

Original Article

Long-Term Follow-Up of Cluster-Based Diisocyanate Asthma Phenotypes

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What is already known about this topic? Asthma is a heterogeneous disease consisting of different phenotypes. In a previous study, we found that occupational asthma (OA) due to diisocyanates is also heterogeneous at diagnosis and is broken into 3 clusters. There are no data on the outcome of cluster-based diisocyanate asthma phenotypes.

What does this article add to our knowledge? Age at diagnosis is the strongest predictor of diisocyanate OA remission. Cluster allocation at diagnosis seems to have an impact on the outcome of diisocyanate OA because patients in cluster 2 showed the most favorable prognosis.

How does this study impact current management guidelines? Age at diagnosis could be used as a prognostic tool in OA. Therefore, early recognition of the disease by specific inhalation challenge should be encouraged.

BACKGROUND: Data on the outcome of occupational asthma (OA) are heterogeneous.

OBJECTIVE: To assess the impact of being part of a specific cluster at diagnosis on the long-term outcome of diisocyanate-induced OA.

METHODS: We collected data from 56 patients who had a diagnosis of OA confirmed by a positive specific inhalation challenge. Patients sensitized to toluene diisocyanate were allocated to cluster 1 or 2 based on a tree analysis, using the 3 variables relevant for cluster segregation identified in a previous study: age, body mass index, and forced expiratory volume in 1 second/forced vital capacity at diagnosis. Patients sensitized to methylene diisocyanate were allocated to cluster 3, as in previous study. We defined OA remission when a patient had met a total of 3 criteria: no asthma symptoms and no antiasthma therapy for the last year, as well as having normal lung function.

RESULTS: At follow-up, 16 patients showed OA remission. They exhibited better lung function, less bronchial hyperreactivity, as well as younger age at diagnosis. Twenty-eight patients were allocated to cluster 1, 10 to cluster 2, and 18 to cluster 3. The percentage of patients with OA remission was higher in cluster 2 (50% vs 25% in cluster 1 and 22.5% in cluster 3),

although the difference was not statistically significant ($P = .2789$).

CONCLUSIONS: Age at diagnosis was a strong predictor of OA remission. The outcome of diisocyanate OA tended to be more favorable for patients with toluene diisocyanate OA allocated in cluster 2, but this finding needs to be validated by further data. © 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2021;■:■-■)

Key words: Cluster analysis; Diisocyanates; Phenotypes; Outcome; Long-term follow-up; Occupational asthma prognosis

INTRODUCTION

Occupational asthma (OA) is a type of asthma in which the inciting agent is well known. The causes of OA can be classified as high-molecular-weight and low-molecular-weight agents. Among the latter, diisocyanates are the most common low-molecular-weight causal chemicals in industrialized countries, as documented by the recent European study E-PHOCAS (European network for the PHenotyping of OCcupational Asthma).¹ For years, non-OA has been recognized as being a heterogeneous disease, with 4 or 5 phenotypes identified by different clustering techniques.²⁻⁴ The clinical relevance of clusters in adult-onset asthma remains undetermined because it is unknown whether the long-term outcome of the disease depends on cluster allocation.^{5,6} In 2 previous studies,^{7,8} we found that OA due to diisocyanates is heterogeneous at diagnosis and is composed of 3 clusters, which is fewer than usually described in patients with non-OA. Even though many studies have been performed, the evolution of OA many years after diagnosis is still unclear. Here, we present a long-term follow-up to assess the evolution of OA and to investigate whether being part of a specific cluster at diagnosis has any implication on the outcome of diisocyanate OA.

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Abbreviations used

BMI- Body mass index
 FeNO- Fractional exhaled nitric oxide
 FEV₁- Forced expiratory volume in 1 second
 FVC- Forced vital capacity
 HDI- Hexamethylene diisocyanate
 IgE- Immunoglobulin E
 MDI- Methylene diisocyanate
 OA- Occupational asthma
 PD₂₀- Provocative dose causing a 20% fall
 SIC- Specific inhalation challenge
 TDI- Toluene diisocyanate

METHODS**Subjects**

Patients included in the study had a diagnosis of OA induced by diisocyanates as confirmed by a positive specific inhalation challenge (SIC) between 1988 and 2016. At diagnosis, exclusion criteria were a history of upper respiratory infection, asthma exacerbation, use of inhaled or oral corticosteroids in the 4 weeks prior to the study, or use of long-acting bronchodilators within 48 hours of the test. Between 2013 and 2018, patients were asked to come to our center for a follow-up visit. All subjects signed informed consent documents prior to participation and the study received ethics approval (Comitato Etico per la Sperimentazione, Azienda Ospedaliera di Padova, project approval number 3930/AO/16).

Study design

At the time of diagnosis, patients were assessed on 4 consecutive days. On day 1, clinical and occupational data were collected, skin prick tests with common aeroallergens were performed, as well as pulmonary function tests (including fractional exhaled nitric oxide [FeNO] measurements). Atopy was defined as at least 1 positive response (wheal mean diameter ≥ 3 mm, after subtracting negative control) to aeroallergens. On day 2, a single-blind sham inhalation challenge was conducted. On day 3, the subjects underwent SIC with the appropriate agent (ie, toluene diisocyanate [TDI], methylene diisocyanate [MDI], or hexamethylene diisocyanate [HDI]). The type of agent to test was chosen according to each subject's occupational exposure, safety data sheet, and medical history. Lung function was monitored for 7 hours after sham and exposures to occupational agents. At the end of sham and active challenges, airway responsiveness to methacholine was assessed. On day 4 (24 h after SIC), lung function and FeNO were assessed again. Sputum induction was performed on days 1 and 4. Subjects did not return to work until the end of the study.

At least 2 years after the diagnosis of OA, patients were contacted for a follow-up visit, in which clinical and occupational data were retrieved, and forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), and FeNO were measured. When appropriate (ie, in the case of absence of moderate to severe obstruction), airway responsiveness to methacholine aerosol was assessed. All patients with an ongoing therapy for asthma paused treatment (inhaled corticosteroid and/or long-acting bronchodilators) within the preceding 48 hours. Asthma control was defined by the Asthma Control Test (ACT) score (range, 5-25). This questionnaire involves 5 questions regarding activity limitations, frequency of symptoms, and frequency of use of quick-relief medication in the past 4 weeks.⁹

As per the Expert consensus framework for asthma remission,¹⁰ asthma was considered in complete remission (off-treatment) at follow-up when 3 criteria were met in the previous year: no asthma

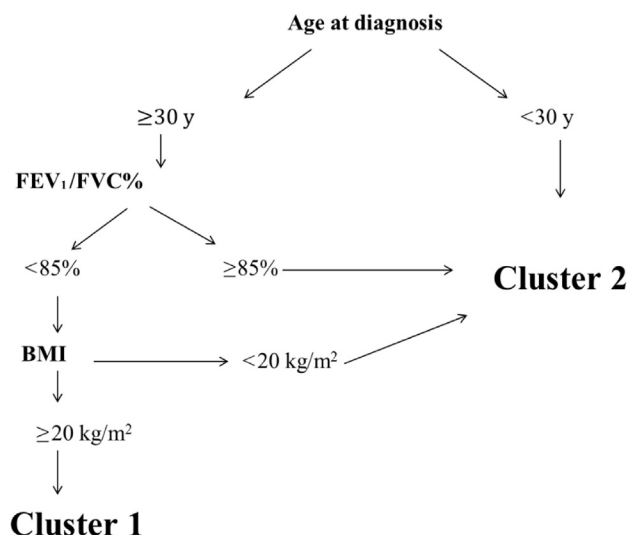


FIGURE 1. Tree analysis. Using 3 variables (age at diagnosis, FEV₁/FVC%, and BMI) subjects with TDI asthma were assigned to cluster 1 or 2.

symptoms (including an ACT score ≥ 20), no antiasthma therapy, and normal lung function.

Lung function and bronchial challenges

We assessed FVC, FEV₁, and airway responsiveness to methacholine aerosol as described previously.¹¹ Bronchial hyperreactivity results were expressed as the cumulative provocative dose causing a 20% fall in FEV₁ (PD₂₀FEV₁ in micrograms of methacholine). Online measurements of FeNO were made according to American Thoracic Society (ATS) recommendations using a chemiluminescence analyzer (NIOX MINO Aerocrine) at the standard expiratory rate of 50 mL/s. We performed SICs with isocyanates as described previously.¹¹ Briefly, subjects were exposed to isocyanate vapor (mean $\pm$ standard error concentration, 10 ± 3 ppb) for a maximum of 60 minutes in an exposure chamber. The concentration of isocyanate was continuously monitored (Single Point Monitor; Zellweger Analytics Inc, Lincolnshire, Ill). An asthmatic reaction was considered to occur when FEV₁ decreased by at least 20% from baseline within 1 hour (early reaction) or greater than 1 hour (late reaction) or both (dual reaction) after exposure to inciting agents. The SIC was also considered positive when the fall in FEV₁ did not reach 20% or greater but there was at least a 2-fold decrease in PD₂₀ compared with the baseline value, as suggested by the European Respiratory Society (ERS) Task Force Report on OA.¹²

Previous cluster analyses and our new cluster allocation algorithm

In our previous study,⁸ we applied an unsupervised cluster analysis to a cohort of 187 patients with diisocyanate-induced OA. This identified 3 clusters: clusters 1 and 2 included patients sensitized to TDI, and cluster 3 included patients sensitized exclusively to MDI.

We found that the allocation of TDI-sensitized patients in cluster 1 or 2 was determined by 3 main variables: age at diagnosis, body mass index (BMI), and FEV₁/FVC% at baseline.

In the present follow-up study, we analyzed 17 patients who were not included in the previous study and, thus, not

TABLE I. Characteristics of the 56 subjects at diagnosis and follow-up*

Variable*	Diagnosis	Follow-up	P value
Age at diagnosis (y)	42 (15-57)	51.5 (22-76)	<.0001
Age at onset of OA symptoms (y)	36 (14-57)	-	
Months of exposure before onset of symptoms	42 (1-348)	-	
Months of symptomatic exposure before diagnosis	36 (1-264)	-	
Gender (M/F)	43/13	-	
BMI (kg/m ²)	24 (17.2-32.9)	25.1 (19.4-33.2)	<.0001
Smoking status (yes/no/ex)	11/26/19	6/26/24	.1718
Atopy	23	-	
Eosinophils in blood ($\times 10^{-9}/L$)	0.3 (0.1-1.2) (21 patients)	-	
Agent (TDI/MDI/HDI)	37/18/1	-	
FVC% predicted	96 (77.7-122.8)	94 (76.1-127)	.1382
FEV ₁ % predicted	92.6 (61.1-128.3)	93.5 (64-128.5)	.3421
FEV ₁ /FVC%	73.7 (52-92.5)	74 (50-100)	.1370
PD ₂₀ (μ g)	529 (49-1440)	1440 (67-1440)	<.0001
FeNO (ppb)	46.4 (4.8-183.3)	27 (5-123)	.0023
ICS intake, μ g/d	0 (0-1000)	86 (0-1500)	.3838

ICS, inhaled corticosteroids.

*Values are given as n, %, or median (range).

TABLE II. Characteristics of patients with OA remission versus patients with active OA

Variable*	OA remission (n = 16)	Active OA (n = 40)	P value
Age at diagnosis (y)	35.5 (15-49)	43 (19-57)	.0089
Duration of follow-up (y)	11.5 (2-29)	9 (2-28)	.1783
Months of exposure before onset of symptoms	24 (1-348)	48 (3-288)	.1444
Months of symptomatic exposure before diagnosis	24 (3-84)	36 (1-264)	.0897
Gender (M/F)	13/3	30/10	.7367
BMI at diagnosis (kg/m ²)	23.5 (17.2-30.2)	24 (19.2-32.9)	.2277
BMI at follow-up (kg/m ²)	25.5 (19.5-30.2)	25 (19.4-33.2)	.3856
Smoking status at diagnosis (yes/no/ex)	6/7/3	5/19/16	.0746
Smoking status at diagnosis (yes/no/ex)	3/7/6	3/19/18	.4819
Atopy (n)	8	15	.3591
Eosinophils in blood at diagnosis ($\times 10^{-9}/L$)	0.2 (0.1-0.3) (3 patients)	0.3 (0.1-1.2) (18 patients)	.3141
Agent (TDI/MDI/HDI)	12/4/0	25/14/1	.6689
FVC% predicted, at diagnosis	101.4 (80-122.5)	94 (77.7-120)	.0214
FEV ₁ % predicted, at diagnosis	102.8 (81-122.5)	91.3 (61.1-128.3)	.0100
FEV ₁ /FVC%, at diagnosis	78.1 (70-91.6)	72.5 (52-92.5)	.0049
PD ₂₀ (μ g), at diagnosis	1,429 (68-1,440)	360 (23-1,440)	.0310
FeNO (ppb), at diagnosis	44 (7-60)	55 (4.8-183.3)	.3930
ICS intake, μ g/d at diagnosis	0 (0-0)	200 (0-1,000)	.0088
FVC% predicted, at follow-up	96.8 (84.7-120.7)	94 (72-127)	.1616
FEV ₁ % predicted, at follow-up	103 (78.9-128.5)	90.8 (64-119)	.0013
FEV ₁ /FVC%, at follow-up	78 (62-91)	71 (50-100)	.0266
PD ₂₀ (μ g), at follow-up	1,440 (1,440-1,440)	1,440 (67-1,440)	.0062
FeNO (ppb), at follow-up	13.5 (5-27)	32 (8-141)	.0011
ICS intake, μ g/d at follow-up	0 (0-0)	92 (0-1500)	.0001
ACT score at follow-up	25 (20-25)	23 (9-25)	.0001

*Values are given as n, %, or median (range).

ACT, Asthma challenge test; ICS, inhaled corticosteroids.

yet allocated to a specific cluster at diagnosis. Considering our aim to assess whether patient cluster affects diisocyanate OA outcome, we performed a tree analysis to allocate the new patients, as in the Severe Asthma Research Program (SARP) study.³ We used the 3

variables previously described⁸ for allocation to cluster 1 or 2 (Figure 1). This simplified algorithm assigned 96.7% of TDI subjects in the previous cohort (151 out of 156) to the appropriate cluster.

TABLE III. Characteristics of the clusters of diisocyanate-induced asthma at diagnosis and follow-up*

Variable*	Diagnosis			Follow-up			P value between clusters	P value between clusters
	1 (n = 28)	2 (n = 10)	3 (n = 18)	1 (n = 28)	2 (n = 10)	3 (n = 18)	Diagnosis	Follow-up
Cluster number	1 (n = 28)	2 (n = 10)	3 (n = 18)	1 (n = 28)	2 (n = 10)	3 (n = 18)		
Age at diagnosis (y)	45 (40-48)	27.5 (25-29)	41 (37-45)				.0003	
Duration of follow-up (y)				10.5 (5-14)	16.5 (10-25)	7.5 (3-9)		.0463
Months of exposure before onset of symptoms	66 (12-120)	21 (9-54)	30 (12-132)				.1779	
Months of symptomatic exposure before diagnosis	42 (24-84)	15 (9-30)	30 (12-60)				.0824	
Gender (M/F)	21/7	7/3	15/3				.7637	
BMI (kg/m ²)	24.7 (23-26.3)	22 (20.4-24.6)	23.2 (20.9-26.7)	25.6 (23.8-27.5)	25.7 (22.7-28.6)	23.9 (21.2-26.5)	.0691	.2282
Smoking status (yes/no/ex)	4/10/14	2/7/1	5/9/4	1/10/17	1/7/2	4/9/5	.1122	.0405
Atopy (n)	14	4	5				.6235	
Agent (TDI/MDI/HDI)	27/0/1	10/0/0	0/18/0				<.0001	
Exposure to the inciting agent at follow-up (n)				6	0	1		.6524
FVC% predicted	96.3 (89.8-106.8)	89.9 (82-94)	99 (90-100.9)	94 (88.2-100.9)	94.2 (85-98.6)	96 (92-104)	.1587	.7571
FEV ₁ % predicted	91.6 (84.1-100.5)	93.2 (87.4-110.8)	94.7 (84.5-105)	93 (80-103)	96.6 (90-100)	92.5 (86.7-102)	.8023	.8010
FEV ₁ /FVC% observed	72.1 (67.2-77.4)	84 (76.6-86.6)	75.5 (62-80)	71.5 (64.8-76)	79.3 (77-81)	73.5 (66-79)	.0242	.0148
PD ₂₀ (μg)	431 (86.2-1,273)	1,418 (317.2-1,440)	1,003 (297.2- 1,440)	1,440 (881-1,440)	1,440 (1,084.5-1,440)	1,440 (1,440-1,440)	.1997	.4987
FeNO (ppb)	57.5 (36-81)	62.5 (30-95)	46.3 (11-86)	27 (18.2-44.5)	36 (12-100)	27 (16-30)	.5928	.9216
Type of reaction to SIC (early/late/dual/increase in NSBHR/unknown)	1/16/7/4/0	1/2/5/2/0	6/6/3/1/2				.0324	
ICS intake mcg/d	0 (0-200)	0 (0-50)	200 (0-400)	0 (0-160)	0 (0-92)	0 (0-160)	.1535	.6299
Exacerbations (n)				0 (0-0)	0 (0-0)	0 (0-0)		.3323
ACT score at follow-up				23 (20-25)	23 (20-24)	23 (22-25)		.6329
OA remission at follow-up (n)				7	5	4		.2789

ACT, Asthma challenge test; ICS, inhaled corticosteroids; NSBHR, non-specific bronchial hyper-responsiveness.

*Values are given as n, %, or median (range).

This new algorithm was thus applied to allocate all current patients diagnosed with TDI OA to cluster 1 or 2.

All patients sensitized to MDI were allocated to cluster 3, considering that they were clearly segregated in the previous study.

As done previously,⁸ we preferred not to remove an HDI-exposed patient. The subject was included in the TDI-exposed group because she performed the same job as the TDI-exposed patients (spray painters) and because the vapor pressure of HDI is closer to that of TDI than MDI.

Statistical analysis

Continuous variables were reported as median and range, and categorical variables were reported as numbers or percentages. A nonparametric Wilcoxon signed-rank test and paired Student *t* test were used to compare clinical variables at the same time-points in all patients. The comparison between those with OA remission and those without were tested with an unpaired Student *t* test for quantitative variables, and a chi-squared test for categorical/qualitative variables. A backward logistic analysis was conducted on relevant variables to identify discriminant characteristics associated with persistence or remission of the disease.

Differences between clusters at baseline and follow-up were analyzed with chi-squared test for categorical variables and a Kruskal-Wallis test for nonparametric continuous variables. The analysis was performed using a statistical software package (Statview, version 9.1; SAS Institute Inc, Cary, NC). A *P* value of less than .05 was considered significant.

RESULTS

Between 1988 and 2016, 204 patients in our center were diagnosed with OA induced by diisocyanates with a positive SIC. We excluded 148 patients because we were unable to retrace them or they refused to participate in the follow-up visit. Only patients with at least 2 years of follow-up since OA diagnosis were included in the study.

The clinical features of the participants both at the time of diagnosis and follow-up are shown in Table I. Thirteen females and 43 males with a mean age at diagnosis of 42 years (15-57 years) were included in the study. The average duration of follow-up was 9.5 years (2-29 years), and the most represented causal agent was TDI (66.1%).

A low percentage of patients (12.5%) were still exposed to the inciting agent at the follow-up visit, but in all cases, the exposure was reduced (compared with that at diagnosis) by product replacement for most working hours (eg, isocyanate-free varnish instead of solvent-based varnish). At diagnosis, 16 patients exhibited airflow obstruction (13 mild, 3 moderate). At follow-up, 19 subjects showed an obstruction (16 mild, 3 moderate); 33 patients underwent methacholine challenge. The degree of bronchial hyperreactivity was lower than that at diagnosis ($P < .0003$), and FeNO was significantly reduced at follow-up ($P = .0023$) (Table I).

At follow-up, 39.3% of patients were free from therapy: 30.4% of patients used a combination of inhaled bronchodilators and inhaled steroids, 12.5% used inhaled steroids, 14.3% used short-acting bronchodilators as needed, and 3.5% used exclusively long-acting and short-acting bronchodilators. A small but statistically significant increase in BMI was observed at follow-up ($P < .0001$).

The clinical features of the participants with a remitted or active OA at follow-up are shown in Table II. Sixteen patients

were in OA remission at follow-up. They were younger at diagnosis ($P = .0089$) and showed better lung function expressed with a higher FVC% predicted ($P = .0214$), FEV₁% predicted ($P = .0100$), FEV₁/FVC% ($P = .0049$), and with less bronchial hyperreactivity at baseline ($P = .0310$), than those with active OA. No differences were detected in terms of FeNO ($P = .3930$) or among the other variables analyzed, gender included.

A logistic regression analysis was performed to evaluate the effects of the covariates, which were significantly different between patients with asthma remission and those with active OA in the univariate model, for the dependent variable OA remission at follow-up. Age at diagnosis was a significant predictor of OA remission ($P = .0550$; odds ratio 1.073; 95% confidence interval 1.002-1.160).

The clinical features of patients in the 3 clusters both at the time of diagnosis and at follow-up are shown in Table III. We found some differences among clusters of diisocyanate-induced asthma in terms of duration of follow-up ($P = .0463$), FEV₁/FVC% ($P = .0148$), and smoking habit ($P = .0405$). Cluster 2 patients had the longest duration of follow-up and exhibited better lung function both at diagnosis and at follow-up. Cluster 3 included more active smokers than clusters 1 and 2. The percentage of patients with OA remission was higher in cluster 2 (50% vs 25% in cluster 1 and 22.5% in cluster 3, respectively), but the difference between the 3 clusters was not statistically significant ($P = .2789$). Latency (ie, months of exposure before onset of symptoms), symptomatic exposure period, gender, BMI, atopy, exposure to the inciting agent at follow-up, inhaled corticosteroid daily intake (micrograms), FVC (% predicted), FEV₁ (% predicted), PD₂₀FEV₁, and FeNO values were not significantly different between clusters.

DISCUSSION

Our study shows that younger age at diagnosis is the main predictor of a good diisocyanate OA prognosis. Furthermore, we demonstrated that being part of a specific cluster of TDI-induced OA at diagnosis is associated with some differences at follow-up in terms of lung function and remission of the disease, although these differences were not statistically significant.

A systematic review¹³ including 35 articles concluded that the contributors of a worse outcome of OA are older age, high-molecular-weight agents, impaired lung function, and longer duration of exposure to the offending agent at time of diagnosis. Our results highlight the relevance of younger age at diagnosis and better lung function as important determinants of diisocyanate OA outcome, as was also observed in non-OA.¹⁴⁻¹⁷ In subjects with OA, it is generally accepted that continuation of work exposure to causal agents is associated with increased loss of lung function.¹³ In contrast, whether an accelerated decline in FEV₁ occurs after cessation of exposure is still controversial. In our study, in which most patients ceased their exposure to inciting compounds, we did not detect an excessive decline in FEV₁% predicted after a long-term follow-up.

Previous reviews¹⁸⁻²¹ suggested that removal from exposure represents the appropriate management for a best medical outcome, but whether cessation of exposure is more effective in improving the overall OA outcome than reduction of exposure is still a controversial issue, especially when taking into account the socioeconomic burden of an OA diagnosis. Our study cannot

shed light on this topic owing to the low number of workers with continued exposure.

Our patients exhibited a lower degree of bronchial hyperactivity at follow-up than at diagnosis, which is in agreement with previous investigations.^{22,23}

The rate of recovery of the disease in our cohort (28.5%) is also in accordance with previous OA studies,^{22,24} and is higher than that detected (15%) in adult-onset asthma (≥ 20 years) in a large Italian cross-sectional study.²⁵ Reduced exposure to the inciting agent may be one of the reasons for a better outcome of OA compared with non-OA, where total avoidance is generally not feasible given that the inciting agents are ubiquitous or unknown.

Some previous studies on OA investigated the changes in airway inflammation at diagnosis and follow-up by means of induced sputum analysis,^{26,27} which is a reliable tool but more difficult to assess than FeNO measurements. In our cohort, the success rate of sputum induction was often inadequate, so we relied on FeNO levels as a surrogate biomarker of eosinophilic airway inflammation. The FeNO changes in OA over multiple years have not been assessed previously. Intriguingly, FeNO levels at diagnosis were similarly high among patients who afterward went into OA remission and those who did not. However, FeNO levels at follow-up normalized in patients whose OA remitted but remained elevated in patients with active OA. The interference of treatment on airway inflammation and nitric oxide synthase activity was excluded because the subjects were free from corticosteroids for at least 48 hours at the time of diagnosis and follow-up. Thus, eosinophilic inflammation might have more impact on the evolution of the disease than on its inception.

The present study is the first to propose a tree diagram, using 3 clinical variables, to phenotype TDI asthma subclasses. This method showed a higher percentage of appropriate assignment (96.7%) than that described by Moore et al in the SARP study (80%).³

To our knowledge, this is also the first investigation to directly compare whether being part of a specific OA cluster at diagnosis is associated with a different outcome after a long-term follow-up.

It is interesting to note that no differences in outcome were detected between MDI- and TDI-sensitized patients. Thus, the role of subtle chemical differences within the same class of agents might be relevant for diagnosis and cluster differentiation, but not for disease progression.

Although bronchial hyperresponsiveness and FeNO levels improved more in cluster 1, the proportion of patients with OA remission was higher in cluster 2. This is reasonable because patients in cluster 2 exhibited all the features that were related to better OA outcome in previous studies: younger age at diagnosis, shorter symptomatic exposure, better lung function at baseline, and not being exposed to the causal agent at follow-up. Other plausible differences between clusters 1 and 2 were not detected. Indeed, the proportion of patients diagnosed between 1988 to 2001 and 2002 to 2016 was not significantly different between the 2 clusters. In addition, most of the patients were spray painters in both clusters (cluster 1: 71%; cluster 2: 80%), which negates the hypothesis that the 2 clusters represent chronologically distinct occupational periods or different types of exposure at work.

In a previous follow-up study on diisocyanate OA,²⁸ the patients having displayed at diagnosis immunoglobulin E (IgE) antibodies to isocyanates used less medication and had fewer symptoms of asthma than the IgE-negative ones. A more

favorable outcome was associated with IgE mediation and also with HDI inducement. Unfortunately, our study cannot shed light on this topic because we did not determine specific IgE antibodies to diisocyanates and only 1 patient showed HDI asthma.

Our study has several strengths. Firstly, the gold standard SIC was used for diagnosis of OA and identification of the causal agent, thus avoiding the risk of misclassification of the disease. Second, the follow-up was sufficiently long and was based on clinical and functional assessments made in the same center with the same methodology. Third, all patients were exposed to the same class of chemicals (diisocyanates), thus avoiding the interference of stimuli acting through different mechanisms. In addition, major interference of treatment on lung function at diagnosis was excluded because the subjects had been free from corticosteroids for at least 4 weeks at diagnosis and 48 hours at follow-up.

Some limitations include the lack of information on the levels of exposure, which is frequently missing in retrospective occupational studies. Furthermore, we unfortunately have insufficient data on sputum cells and other inflammatory biomarkers that could help identifying possible OA endotypes. Although the size of our cohort is comparable with many previous studies on OA, we were able to obtain follow-up data from only 56 of the 204 potentially eligible patients and cannot exclude some selection bias. Lastly, this study lacks a severe asthma phenotype. However, we believe that this is a feature of OA and not an effect of exclusion of individuals from completing the SIC diagnostic process; in fact, not performing SIC owing to severe airway obstruction has been a rare event in our center for many years.

The findings of this analysis suggest that the prompt recognition and diagnosis of OA are essential for a good prognosis of the disease, with younger age and better lung function at diagnosis being the most relevant characteristics of patients with disease remission.

The results also suggest that patients should be encouraged by health professionals to discuss the possible association between their respiratory symptoms and work. Indeed, communication with occupational physicians might help with early identification and avoiding exposure in the workplace and, thus, with better management of the disease.²⁹

We also underlined that the use of cluster analysis in asthma due to diisocyanates at diagnosis might help in predicting its long-term outcome, but more data are needed to confirm this.

Further immunological studies are also necessary to clarify whether these phenotypes indeed represent distinct endotypes of OA.

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