic workers with positive platinum skin tests. Thus, it can be suggested that ultrasonic fog, a test also proved to be more specific than methacholine in nonoccupational asthma,¹⁹-²² could be of clinical help in assessing TDI-asthma in symptomatic exposed subjects. At present we are unaware of studies with ultrasonic fog in TDI-asthma.

In this study, we first aimed to determine the validity of UNDW challenge, in comparison to methacholine, in the assessment of TDI-asthma diagnosed with the specific challenge as the gold standard. Second, we attempted to evaluate whether the combination of methacholine and UNDW could be of any advantage for the same goal.

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PD15FEV₁ = methacholine dose capable of causing a 15 percent fall in FEV₁; TDI = toluene diisocyanate; UNDW = ultrasonic nebulization of distilled water
Materials and Methods

Subjects
We examined 75 patients (64 male and 11 female) referred to our units because of probable occupational asthma (Table 1).

All subjects had a history of occupational exposure to TDI and reported respiratory symptoms (shortness of breath, wheezing, chest tightness, or dry cough) related in some way to exposure. No subject experienced such symptoms before exposure to isocyanates. Most subjects were employed in the polyurethane plastic industries or were self-employed carpenters; a smaller fraction were workers employed in the furniture industry or were self-employed in auto body repair.

Each subject underwent clinical interview to establish type of job and exposure, symptoms, relationships between occupational exposure and symptoms, duration of occupational exposure, 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 three pack-years, and nonsmokers.

At the time of the diagnosis of occupational asthma, each patient underwent pulmonary function tests and bronchial challenge with methacholine, UNDW, and TDI.

All subjects had been free of clinical symptomatic respiratory infection for at least 2 weeks. No subject took anticholinergic, adrenergic bronchodilator, or sodium cromoglycate within the 24 h preceding each study day, nor did they take slow-release theophylline or nonsteroidal anti-inflammatory drugs within 48 h. No patient was taking steroids at the time of testing.

Forty-one subjects underwent intradermal skin tests with a panel of 11 common allergens extracts (Phleum pratense, Dactylis glomerata, Rhietaeta officinalis, Rhietaeta judaica, Artemisia vulgaris, Olea europea, Dermatophagoides pteronyssinus, Dermatophagoides farinae, Aspergillus fumigatus, Alternaria tenuis, and cat dander).

A subject was considered to be atopic if there was a personal history of eczema or allergic rhinitis, or a positive skin reaction (wheat >5 mm) to one or more allergens.*

Pulmonary Function Tests
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 1 s (FEV1) were taken as the best of three satisfactory respiratory tracings.

Bronchial Challenge With Methacholine
The challenge was performed as previously described9 on a day before "fog" challenge, within the same week.

Briefly, methacholine (Lofarma Allergeni, Milan, Italy) was delivered by a nebulizer (Mefar, Brescia, Italy) connected to a dosimeter. The nebulizer was filled with 2 ml of methacholine solution 0.2 percent or 1 percent) or diluent control, after its passage through a 0.2 polymer (Millipore) filter. Five inhalations were administered with phosphate-buffered saline solution and for each increasing, doubling dose of methacholine, administered at 2-min intervals, from 30 to 3,200 μg maximal cumulative dose until a fall in FEV1 ≥15 percent from the highest postsaline solution control level was observed. FEV1 was measured 2 min after each series of inhalations. The final datum obtained was the methacholine dose causing a 15 percent fall in FEV1.10 A subject was considered to be hyperresponsive if he had a PD15FEV1 <850 μg.10

Bronchial Challenge With UNDW
We measured FEV1 before and 5, 15, and 30 min after inhalation of UNDW generated by an ultrasonic nebulizer (Devillbis 65 Ultrasonic Nebulizer, Devilbliss, Somerset, PA) at a flow of 4 L/min.16,17 Patients inhaled through a mouthpiece connected to a three-way valve breathing at tidal volume for 5 min. The output of the nebulizer was measured by simulating ventilation by means of a pump. The pump was connected to the nebulizer through a three-way valve. We measured the difference in weight of the cannister containing distilled water, tubing, and valve before and after simulated ventilation. We carried out ten determinations at different levels of simulated ventilation. The mean difference in weight was 1.35 g/L of airflow (SEM 0.14).

FEV1 was measured using a computerized (M 24 Olivetti) water-sealed spirometer (Biomedin, Padova, Italy). Each time FEV1 was taken as the best of three satisfactory tracings.

FEV1 values after challenge were expressed as the percent FEV1, variations as compared with baseline (100 × [post – pre]/pre).

The test was considered positive when a fall of at least 15 percent in FEV1 after challenge was recorded.

Specific Bronchial Challenge
The TDI challenge4 was carried out by exposure to an atmosphere containing 0.011 ppm (SD 0.008) TDI in a 7.2-m3 exposure chamber.4 Exposure was terminated after 30 min or the onset of asthma, whichever occurred first. The TDI atmosphere was generated by controlled evaporation of 2 μl of pure TDI in a steel pot. A fan in the chamber ensured adequate mixing and circulation. Quantitation of TDI atmosphere was accomplished by a TDI monitor (model 7000, UEL, Poole, England). Respiratory function was monitored measuring the FEV1, before (0) and at 15, 30, min, then hourly after exposure for the next 8 h. The test was considered positive when a decrease in FEV1, of at least 15 percent from baseline was observed within 30 min (immediate response or immediate component of a dual response), and 1 or more h (late response or late component of a dual response).

To exclude a spontaneous variation of pulmonary function, FEV1, was recorded at the same time points of specific challenges on a nonchallenge control day in each patient.

Expression and Analysis of Data
The methacholine dose capable of causing a 15 percent fall in FEV1, (PD15FEV1), was calculated by interpolating on a semilogarithmic dose-response curve plot the point corresponding to the dose causing the FEV1, fall just below 15 percent with the point corresponding to the dose causing the FEV1, fall just above it.

FEV1 values after UNDW challenge were expressed as the percent FEV1, variation as compared with the baseline (100 × [post – pre]/pre). The test was considered positive when a decrease in FEV1, of at least 15 percent as compared with the baseline was observed.

Sensitivity (percent of patients 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 with TDI as the "gold standard." The two tests were also combined to enhance sensitivity or specificity.* Combination in series was considered a subject positive if he tested positive to both methacholine and UNDW, negative if he tested negative to any; combination in parallel labeled a subject positive if he tested positive to any 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 χ2 test where it was appropriate. A level of greater than 95 percent probability was considered to be significant.

Results
Thirty of 75 subjects (40 percent) developed a bronchoconstrictive response to TDI inhalation (reactors), 45 subjects (nonreactors) did not (Table 1). Between TDI reactors and nonreactors, no significant
Table 1—Clinical Features of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>Reactors</th>
<th>Nonreactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>No. male</td>
<td>26</td>
<td>38</td>
</tr>
<tr>
<td>No. female</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr*</td>
<td>37.3 ± 2.0</td>
<td>42.0 ± 1.5</td>
</tr>
<tr>
<td>FEV₁, % of predicted*</td>
<td>90.0 ± 3.2</td>
<td>87.8 ± 2.1</td>
</tr>
<tr>
<td>Atopy, %†</td>
<td>45</td>
<td>38.1</td>
</tr>
</tbody>
</table>

Smoking habits
- Current smokers, %: 8.3‡, 45.2‡
- Nonsmokers, %: 50.0, 38.7
- Exsmokers, %: 41.7, 16.1

Duration of symptoms before diagnosis, yr*:
- Immediate: 20.6 ± 3.6, 18.8 ± 5.6
- Late: 8 (26.7), 15 (50.0)
- Dual: 7 (23.3)

Duration of exposure, yr*:
- FEV₁ maximal fall after UNDW, %: 15.9 ± 3.3¶, 7.31 ± 1.6¶

*Mean ± SEM.
†Data relative to 41 subjects.
‡p<0.01, χ² test.
§p<0.05, χ² test.
¶p<0.01, Mann-Whitney U test.
*Data relative to 27 subjects.
tp<0.05, Kruskal-Wallis analysis of variance.

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

<table>
<thead>
<tr>
<th></th>
<th>Immediate Response</th>
<th>Late Response</th>
<th>Dual Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>8</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>No. male</td>
<td>8</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>No. female</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Age, yr*</td>
<td>33.6 ± 4.3</td>
<td>36.5 ± 2.9</td>
<td>43.0 ± 1.6</td>
</tr>
<tr>
<td>FEV₁, % of predicted*</td>
<td>98.2 ± 3.5‡</td>
<td>92.5 ± 4.4‡</td>
<td>75.5 ± 6.2‡</td>
</tr>
<tr>
<td>Atopy, %†</td>
<td>40.0</td>
<td>54.5</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Smoking habits
- Current smokers, %: 14.3, 8.3, 0.0
- Nonsmokers, %: 57.1, 41.7, 60.0
- Exsmokers, %: 25.6, 50.0, 40.0

Duration of symptoms before diagnosis, yr*:
- FEV₁ maximal fall after UNDW, %: 25.0 ± 8.7, 9.8 ± 3.3, 18.4 ± 5

*Mean ± SEM.
†Data relative to 27 subjects.
tp<0.05, Kruskal-Wallis analysis of variance.

The frequency and severity (maximal mean percent decrease in FEV₁ after UNDW challenge) of the response to UNDW were significantly (p<0.05) higher in TDI reactors than in nonreactors (Table 1, Fig 1), whereas no difference was found among the three groups of reactors with different pattern of response to TDI (Table 2). Comparing each pattern of response to TDI with the group of nonreactors to TDI, we found that the subjects with an immediate component of the response to TDI (immediate plus immediate component of a dual response considered together) significantly differed from nonreactors both in the frequency (53.3 percent vs 17.8 percent) and severity (8.3 percent vs 4.3 percent) of the response to UNDW.

Figure 1. Distribution of the responses to ultrasonic nebulization of distilled water (UNDW) challenge in toluene diisocyanate (TDI) reactors (TDI +) and nonreactors (TDI −).
percent, p<0.02) and in severity of response to UNDW challenge (FEV₁ percent variation = 21.9 [SEM 5.3] vs -7.3 [SEM 1.6], p<0.02). A significant correlation was found between the PD15FEV₁ of methacholine and the maximal decrease in FEV₁ after UNDW inhalation (Spearman correlation coefficient = 0.463, p<0.001). The concordance between the two nonspecific bronchial challenges was 58.7 percent.

Combination of positive and negative responses to methacholine and UNDW had a different distribution in reactors and nonreactors (Table 3), but no difference was found among the three groups of patients with different patterns of response to TDI.

The sensitivity and specificity of UNDW and methacholine in the diagnosis of TDI-asthma with TDI challenge test as the gold standard are illustrated in Figure 2. The UNDW challenge offered the best specificity (82.2 percent), but it lacked sensitivity (40 percent). Methacholine challenge proved to be more sensitive (76.7 percent), but it was less specific (51.1 percent).

We also determined the sensitivity and specificity of combination of UNDW and methacholine. Combination in series (both tests positive) of methacholine and UNDW had considerably greater specificity (86.7 percent) than methacholine alone, and combination in parallel (positivity to any of the two tests) did not substantially change sensitivity, which was similar to methacholine alone (80 percent vs 76.7 percent) (Fig 2).

**Discussion**

The results of our study show that UNDW challenge is a more specific method than methacholine challenge in assessing TDI-asthma diagnosed with the specific challenge as the gold standard, but it is less sensitive. The combination in series of the two tests (both tests positive) substantially enhances specificity and improves our ability in detecting subjects with TDI-asthma. Even combining the two challenges, however, sensitivity and specificity figures are not enough to avoid the specific challenge for the definitive diagnosis.

The clinical features of present cases confirm our previous findings⁴ in a more consistent group of symptomatic subjects occupationally exposed to TDI. Only a proportion of subjects had asthma due to TDI, diagnosed on the basis of a positive response to TDI challenge. This group of patients did not differ from the subjects with a negative response to the specific challenge (nonreactors) in age, sex, or in the characteristics of the occupational history. Atopy did not seem to influence the development of TDI-asthma. In the reactors, respiratory function was not impaired (only 9 reactors had baseline FEV₁ lower than 80 percent of predicted). Asthmatic responses to TDI inhalation were immediate, late, and dual and the late component appeared as a common feature (73.3 percent of the subjects).⁵,⁶ Among the three groups of patients, no difference was found in age, sex, or occupational history. By contrast, baseline FEV₁ was impaired (mean, 75.5 percent of predicted, Table 2) only in the subjects with a dual response, thus supporting the suggestion that a dual response may be a feature of the most advanced stages of asthma.⁴,⁷,⁸,²⁸ Current smokers were all distributed in the groups of patients who developed an immediate or a late response.

The distribution of bronchial hyperresponsiveness to methacholine was significantly different in TDI reactors and nonreactors, without difference regarding the type of asthmatic response. A small group of TDI reactors (7 patients, 23.3 percent) had a normal degree of bronchial responsiveness to methacholine, confirming that a negative challenge cannot exclude the presence of TDI-asthma,¹⁰,¹¹ whereas a high percentage (48.9 percent) of TDI nonreactors had hyperresponsiveness to the pharmacologic stimulus,⁶⁹ confirming that the test lacks specificity.

The features of bronchial responsiveness to UNDW inhalation were interesting. The frequency and severity of positive responses to UNDW inhalation were significantly higher in TDI reactors, as compared with nonreactors, and in the proportion of TDI reactors who showed an immediate component of the asthmatic

**Table 3—Distribution of Combination of Positive and Negative Responses to Methacholine and UNDW in TDI Reactors and Nonreactors**

<table>
<thead>
<tr>
<th></th>
<th>Reactors†</th>
<th>Nonreactors†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mch + , UNDW + (%)</td>
<td>11 (36.7)</td>
<td>6 (13.3)</td>
</tr>
<tr>
<td>Mch + , UNDW - (%)</td>
<td>12 (40.0)</td>
<td>16 (35.6)</td>
</tr>
<tr>
<td>Mch - , UNDW + (%)</td>
<td>1 (3.3)</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td>Mch - , UNDW - (%)</td>
<td>6 (20.0)</td>
<td>21 (46.7)</td>
</tr>
</tbody>
</table>

*UNDW = ultrasonic nebulization of distilled water; Mch = methacholine; TDI = toluene diisocyanate.
†p<0.05, χ² test.

![Figure 2](http://journal.publications.chestnet.org/pdaccess.ashx?url=/data/journals/chest/21676/)

**Figure 2.** Sensitivity and specificity of methacholine (Mch) and ultrasonic nebulization of distilled water (UNDW), alone and in combination.
response to TDI (immediate plus immediate component of the dual considered together), as compared with TDI nonreactors. By contrast, the frequency and severity of positive responses to UNDW did not differ comparing the three patterns of response to TDI (immediate, dual, and late) with each other. These findings suggest that a positive response to UNDW is in some way correlated to a positive response to TDI with an immediate component (immediate alone or dual). Similarly to nonoccupational asthma, \textsuperscript{19-22} UNDW was found less sensitive but more specific than methacholine in assessing TDI-asthma (Table 1). The poor concordance (58.7 percent) between the response to methacholine and UNDW, despite a significant correlation between the degree of the response, suggests that the two challenges have different mechanisms or pathways leading to bronchoconstriction. Unlike methacholine, which acts directly on smooth muscle to cause contraction, UNDW appears to invoke an intermediate event, which is presently thought to be the release of mediators from mast cells in the airway lumen and submucosa.\textsuperscript{13-17,20}

Combination in parallel of methacholine and UNDW challenge did not increase sensitivity to a great extent (80 percent vs 76.7 percent, tests in parallel), whereas combination in series substantially enhanced specificity (86.7 percent vs 51.1 percent, test in series) in identifying subjects with TDI-asthma. This might suggest the following procedure in the assessment of TDI-asthma. In the presence of a suggestive history, methacholine challenge should be performed at first. In case of positivity of this challenge, UNDW can be usefully carried out since, when positive, it improves our ability in identifying subjects with TDI-asthma and constitutes a first objective confirmation of the history. In case of negative methacholine challenge, carrying out UNDW does not substantially improve diagnostic accuracy. However, even when both tests are positive, sensitivity and specificity figures of around 80 percent are not high enough to permit avoiding further diagnostic steps,\textsuperscript{20} in particular the specific challenge, especially for medicolegal purposes.

In conclusion, in the assessment of TDI-asthma, UNDW challenge alone proves more specific than methacholine, but it is not sensitive enough to be recommended. Instead, combining UNDW and methacholine challenge, when methacholine is positive, improves our ability in identifying subjects with TDI-asthma diagnosed with the specific challenge as the gold standard. To our knowledge, this procedure constitutes a first objective confirmation of a suggestive history of TDI-asthma which is useful for clinical purposes. However, especially for medicolegal purposes, the definitive diagnosis requires the specific challenge.

**References**

Anesthesiology (part VI)

The Department of Anesthesia, Hospital of the University of Pennsylvania, offers this course November 13-14 at the Penn Tower Hotel, Philadelphia. For information, contact Ms. Janice Ford, Hospital of the University of Pennsylvania, 1 Silverstein Building, 3400 Spruce Street, Philadelphia 19104 (215:662-6904).

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