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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13645

Clustering analysis in outpatients with allergic rhinitis in clinical practice

To the Editor,

It is well known that allergic rhinitis (AR) may represent a main risk factor for asthma onset and/or worsening.¹ Actually, both disorders share similar pathophysiological mechanisms, including airways inflammation, type 2 immune response, airflow limitation and impaired quality of life.^{2,3} In this regard, some AR patients may have bronchial hyperreactivity (BHR) and high levels of fractional exhaled oxide nitrite (FeNO): this condition may suggest possible evolution in asthma.⁴

Cluster analysis is a way of grouping similar participants together. If this involves clinical data, this may provide a description of different phenotypes. If this involves "omic" data, it may provide a description of different endotypes. So, this methodology defines subgroups that are characterized by having common features. Therefore, the current study aimed to identify subgroups of AR patients with possible distinct phenotypes.

This cross-sectional study included 724 consecutive patients (654 males, median age 28 [24-33] years), suffering from AR. They were Navy soldiers who were referred to the Navy Medical Service for mandatory certification of their physical suitability for specific military training. The subjects were enrolled in the study on the basis of a documented diagnosis of AR according to validated criteria.¹ Exclusion criteria were as follows: any prior

documented history of asthma diagnosis or referral for asthma symptoms (including cough, wheezing, breathlessness, chest tightness), presence of acute or chronic upper respiratory infections, nasal polyps, clinically relevant septal deviation, previous or current intensive smoking, such as more 10/pack/year, previous or current specific immunotherapy, and use of nasal or oral corticosteroids, nasal or oral vasoconstrictors, leukotrienes antagonists, and antihistamines during the previous 4 weeks. The Navy Review Board approved the study procedure and written informed consent was obtained from each subject, according to the procedure for guaranteeing data privacy.

The visits included the following parameters: age at visit, smoking habit, clinical examination, respiratory (nasal and bronchial) symptom perception assessed by visual analogue scale (VAS), lung function testing, methacholine (MCH) challenge and FeNO assessment.

Categorical data were reported as absolute frequencies and percentages, and compared by the chi-square or the Fisher's exact test, as appropriate. Quantitative variables (with the exception of MCH PD₂₀) were summarized as medians with first and third quartiles (1st-3rd q), whereas MCH PD₂₀ as geometric mean with minimum and maximum and compared by the nonparametric analysis of variance (Kruskal-Wallis test).

To better depict the relationships among different sociodemographical and clinical variables, ascending hierarchical clustering (AHC) analysis was performed. AHC was determined by using Euclidean distance, and Ward's method was used as aggregation criterion. An automatic truncation option of XLSTAT software was chosen to determine the number of statistically different classes. This automatic truncation, based on the entropy, tries to identify homogeneous groups and decides when to stop in aggregating observations (or groups of observations). All the tests were two-sided, and a P value < 0.05 was considered as statistically significant. Statistica software 9.0 (StatSoft Corp., Tulsa, OK, USA) was used for all the analyses and XLStat 2.0.1 (Addinsoft Co., New York, NY, USA) for the clustering.

Ascending hierarchical clustering analysis produced five clusters of patients (Figure 1): Cluster 2, Cluster 3 and Cluster 5 have peculiar characteristics. As shown in Table 1, patients belonging to Cluster 1 have frequent sensitization to Dermatophagoides, and patients belonging to Cluster 2 have a short disease duration, are almost all nonsmokers, have normal FEF₂₅₋₇₅ values, low FeNO levels, almost no BHR and are sensitized to perennial allergens. Cluster 3 is characterized by a longer disease duration and a high frequency of

sensitization to pollens. Cluster 4 is characterized by frequent sensitization to Grass pollens. Cluster 5 is the smallest cluster: patients have the longest disease duration, are generally smokers, have low FEF₂₅₋₇₅ levels, have BHR and high FeNO levels; they are sensitized to moulds frequently (notably all patients are sensitized to Alternaria) and, even less frequently, to Olea and Alder, and only seldom to Parietaria and Compositae.

The five clusters were similar in terms of FVC and FEV₁ (% of predicted) values.

The current study demonstrates that it is possible to identify different phenotypes in a sample of subjects with AR. In particular, we identified a cluster with a series of impaired parameters, including longer AR duration, smoking, low FEF₂₅₋₇₅ values, frequent BHR, high FeNO levels and impressively sensitization to Alternaria that represent risk factors for evolving asthma.⁵ On the other hand, the other clusters are characterized by a potential low risk for asthma progression. These outcomes could have a clinical relevance as phenotyping/endotyping patients is the premise to the precision medicine, which is finalized to tailor the most adequate management into the single patient (the so called personalized medicine).⁶ In this

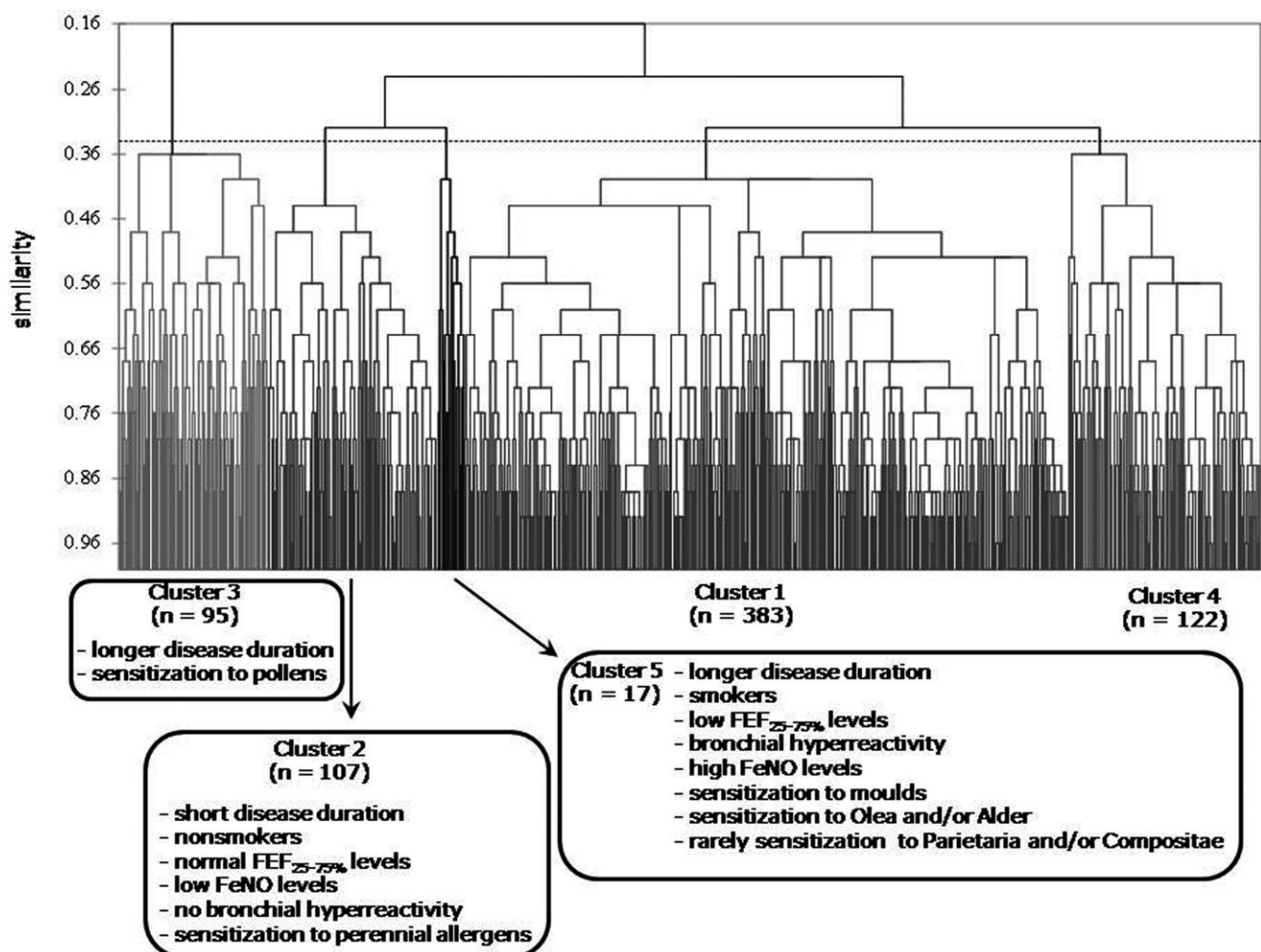


FIGURE 1 Ascending hierarchical clustering analysis of 724 patients with allergic rhinitis

TABLE 1 Demographic and clinical data

	Cluster 1 (n = 383)	Cluster 2 (n = 107)	Cluster 3 (n = 95)	Cluster 4 (n = 122)	Cluster 5 (n = 17)	P	Total (n = 724)
Males	341 (89%)	99 (92.5%)	87 (91.6%)	110 (90.2%)	17 (100%)	0.62 ^a	654 (90.3%)
Age (years)	28 (24-33)	27 (24-31)	27 (23-34)	28 (25-31)	33 (24-37)	0.65 ^b	28 (24-33)
Disease duration (years)	4 (2-7)	3 (2-8)	6 (3-10)	4 (3-8)	7 (4-11)	0.031 ^b	4 (2-8)
Smoking: yes	69 (18%)	9 (8.4%)	16 (16.8%)	36 (29.5%)	7 (41.2%)	<0.0001 ^a	137 (18.9%)
FVC (% pred)	106 (99-114)	105 (98-113)	106 (98-115)	105 (98-116)	108 (103-119)	0.66	
FEV ₁ (% pred)	102 (95-112)	105 (101-117)	107 (96-116)	103.5 (96-112)	105 (92-113)	0.003	
FEV ₁ /FVC	81 (77-86)	87 (82-89)	85 (78-88)	81 (77-86)	79 (73-81)	<0.0001	
FEF ₂₅₋₇₅ (% pred)	89 (73-110)	107 (85-126)	102 (81-120)	90 (74-107)	85 (68-98)	<0.0001	93 (75-114)
FeNO (ppb)	21 (12-36)	19 (11-41)	22 (15-52)	29 (14-45)	41 (28-58)	0.0007 ^b	22 (12-41)
R-VAS	2 (1-3)	2 (1-3)	2 (1-4)	2 (1-4)	3 (2-4)	0.029 ^b	2 (1-3)
B-VAS	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0.45 ^b	0 (0-0)
MCH PD ₂₀ ^d values (mcg)	1200.5 (30-1600)	1096.2 (10-1600)	1180.1 (30-1600)	849.3 (10-1600)	692.7 (190-1600)	<0.0001	1100.6 (10-1600)
BHR: Yes	88 (23.0%)	22 (20.6%)	30 (31.6%)	63 (51.6%)	12 (70.6%)	<0.0001 ^a	215 (29.7%)
Sensitization to							
Perennial allergens							
Dermatophagoides	321 (83.8%)	83 (77.6%)	52 (54.7%)	67 (54.9%)	9 (52.9%)	<0.0001 ^a	532 (73.5%)
Cat	51 (13.3%)	98 (91.6%)	26 (27.4%)	6 (4.9%)	6 (35.3%)	<0.0001 ^a	187 (25.8%)
Dog	17 (4.4%)	84 (78.5%)	18 (18.9%)	5 (4.1%)	4 (23.5%)	<0.0001 ^a	128 (17.7%)
Pollens							
Gramineae	102 (26.6%)	59 (55.1%)	75 (78.9%)	98 (80.3%)	12 (70.6%)	<0.0001 ^c	346 (47.8%)
Parietaria	152 (39.7%)	36 (33.6%)	51 (53.7%)	43 (35.2%)	2 (11.8%)	0.003 ^c	284 (39.2%)
Olea	77 (20.1%)	50 (46.7%)	71 (74.7%)	20 (16.4%)	11 (64.7%)	<0.0001 ^c	229 (31.6%)
Alder	3 (0.8%)	1 (0.9%)	81 (85.3%)	2 (1.6%)	10 (58.8%)	<0.0001 ^a	97 (13.4%)
Hazel	7 (1.8%)	8 (7.5%)	72 (75.8%)	1 (0.8%)	1 (5.9%)	<0.0001 ^a	89 (12.3%)
Betulaceae	4 (1%)	0 (0%)	80 (84.2%)	1 (0.8%)	3 (17.6%)	<0.0001 ^a	88 (12.2%)
Compositae	28 (7.3%)	11 (10.3%)	28 (29.5%)	19 (15.6%)	1 (5.9%)	<0.0001 ^a	87 (12%)
Cypress	20 (5.2%)	17 (15.9%)	27 (28.4%)	11 (9%)	3 (17.6%)	<0.0001 ^a	78 (10.8%)
Moulds							
Alternaria	24 (6.3%)	17 (15.9%)	12 (12.6%)	2 (1.6%)	17 (100%)	<0.0001 ^a	72 (9.9%)
Cladosporium	6 (1.6%)	1 (0.9%)	6 (6.3%)	0 (0%)	3 (17.6%)	<0.0001 ^a	16 (2.2%)
Aspergillus	6 (1.6%)	3 (2.8%)	1 (1.1%)	0 (0%)	0 (0%)	0.45 ^a	10 (1.4%)

The highest frequencies are highlighted.

^aP: Fisher's exact test.

^bP: Kruskal-Wallis test.

^cP: chi-square test.

^dThe dose causing a 20% fall of FEV₁.

regard, Precision Medicine is very useful in the management of patients with severe asthma.⁷ In fact, to select responder to biological medications is a priori requirement from a pharmaco-economic point of view.⁸ Therefore, the identification of biomarkers able to define a specific pheno/endotype represents a keystone in the work-up of these patients.⁹

The present study allows to identify a cluster of subjects with AR with peculiar characteristics that could induce the suspect of a progression towards asthma. Thus, it seems to be fruitful in clinical

practice to consider some parameters, including AR duration, lung function, namely, FEF₂₅₋₇₅, BHR, FeNO, type of sensitization and smoking. Indeed, these variables could, if impaired, suggest the adoption of a personalized treatment, mainly concerning allergen immunotherapy, as it is the unique treatment disease-modifying, that is, able to prevent the progression from rhinitis to asthma.¹ In fact, patients belonging to Cluster 5, such as the most likely predisposed to develop asthma, should early start allergen immunotherapy to hopefully prevent it.

However, the current study has some limitation: the cross-sectional design, the lack of biomarker assessment, the relatively young age, the setting (ie, Navy) and the prevalence of the male gender. Moreover, it has been recently reported that the gender has no relevant impact on asthma, apart from the anxiety and the perception of asthma control.¹⁰ Thus, the present outcomes could be cautiously generalized to the AR patients. Anyway, further studies should be conducted to answer the unmet questions, including validation by prospective studies, long-term follow-up and more general population.

In conclusion, the clustering analysis could be a useful tool in the management of AR patients as it could allow to identify specific clusters of patients potentially at risk of evolving towards asthma.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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DOI: 10.1111/all.13648

Exposure to 3-methyl-4-nitrophenol facilitates development of intestinal allergy

To the Editor,

It is recognized that the environmental pollution is associated with the pathogenesis of a number of diseases.¹ The sources of environmental pollution include multiple aspects, such as the automobile exhaust particles.² 3-methyl-4-nitrophenol (MNP) is one of the components of diesel-exhaust particles.³ Food contaminated by organophosphorus insecticide fenitrothion can be another source of MNP.⁴ It is reported that exposure to MNP induces renal tubule epithelial cell apoptosis and spermatogenic cell apoptosis.^{5,6} Based on the above information, we hypothesize that MNP facilitates the development of intestinal allergy. In this study, mice were exposed to MNP or/and OVA. The exposure to MNP induced intestinal

epithelial cell apoptosis, induced epithelial barrier dysfunction and facilitated the development of intestinal allergy in mice.

1 | MNP FACILITATES THE DEVELOPMENT OF INTESTINAL ALLERGY

Grouped mice were gavage-fed with MNP or/and OVA daily for 3 weeks. The mice were sacrificed 1 day after the last gavage. Samples were collected from the small intestine. The status of intestinal allergy was analyzed. The results showed that exposure of mice to both MNP- and OVA-induced intestinal allergy, including drop of the