Distinct Clinical Phenotypes of Occupational Asthma due to Diisocyanates

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Objective: To assess whether diisocyanate occupational asthma represents a unique phenotype. **Methods:** We studied 187 patients with diagnosis of asthma due to diisocyanates confirmed by a positive specific inhalation challenge. The simplified algorithm from severe asthma research program (SARP) (Moore et al, 2010) was applied to classify patients into five clusters. **Results:** Our patients were allocated in three of the five clusters described in common asthma, since the most severe Clusters (4 and 5) were not represented. Cluster 2 was the most populated, as in common asthma, and included the youngest patients with the shortest duration of exposure to the sensitizers. Cluster 3 included older men patients with worse lung function and longer occupational exposure. **Conclusions:** Diisocyanate asthma is a heterogeneous disease. Differences across clusters include demographic characteristics, lung function, and chronology of diisocyanate exposure.

Diisocyanates represent the major cause of occupational asthma (OA) due to low molecular weight agents in many industrialized countries.[1] Although this type of OA is due to a single class of chemicals there are differences in time of onset, severity, and outcome. This suggests a heterogeneity of this form of OA.

Heterogeneity is well recognized in common asthma and has been described by several studies using unsupervised multivariate mathematical approach.[2–5]

The National Heart Lung and Blood Institute sponsored the severe asthma research program (SARP) to compare subjects with mild to moderate asthma.[2] A cluster analysis of these patients was performed to evaluate the range of phenotypic heterogeneity.[6] The analysis was complex due to the great number of variables considered at baseline. Thirty-four variables were selected and five clusters were identified. Afterwards a tree analysis was performed using subsets of these variables to assess classification of subjects. Using only three of the initial 34 variables, 80% of patients were successfully assigned to the appropriate cluster. These three variables were: baseline % of predicted forced expiratory volume in 1 second (% predicted FEV_1), maximal % predicted FEV_1, and age of asthma onset. The clinical relevance of this simplified algorithm and the fixity of the described five groups have been studied also in asthma within an urban population by Patrawalla et al.[5] Their analysis supported the use of the simplified SARP algorithm by reproducing groups with similar phenotypic characteristics to those resulted in the SARP population. Only one study has been conducted on clusters of OA[7] and little is known on phenotypes of OA due to a single class of chemicals such as diisocyanates. We hypothesized that this type of OA could have different phenotypes as it has been shown in common asthma. This hypothesis was assessed by applying the simplified SARP algorithm in a relatively large cohort of patients with diisocyanate-induced asthma.

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METHODS

Subjects

Patients who had a diagnosis of occupational asthma confirmed by a positive specific inhalation challenge (SIC) with diisocyanates between 1988 and 2013 were included in our study. The positive response to SIC included an early asthmatic reaction, a late asthmatic reaction or both (dual reaction). All subjects signed informed consent to perform the procedures for the diagnosis of OA, including SIC. The local Ethic Committee approved the use of the retrospective data of the patients (n. 3930/AO/16).

Study Design

Subjects were examined on 4 consecutive days as previously described.[8] On day 1, clinical and occupational data were collected, skin prick tests with common aeroallergens, and pulmonary function were performed, including pre- and post-bronchodilator (BD) spirometry. Post-BD spirometry was thus obtained 15 minutes after salbutamol inhalation (200 to 400 μg). Atopy was defined as at least one positive response (wheal mean diameter greater than or equal to 3 mm, after subtracting negative control) to aeroallergen. On day 2, a single-blind sham inhalation challenge was performed. On day 3, the subjects underwent SIC with the appropriate isocyanate (ie, toluene diisocyanate, TDI; methylene diisocyanate, MDI, or hexamethylene diisocyanate, HDI). The type of isocyanate to test was chosen according to each subject's occupational exposure and medical history. Lung function was monitored for 7 hours after sham and occupational agents' exposures. At the end of sham and active challenges airway responsiveness to methacholine was assessed. On day 4 (ie, 24 hours after SIC), lung function was assessed again. Subjects did not return to work until the end of the study. We considered these variables for the analysis of our population: age, gender, body mass index (BMI), smoking status, atopy, FEV_1 over forced vital capacity (FEV_1/FVC)%, baseline FEV_1% predicted, maximal post bronchodilator FEV_1% predicted, airway responsiveness to methacholine, agent, type of reaction to SIC, age at onset of disease, age at first exposure, duration of exposure and latency (the time—expressed in years—between the first exposure to diisocyanates and the beginning of the symptoms).

Lung Function and Bronchial Challenges

FVC and FEV_1 were measured with a spirometer (ESS system; Biomedin, Padova, Italy). The predicted normal values of the Communauté Européenne du Charbon et de l'Acièr (CECA) were used.[9] Airway responsiveness to methacholine aerosol was assessed as previously described[8] and results were expressed as the cumulative provocative dose causing a 20% fall in FEV_1 ($PD_{20}FEV_1$ in milligrams of methacholine). SIC with isocyanates were performed as described previously. Briefly, subjects were exposed to

the isocyanate vapor (mean ± [standard error], concentration, 10 ± 3 parts per billion [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, IL). An asthmatic reaction was considered to occur when FEV_1 decreased by at least 20% from baseline within 1 hour (early reaction) or greater than or equal to 2 hours (late reaction) or both (dual reaction) after exposure to isocyanates.

Application of the Simplified SARP Algorithm for the Asthma Phenotypes

We applied the simplified three variables algorithm described in the Moore et al[6] study to classify our occupational population into clusters. The variables were: baseline % predicted FEV_1, maximal % predicted FEV_1, and age of onset of the disease. Age of asthma onset was established by onset of asthma symptoms. As defined by the simplified SARP algorithm, the five groups were classified as: Group 1, pre-FEV_1 is greater than or equal to 68% predicted, post-FEV_1 is greater than or equal to 108%; Group 2, pre-FEV_1 is greater than or equal to 68%, post-FEV_1 is less than 108%, and asthma onset is less than 40 years; Group 3, pre-FEV_1 is greater than or equal to 68%, post-FEV_1 is less than 108%, and asthma onset is greater than or equal to 40 years; Group 4, pre-FEV_1 is greater than or equal to 65%; Group 5, pre-FEV_1 is less than 68%, post-FEV_1 is less than 65%.

Statistical Analysis

Data are shown as mean (SD). Differences between clusters were analyzed with chi-squared test for categorical variables and Kruskall–Wallis test for quantitative variables. The analysis was performed using a statistical software package (Statview, version 5.0.1; SAS Institute Inc; Cary, NC). A P value of <0.05 was considered significant.

RESULTS

Within all subjects who referred to our department for suspected OA due to isocyanates, 187 had a positive SIC with diisocyanates. Table 1 shows demographic and clinical data of patients.

Mean age was 35 years (±11) and 70.6% of subjects were men. The population was not obese—mean BMI 24 kg/m^2 [±3.5]—and the majority were never smokers (54% vs. 31.6% ex-smokers vs. 14.4% current smokers). Twenty-eight percent of patients were atopic.

TABLE 1. Characteristics of the 187 Patients at Baseline

Parameters	
Age (yrs)	35 (±11)
Gender (M/F)	132/55
BMI (kg/m^2)	24 (±3.5)
Smoking status (yes/no/ex)	27/101/59
Atopy (%)	28
Baseline FEV_1/FVC (%)	79 (±8)
Baseline FEV_1 (% predicted)	100 (±13)
Maximal post-bronchodilator FEV_1 (% predicted)	103 (±13)
Baseline $PD_{20}FEV_1$ (mg)	0.809 (±0.573)
Agent (TDI/MDI/HDI)	151/31/5
Type of reaction (early/late/dual)	38/102/47
Age at onset (yrs)	33 (±11)
Age at first exposure (yrs)	24 (±9)
Duration of exposure (yrs)	11 (±9)
Latency (yrs)	8 (±8)

Data are expressed as N° or mean (±SD). BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; HDI, hexamethylene diisocyanate; MDI, methylene diisocyanate; $PD_{20}FEV_1$, provocative dose causing a 20% fall in FEV_1; TDI, toluene diisocyanate.

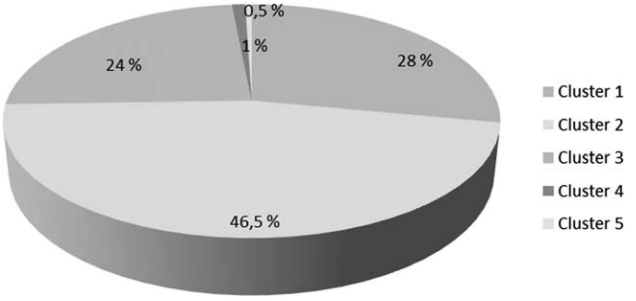

FIGURE 1. Distribution of the clusters obtained by applying SARP algorithm to OA population. OA, occupational asthma; SARP, severe asthma research program.

Lung function at baseline showed a mean FEV_1/FVC% predicted of 79 [±8] and a FEV_1% predicted of 100 [±13]. PD20 pre-SIC was of 0.8 mg [±0.57]. TDI was the most represented agent (80.7%) followed by MDI (16.6%) and HDI (2.7%). More than half population (54.5%) had a late asthmatic reaction. Mean age at onset of asthma symptoms was 33 years [±11] with mean age at first exposure of 24 years [±9] and a mean duration of exposure of 11 years [±9]. There was a latency of 8 years [±8].

In this population the simplified SARP algorithm was applied to create the five groups previously identified. The distribution of patients in the various clusters is shown in Fig. 1. Cluster 2 was the most represented (87 patients, 46.5%), followed by Cluster 1 (52 patients, 28%) and Cluster 3 (45 patients, 24%). Few patients were allocated in Cluster 4 (2 patients, 1%) and in Cluster 5 (1 patient, 0.5%). Table 2 reports the characteristics of subjects in three out of five clusters (ie, excluding the three subjects in Cluster 4 and 5). Men were prevalent in all groups. Cluster 1 included more women than the others (42%) and patients exhibited the mildest functional impairment, less bronchial hyperreactivity, and the lowest BMI. Cluster 2 was the most represented (46.5%) and included the youngest patients at first exposure who had the shortest duration of exposure and latency. Cluster 3 included mostly men, with the highest BMI, older, with the worst lung function, and the longest exposure and latency periods. There were no differences in smoking status neither in atopy across clusters. The type of isocyanate and the pattern of asthmatic reaction upon SIC were not significantly different among clusters.

DISCUSSION

In this study, we demonstrated that OA due to isocyanate is a heterogeneous disease as common asthma. The application of the simplified SARP algorithm for the first time in a selected population affected by OA due to isocyanates identified three distinct clusters. Whereas the previous studies applied SARP algorithm to a population of asthmatic subjects with the disease caused by different factors or unknown triggers, in the present study the inducing agent had been identified as a single class of low molecular weight chemicals containing isocyanate groups. It was not taken for granted that a type of asthma caused by work exposure to the same agent was distinguishable in more groups applying an algorithm developed for common asthma. In the present study, we demonstrated that among individuals susceptible to sensitization and disease, upon exposure to isocyanates at work, there are differences. The factors that explain the heterogeneity of isocyanate-induced asthma are, therefore, independent from the type of inducing agent. These factors may be related to intensity of exposure (environmental concentration and

TABLE 2. Characteristics of the Patients in Cluster 1, 2, and 3

Characteristics	Cluster 1 52 (28%)	Cluster 2 87 (46%)	Cluster 3 45 (24%)	*P*
Age (yrs)	34 (±10)	30 (±6.5)	48 (±5)	<0.0001
Gender (%M)	58	73.5	82	<0.0001
BMI (kg/m^2)	23.5 (±3)	24 (±4)	25 (±3.4)	<0.05
Smoking status (yes/no/ex)	9/27/16	13/53/21	5/20/20	n.s.
Atopy (yes/no)	12/40	26/61	12/33	n.s.
Baseline FEV_1/FVC (%)	84 (±6)	80 (±7.5)	74.5 (±8)	<0.0001
Baseline FEV_1 (% predicted)	114 (±10)	98 (±8)	92 (±8)	<0.0001
Maximal post-bronchodilator FEV_1 (% predicted)	116 (±13)	99 (±9)	98 (±7)	<0.0001
Baseline $PD_{20}FEV_1$ (mg)	0.105 (±0.537)	0.727 (±0.567)	0.712 (±0.556)	<0.0001
Agent (TDI/MDI/HDI)	45/5/2	68/18/1	38/7	n.s.
Type of reaction (early/late/dual)	8/31/13	21/41/25	9/29/7	n.s.
Age at onset (yrs)	32 (±10)	27 (±6)	45 (±8)	<0.0001
Age at first exposure (yrs)	25 (±10)	21 (±6)	29 (±10)	<0.0001
Duration of exposure (yrs)	10 (±9)	5 (±6)	17 (±10)	<0.0001
Latency (yrs)	8 (±9)	5 (±5)	12 (±11)	<0.0001

Data are expressed as N° or mean (±SD).

BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; HDI, hexamethylene diisocyanate; MDI, methylene diisocyanate; n.s., not significant; $PD_{20}FEV_1$, provocative dose causing a 20% fall in FEV_1; TDI, toluene diisocyanate.

peaks, skin absorption) and/or to personal characteristics that need to be identified. However, we were able to assess that atopy, smoking habit, and type of asthmatic reaction are not relevant in differentiating subgroups of occupational asthma. As in SARP analysis[6] and in Patrawalla et al[5] study, the most populated Cluster was number 2. Clusters 4 and 5, that in previous studies included the most severe patients, were substantially not represented in our group of occupational asthmatics. Therefore, the heterogeneity of OA results less than that of common asthma, since subjects with poor lung function were not detected. In an urban population not enriched by severe patients,[5] Clusters 4 and 5 represented the 10.8% and 10.4%, respectively, while 1.5% of isocyanate asthma only was allocated in these two clusters. One reason why Clusters 4 and 5 were not detected in OA can be the relatively short symptomatic period (average 3 years), that did not allow the disease to progress towards a more severe functional impairment. Another possibility is the shorter time of exposure to the specific offending agent, given that it is limited to the working time during adult life. Moreover, the proportion of asthmatic subjects with BMI greater than 30, in Patrawalla et al[5] study, was 36% and in their Cluster 4 and 5 the percentage of obese subjects reached percentages of more than 40%. Whereas in isocyanate-induced asthma a BMI greater than 30 was detected in 8% of the patients. Obesity is generally related to frequent comorbidities, to a poorer prognosis of asthma, and less controlled symptoms than having a normal weight.[10,11] The absence of this condition in our cohort may be an additional factor to explain a milder impairment in OA. Overall, our data suggest that diisocyanate-induced OA is less severe than common asthma. Although there is the possibility of a selection bias of milder patients in order to perform SIC, the exclusion of individuals from completing the diagnostic process due to too poor lung function was a rare event.

There are similarities in lung function between our clusters and those identified previously with SARP algorithm.[5,6] Cluster 1 patients had the best spirometry, whereas Cluster 3 patients showed mild reduction in baseline lung function. A major difference compared with clusters derived by general population regards sex distribution. The predominance of men in isocyanate-induced asthma is reasonably explained by the higher number of men subjects at risk. However, we did not observe predominantly women subjects in Cluster 3 as in the Patrawalla et al[5] cohort.

The information we provide is new since phenotyping of OA was performed previously only by Lemiere et al.[7] That investigation had a different aim, that is, to assess whether an increase in fractional exhaled nitric oxide following a positive SIC with various agents was restricted to particular phenotypes of patients. Our results are difficult to compare with Lemiere et al study since they used a different method of analysis and mixed causes of OA were included. Indeed, the type of occupational agent, high or low molecular weight, was one of the strongest determinants of the clusters. Accordingly, atopy was significantly different among the three clusters in Lemiere et al cohort.

Our data confirmed that atopy is not relevant isocyanate-induced asthma, since it was equally distributed among clusters and the percentage of atopics was even less than that observed in the general population[12] and in previous studies in asthma using SARP algorithm.[5,6]

Our work has some strengths. We collected a relatively large cohort considering that our study focused on OA due isocyanates only. The inclusion criteria required a diagnosis of OA confirmed by SIC. The patients were exposed to a single class of LMW chemicals, avoiding the interference of stimuli acting through different mechanisms and the influence of environmental exposure, since isocyanates are encountered exclusively in the workplace. The confounder of treatment was avoided since the subjects had been free from corticosteroids for at least 4 weeks.

There may be a concern that some variables relevant for OA that are not considered in SARP algorithm. These include the type of causing agent, the type of reaction, the duration and intensity of exposure, and latency. Therefore, an unsupervised cluster analysis including a set of appropriate variables might give different results. The three clusters differentiated by several temporal variables, including age and duration of exposure. Since some of these time variables may be related to each other, their relative weight remains to be determined. It is unclear if being part of a specific cluster is relevant to the outcome of the disease and if it is reasonable to provide a different management of the disease according to cluster allocation. More studies are needed to determine if cluster differentiation has a clinical application.

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