

Accepted Manuscript

Cluster Analysis Of Occupational Asthma Due to Isocyanates

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PII: S0091-6749(18)31210-7

DOI: [10.1016/j.jaci.2018.08.018](https://doi.org/10.1016/j.jaci.2018.08.018)

Reference: YMAI 13589

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 2 February 2018

Revised Date: 20 August 2018

Accepted Date: 24 August 2018

Please cite this article as: Mason P, Frigo AC, Scarpa MC, Maestrelli P, Guarnieri G, Cluster Analysis Of Occupational Asthma Due to Isocyanates, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.08.018.

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TITLE PAGE

CLUSTER ANALYSIS OF OCCUPATIONAL ASTHMA DUE TO ISOCYANATES

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This work was supported by grants from the Italian Ministry of Education, University and Research; the University of Padova; and Associazione Ricerca Cura Asma (ARCA), Padova, Italy.

2 CAPSULE SUMMARY

3 Asthma due to isocyanates is a heterogeneous disease, which can be separated in 3 distinct
4 phenotypes by unsupervised cluster analysis. We established that the causing agent is not sufficient
5 to explain the heterogeneity of OA. Differences across clusters suggest that individual susceptibility
6 and/or intensity of exposure are implicated.

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8 KEY WORDS

9 phenotype, airway disease, sensitization, inhalation challenge, chemical exposure

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CLUSTER ANALYSIS OF OCCUPATIONAL ASTHMA DUE TO ISOCYANATES

To the Editor

Asthma is a complex disease that includes different phenotypes. Significant advances in understanding asthma heterogeneity have been made using unbiased multivariate approaches to group asthmatics in distinct clusters [1-4]. Whether such well-defined asthma phenotypes provide a better understanding of the aetiology of the disease remains undetermined since inciting agents in common asthma are often unknown. Occupational asthma (OA) is a form of asthma in which the causing agent is recognized. The causes of OA can be classified as high molecular weight agents, and low molecular weight agents such as diisocyanates which are the major cause of OA in many industrialized countries [5].

Very few investigations have been performed on clusters of OA [6]. In the present study we sought to assess whether different clusters can be identified in OA due to a single class of chemicals such as diisocyanates, using variables more appropriate for OA than those of the Severe Asthma Research Program [2,7].

Patients who had a diagnosis of OA confirmed by a positive Specific Inhalation Challenge (SIC) with diisocyanates from 1988 to 2013 were included in our study. The positive responses to SIC included an early asthmatic reaction, a late asthmatic reaction or both (dual reaction) [8]. All subjects signed informed consent to perform the procedures for the diagnosis of OA, including SIC. The local ethics committee approved the use of the retrospective data of the patients (n. 3930/AO/16).

The cluster analysis was performed using the variables reported in Table 1. The number of clusters was derived both from the dendrogram obtained using Ward's method and from the statistics Cubic Clustering Criterion (CCC), pseudo F and pseudo t^2 . Afterwards we applied the k-means method to classify subjects into the clusters. More details of the methods are described in this article's Online Repository at www.jacionline.org.

Table 1 shows the characteristics of the 187 subjects included in the study. In a preliminary cluster analysis the 2 HDI-exposed patients were segregated separately from TDI and MDI groups. Since we preferred not to remove these two patients, they were forced into TDI-exposed group in the cluster analysis presented in Table 2. The reasons for it are that the job was the same than TDI-exposed (spray painters) and the vapour pressure of HDI is closer to that of TDI than to that of MDI. Furthermore, we performed an additional analysis, including variables with missing data and without forcing HDI patients into TDI group, which is shown in table E1 of the online repository of the manuscript. The largest Cluster (No 1) contained 80 patients (43.0%), followed by Cluster 2 (n 76, 40.5%) and Cluster 3 (n 31, 16.5%). Cluster 1 and Cluster 2 included patients sensitized to TDI, mostly with a late SIC response. Cluster 1 patients were predominantly males and non-smokers, and had younger age and higher BMI than Cluster 2 patients. Cluster 2 patients exhibited shorter exposure than patients in Cluster 1. Cluster 3 included patients sensitized to MDI, mostly with an early SIC response. Atopy, pre and post SIC methacholine PD₂₀ FEV₁, eosinophils and neutrophils percentages in peripheral blood were not significantly different among Clusters.

The number of clusters we detected is smaller than that usually identified in common asthma (4 or 5). Thus, OA seems less heterogeneous than common asthma. One possible explanation may be the obvious lack of the early onset phenotype. In addition, we did not identify clusters characterized by severe lung function impairment, as found in some studies of common asthma [1-4]. One reason why the severe phenotypes were not detected in OA might be the short symptomatic period (average 3 years), that may have prevented the disease progressing towards more severe functional impairment. Another possibility is the relatively short time of exposure to the specific offending agent, given that relevant exposure is limited to the working hours.

Among the different parameters considered by previous investigations on asthma heterogeneity, some variables were particularly relevant across the studies for the allocation of patients to the clusters, such as age at onset of asthma, sex, atopy and baseline lung function. Age at onset of

disease and baseline lung function were significantly different also within our OA clusters, whereas atopy was not. Although sex was significantly different among our clusters, we cannot tell whether this is due to sex *per se* or to a different distribution of males and females among jobs with isocyanate exposure.

To our knowledge, the results we provide are new since a cluster analysis of OA was performed previously only by Lemière et al. [6], who identified 3 clusters in a smaller occupational cohort. Moreover, their study had a different aim (i.e. role of exhaled NO in the response to specific inhalation challenge), mixed causes of OA were included and a different method of analysis was used.

The chief limitations of our study are (a) the smaller number of patients compared to the cohorts recruited in cluster studies of common asthma and (b) the lack of workplace measurements of isocyanate exposure, including information on the occurrence of peak exposures or skin contact. Different routes and levels of exposure could explain the differences among Clusters, particularly between Cluster 1 and 2. Finally, we had few data on inflammatory biomarkers (*e.g.* exhaled nitric oxide values and induced sputum cell pattern) which could be informative about possible different disease pathways [8] and which were applied in defining phenotypes in common asthma [1].

The novelty of this study is the finding that OA due to isocyanates is a heterogeneous disease. In both Cluster 1 and 2 subjects were sensitized to TDI and showed a late component of the asthmatic reaction to SIC. Cluster 2 contained patients who were younger at diagnosis, with better lung function, and younger age at onset than patients in Cluster 1. Since the proportion of patients diagnosed in the two periods, between 1988-1999 and 2000-2013, was similar in the two clusters and in addition most of the patients were spray painters in both Clusters (Cluster 1: 91%; Cluster 2: 93%), the hypothesis that these two clusters represent two chronologically distinct occupational histories in different industries both using TDI is unlikely. According to multivariate logistic regression model with backward stepwise selection considering the variables statistically significant

in Table 2, the variables which predicted the separation between Clusters 1 and 2 were: age at diagnosis ($p < 0.0001$, OR=1.39 95%CI: 1.20-1.62), BMI ($p = 0.0009$, OR=2.01 95%CI: 1.34-3.04) and FEV1/FVC% predicted at baseline ($p = 0.005$, OR=0.678 95%CI: 0.55-0.84) with a c-statistic=0.98.

Patients in Cluster 3 were sensitized exclusively to MDI. Thus, we demonstrated that little changes of the isocyanate chemical structure play a critical role in cluster differentiation. Our results are consistent with mouse models showing the lack of cross-reactivity across MDI and TDI [9]. This observation may suggest a different pathogenesis of the disease based on the type of molecule. In addition, we established that the causing agent is not sufficient to explain the heterogeneity of OA, since TDI-induced asthma can be differentiated in two clusters, suggesting that individual susceptibility and/or intensity of exposure are implicated. It remains to be investigated whether being part of a specific cluster has any bearing on disease outcome or whether it is appropriate to target management according to the cluster allocation.

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112 This work was supported by grants from the Italian Ministry of Education, University and
113 Research; the University of Padova; and Associazione Ricerca Cura Asma (ARCA), Padova, Italy.

114 The sponsor had no role in the design of the study, the collection and analysis of the data, or in the
115 preparation of the manuscript.

116

117 Financial/non financial disclosures: The authors have reported to JACI that no potential conflicts of
118 interest exist with any companies/organizations whose products or services may be discussed in this
119 article.

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Table 1: Characteristics of the subjects with OA ($n= 187$) according to the 13 variables analysis

Age (yrs)	35 (± 10.8)
Gender (M/F)	132/55
BMI (kg/m^2)	24 (± 3.5)
Smoking status (yes/no/ex)	27/101/59
Atopy ($n, \%$)	52 (28%)
FEV1/FVC % predicted, baseline	79 (± 8)
FEV1 % predicted, baseline	100 (± 13)
PD ₂₀ pre SIC (μg)	809 (± 573)
Agent (TDI/MDI/HDI)	154/31/2
Eosinophils in peripheral blood (%)	3.9 (± 2.8)
Neutrophils in peripheral blood (%)	55.8 (± 11.2)
Type of reaction (early/late/dual)	38/102/47
Age at onset (yrs)	32 (± 10.1)

BMI, Body Mass Index; HDI, hexamethylene diisocyanate; MDI, methylene diisocyanate; PD₂₀, provocative dose causing a 20% fall in FEV1; SIC, specific inhalation challenge; TDI, toluene diisocyanate.

Values are given as n and %, or mean ($\pm$ standard deviation).

Table 2: Characteristics of the 3 Clusters

	Cluster 1 <i>n</i> = 80 (43%)	Cluster 2 <i>n</i> = 76 (40.5%)	Cluster 3 <i>n</i> = 31 (16.5%)	p
Age at diagnosis (yrs)	43 (36.5, 48.5)	25 (22, 30)	38 (30, 45)	< 0.0001
Gender (M/F), <i>n</i> -%	66/14- 83/18%	42/34- 55/45%	24/7- 77/23%	0.0006
BMI (kg/m ²)	25.3 (23.3, 28.3)	21.9 (20.3, 23.8)	24.8 (21.5, 26.8)	< 0.0001
Smoking status, (yes/no/ex)	6/29/45	18/54/4	3/18/10	< 0.0001
Atopy (<i>n</i>)	28/52	16/60	8/23	0.1458
FEV1/FVC % predicted, baseline	76.8 (71.5, 80.2)	86.2 (79.6, 89.2)	77.2 (68.5, 83.5)	< 0.0001
FEV1 % predicted, baseline	98.3 (92.2, 107.8)	102.6 (94.6, 113)	95 (85.4, 105.6)	0.0078
pre SIC PD ₂₀ (μg)	669 (196, 1440)	676 (273, 1440)	1003 (275, 1440)	0.7979
Agent (TDI/HDI/MDI)	79/1/0	75/1/0	0/31	< 0.0001
Eosinophils in peripheral blood (%)	3.8 (2, 5)	4 (2, 5.4)	2.9 (1.7, 5)	0.5527
Neutrophils in peripheral blood (%)	58.1 (51, 63)	55 (52, 60)	58 (51.6, 62)	0.2162
Type of reaction, (Early/Late/Dual)	12/48/20	10/45/21	16/9/6	0.0001
Age at onset (yrs)	40 (34, 47)	23 (21, 29)	33 (27, 41)	< 0.0001

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175 BMI, Body Mass Index; HDI, hexamethylene diisocyanate; MDI, methylene diisocyanate; PD₂₀,
 176 provocative dose causing a 20% fall in FEV₁; SIC, specific inhalation challenge; TDI, toluene
 177 diisocyanate.

178 Values are given as *n* and %, or median (IQR).

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Table E1: Cluster analysis of the 187 subjects including variables with missing data and without forcing HDI-exposed subjects into TDI-exposed group.

	HDI-exposed <i>n</i> = 2	Cluster 1 <i>n</i> = 83	Cluster 2 <i>n</i> = 65	Cluster 3 <i>n</i> = 37
Age at diagnosis (yrs)	32.5 (25, 40)	43 (37, 48)	24 (22, 27)	37 (29, 42)
Gender (M/F), <i>n</i>	0/2	67/16	35/30	30/7
BMI (kg/m ²)	24.6 (21.9, 27.3)	24.7 (23.1, 27)	21.7 (20.3, 23.6)	25.2 (22.3, 27.4)
Smoking status, (yes/no/ex)	1/0/1	7/35/41	16/44/5	3/22/12
Atopy (yes, <i>n</i>)	0	26	13	13
FEV1/FVC % predicted, baseline	86.7 (84.1, 89.3)	77.3 (71.9, 81)	86.5 (79.8, 89.3)	77.8 (69.2, 83)
FEV1 % predicted, baseline	97.7 (88.8, 106.5)	98.5 (92.7, 108.9)	103.1 (95.1, 111.7)	95 (87.1, 103)
pre SIC PD ₂₀ (μg)	239 (118, 360)	517 (236, 1440)	1198 (359, 1440)	676 (186, 1440)
Agent (TDI/MDI/HDI)	0/0/2	83/0/0	65/0/0	6/31/0
Eosinophils in peripheral blood (%)	6.6 (5.4, 7.8)	3.1 (2, 5)	4 (2, 5.4)	3.3 (2, 5.7)
Neutrophils in peripheral blood (%)	48.1 (44.1, 52.2)	58 (52.2, 63)	56 (52, 60)	57.5 (49.1, 60.8)
Type of reaction, (Early/Late/Dual)	0/2/0	8/54/21	8/37/20	22/9/6
Age at onset (yrs)	32 (24, 40)	39 (34, 44)	22 (20, 25)	30 (27, 39)
Age at first exposure (yrs)	30.5 (22, 39)	23 (18, 35)	19 (15, 21)	27 (19, 33)
Duration of exposure	2 (1, 3)	17 (9, 23)	4 (3, 8)	7 (4, 12)

BMI, Body Mass Index; HDI, hexamethylene diisocyanate; MDI, methylene diisocyanate; PD20, provocative dose causing a 20% fall in FEV1; SIC, specific inhalation challenge; TDI, toluene diisocyanate.

Values are given as *n* and %, or median (IQR)

CLUSTER ANALYSIS OF OCCUPATIONAL ASTHMA DUE TO ISOCYANATES

On line repository

METHODS

Subjects

Patients who had a diagnosis of OA confirmed by a positive 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 ethics 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 [E1]. On day 1, clinical and occupational data were collected, skin prick tests with common aeroallergens and pulmonary function were performed. On day 2, a single-blind sham inhalation challenge was performed. On day 3, the subjects underwent SIC with the appropriate isocyanate (i.e. TDI, MDI or HDI). The type of isocyanate to test was chosen according to individual occupational exposure and medical history. Lung function was monitored for 7 hours after sham and occupational agent exposures. At the end of sham and active challenges, airway responsiveness to methacholine was assessed. On day 4 (i.e. 24 h after SIC), lung function was assessed again. Subjects did not return to work until the end of the study. Exclusion criteria were a history of upper respiratory infection, an asthma exacerbation or use of inhaled or oral corticosteroid in the four weeks prior to the study or long acting bronchodilators within preceding 48 h.

Lung Function, Methacholine Bronchial Challenge and Specific Inhalation Challenge

Forced Vital Capacity (FVC) and Forced Expiratory volume in 1 second (FEV₁) were measured with a spirometer (ESS system; Biomedin, Padova, Italy). The predicted normal values of the Communauté Européenne du Charbon et de l'Acier (CECA) were used [E2]. Airway responsiveness to methacholine aerosol was assessed as previously described [E1] and results were expressed as the cumulative provocative dose causing a 20% fall in FEV₁ (PD₂₀ FEV₁ in µg of methacholine). SIC with isocyanates were performed as described previously. Briefly, subjects were exposed to the isocyanate vapour (mean ± [standard error, SE], concentration, 10±3 parts per billion [ppb]) for a maximum of 60 min in an exposure chamber. The concentration of isocyanate was continuously monitored (Single Point Monitor; Zellweger Analytics Inc; Lincolnshire, IL, USA). An asthmatic reaction was considered to occur when FEV₁ decreased by at least 20% from baseline within 1 h (early reaction) or ≥2 h (late reaction) or both (dual reaction) after exposure to isocyanates.

Statistical analysis

Results are expressed as mean (± standard deviation, SD) or median (Interquartile Range, IQR). The analysis was performed using these variables: age, sex, Body Mass Index (BMI), smoking status, atopy, FEV/FVC % at baseline, FEV₁ % predicted at baseline, pre SIC methacholine PD₂₀ FEV₁, percentages of eosinophils and neutrophils in peripheral blood at baseline, causing agent, type of reaction to SIC, age at onset of symptoms.. The number of clusters was derived both from the dendrogram obtained using Ward's method and from the statistics Cubic Clustering Criterion (CCC), pseudo F and pseudo t². These metrics were provided by SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and represent measures for choosing the optimal number of groups [E3]. Afterwards we applied the k-means method to classify subjects into the clusters.

Differences among clusters were analyzed using χ^2 for categorical variables, ANOVA test for normal distributed quantitative variables and the non parametric Kruskal-Wallis test for the quantitative variables not normally distributed (verified using Shapiro-Wilk test). All the analysis were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

P values ≤ 0.05 were considered statistically significant.

Since the number of subjects with lack of data for some potentially relevant covariates was rather elevated, we adopted a single imputation method with predictive mean matching for continuous variables. This method is similar to the regression method except that it imputes a value randomly from a set of observed values whose predicted values are closest to the predicted value for the missing value from the simulated regression model. In a further cluster analysis, we were able to include 2 more variables (age at first exposure and duration of exposure) (Table E1). The findings are quite similar to those we obtained in the original analysis. Indeed, Cluster 1 and 2 are the most represented and include exclusively TDI-exposed patients. In Cluster 1 patients are older at diagnosis, predominantly males, mostly non-smokers, with a worse baseline lung function and a longer duration of exposure than in Cluster 2. Cluster 3 included all the patients exposed to MDI (81%), with an older age at first exposure and mostly with an early SIC response.

One may argue that the binary nature of the TDI/MDI variable will dominate the clustering process. This argument is rather weak since a) other binary variables, such as sex and atopy, included in the analysis resulted to have little impact on cluster differentiation; b) the number of clusters identified was 3, not 2 as expected if the binary variable was so relevant; c) the additional analysis using TDI/MDI/HDI variable (Table E2) confirmed that MDI-exposed workers grouped in a single cluster.

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