TITLE: Identification of asthma phenotypes in the Spanish MEGA cohort study using cluster Analysis

ABSTRACT

Introduction: The definition of asthma phenotypes has not been fully established, neither there are cluster studies showing homogeneous results to solidly establish clear phenotypes. The purpose of this study was to develop a classification algorithm based on unsupervised cluster analysis, identifying clusters that represent clinically relevant asthma phenotypes that may share asthma-related outcomes.

Methods: We performed a multicentre prospective cohort study, including adult patients with asthma (N=512) from the MEGA study (Mechanisms underlying the Genesis and evolution of Asthma). A standardised clinical history was completed for each patient. Cluster analysis was performed using the kernel k-groups algorithm.

Results: Four clusters were identified. Cluster 1 (31.5% of subjects) includes adult-onset atopic patients with better lung function, lower BMI, good asthma control, low ICS dose, and few exacerbations. Cluster 2 (23.6%) is made of adolescent-onset atopic asthma patients with normal lung function, but low adherence to treatment (59% well-controlled) and smokers (48%). Cluster 3 (17.1%) includes adult-onset patients, mostly severe non-atopic, with overweight, the worse lung function and asthma control, and receiving combination of treatments. Cluster 4 (26.7%) consists of the ederly-onset patients, mostly female, atopic (64%), with high BMI and normal lung function, prevalence of smokers and comorbidities.

Conclusion: We defined four phenotypes of asthma using unsupervised cluster analysis. These clusters are clinically relevant and differ from each other as regards FEV1, age of onset, age, BMI, atopy, asthma severity, exacerbations, control, social class, smoking and nasal polyps.

KEYWORDS

Asthma; asthma phenotypes; asthma endotypes; clustering analysis.

INTRODUCTION

The classification of asthma severity in the guidelines establishes levels based on the symptoms, treatment, lung function, and the control of the disease. Using this classification, a similar treatment was proposed for all patients in each level, without taking into account the heterogeneity of the individuals in each severity level (1,2). This approach has some weaknesses when applied in routine clinical practice, since it is unable to identify which patients respond to a particular treatment (3,4), or who is at risk of developing life-threatening exacerbations, which may even be observed in patients classified as 'mild' (5).

The need to better classify such a heterogeneous group of patients has seemingly become clearer, which may be because there is access to best tools in order to identify diverse characteristics of the disease and due to the need to use new treatments efficiently (6-9). Identifying asthma phenotypes could improve knowledge of the disease's underlying pathophysiology and the prognostic factors, as well as optimise the personalisation of the treatment, which is associated with high socioeconomic costs (6,10,11).The definition of these phenotypes has not yet been fully established, and has even been modified at short notice, gaining expert approval. Thus, the latest Spanish Guidelines for the Management of Asthma (GEMA) have modified the classification into severe asthma phenotypes, rejecting the one associated with obesity, which was consolidated in the previous version (2).

Cluster analysis uses a group of variables to define patient subgroups that share specific critical characteristics. However, there are still no cluster studies available up until now that have shown homogeneous results with the ability to solidly establish clear phenotypes. With this in mind, it seems reasonable to advocate the need to continue with research in this area by including more variables which allow the definition of identified clusters and its clinical applicability to improve (8,9,12).

The MEGA Study (Mechanisms underlying the Genesis and evolution of Asthma) is an ongoing multicentre study carried out in Spain within the Biomedical Research Centre Network /Centro de Investigación Biomédica en Red (CIBER) framework for Respiratory Diseases (13). Previous results for the MEGA Study supported the greatest prevalence of chronic rhinosinusitis with nasal polyps (CRSwNP), severe rhinitis, anxiety, depression, gastroesophageal reflux and bronchiectasis in patients with severe asthma (14). Similarly, some treatable traits such as obesity and anxiety seem to have a decisive impact on asthma control and quality of life (15).

The aim of our study is to gain more of an understanding of asthma's heterogeneity and to develop a classification algorithm based on unsupervised cluster analysis, identifying and characterising clusters that may represent clinically relevant asthma phenotypes. Furthermore, we have tested the hypothesis that patients included in a given cluster share asthma-related health outcomes.

METHODS

We have conducted a multicentre prospective cohort study including consecutive patients with asthma from eight hospitals in Spain. Standard data collection methods were used in all of the participating centres with an electronic database (13,14).

We included patients, via the outpatient clinic of the participant institutions, with asthma with 18–75 years old who were diagnosed at least 1 year before inclusion based on the Global Initiative for Asthma (GINA) criteria. Patients were excluded if they had other acute or chronic active lung disorders. All patients signed an informed consent form (1,13,14).

A standardised clinical history was completed for each patient (13,14). Validated Spanish versions of the following questionnaires were administered: the Asthma Control Test (ACT) (13), the

Asthma Quality of Life Questionnaire (Mini- AQLQ) (13), the Sino-Nasal Outcome Test 22 (SNOT- 22) (13) and the Hospital Anxiety and Depression Scale (HADS) (13).

All study subjects underwent a detailed clinical examination, including body mass index (BMI) and respiratory function tests (baseline spirometry, the bronchodilator test, lung volume measurement by plethysmography, fraction of exhaled nitric oxide [FeNO], and the CO transfer test [DLCO] using the single- breath method), in accordance with the recommendations of the European Respiratory Society (16,17). The methacholine challenge (PC20) was performed at baseline (13,18). Chest x-ray and skin prick tests (SPT) with common aeroallergens were performed at the beginning of the study (13). Atopy was defined as the presence of at least one positive SPT or aeroallergen- specific immunoglobulin E (IgE) in serum (13,14).

The social class was classified into 6 levels in accordance with the criteria set by the Spanish Society of Epidemiology: class I (professionals and staff in managerial positions in companies with 10 or more employees), class II (staff in managerial positions in companies with fewer than 10 employees and intermediate professions), class III (qualified non-manual workers), class IVa (skilled manual workers), class IVb (semiskilled manual workers) and class V (unskilled manual workers) (13).

In order to define the clusters, we selected seven variables that were considered to be of special relevance by a multidisciplinary team of project investigators (Table 1). We also added variables that we classified into 4 groups associated with asthma disease, social aspects, comorbidities and treatment (Tables 2-5).

This research project has been approved by the Clinical Research Ethics Committee of all the hospitals participating in the study..

STATISTICAL ANALYSIS:

The cluster analysis was carried out using the kernel k-groups algorithm, a calculation of the similarity between the elements of each cluster using the energy distance. The Alpha coefficient was selected with a value equal to 1, and the number of clusters was fixed at k=4. The variables introduced in the algorithm to define the clusters can be seen in Table 1. Next, we described the characteristics of the new phenotypes in a wider group of variables (Tables 2-5). To do this, we carried out a descriptive analysis whereby the mean and variance of the patients of each cluster were calculated in the continuous variables, and the percentage of each category was reported as categorical variables. Using these variables with the distance components (DISCO) test, we tried to establish whether there were any statistically significant differences in the 4 clusters formed by each one of the mentioned variables.

Finally, to obtain a set of clinical decision rules that should explain the differences between the patients that make up the different clusters, a C.50 classification tree was fitted using the variables that defined the clusters (19).

All the statistical analyses were carried out using the R 3.4.5 statistics software. The k-groups algorithm, in addition to the DISCO test, can be freely used from the R energy package, while the C.50 classification algorithm has been set using the R C.50 package.

RESULTS:

A total of 512 patients were included in the MEGA cohort, with a mean age of 47.3 years, 66.15% of whom were female (Table 1). For the cluster study, we included 292 patients for whom all the data required for the study was available.

We identified 4 clusters which were all significantly different from each other as regards FEV_1, age of asthma onset, current age of patients, BMI and atopy. No major variations were observed between clusters either for gender or for blood eosinophil count (BEC), exceeding 300 cells/μL in all groups (Table 1). Nevertheless, notable differences were found among the clusters for certain asthma characteristics such as severity, exacerbations and asthma control. There were also widespread dissimilarities for other variables such as level of studies, social class, smoking and nasal polyps as well as other comorbidities (Tables 2-5).

Cluster 1 was the largest group with 32.5% of the patients. It was noted for adult-onset asthma, better lung function, more atopy and less overweight. Most of cluster 1 patients were university graduates, less likely to be smokers, in a lower social class in childhood, with better asthma control and fewer exacerbations. They were also prescribed lower doses of inhaled corticosteroids (ICS) and had a better quality of life.

Cluster 2 included 23.6% of the patients, with younger age, adolescent onset asthma, normal lung function, and a high percentage of atopy, 59% being well-controlled. Like in cluster 1, they were brought up in a lower social class as children, but formed the highest percentage of individuals with tertiary education qualifications. Most of these patients were smokers and 48% were obese, but only 17% had CRSwNP. As for treatment, it was the cluster with the lowest use of long-term anticholinergics and systemic corticosteroids and with the least adherence to treatment.

Cluster 3 was the smallest cluster and included 17.1% of the all the patients, with worse lung function, less atopy, who were of advanced age, with adult-onset asthma and were overweight. These were the patients that had had asthma for the longest, with the highest figures in the following areas: percentage belonging to low social classes, prevalence of CRSwNP, severity of asthma, exacerbation rates, admissions to the intensive care unit, adherence to treatment and use of long-acting muscarinic antagonists (LAMA), systemic corticosteroids (SCSs), ICS and long-acting beta-agonist (LABA) combinations. They also had the lowest quality of life and the least control of the disease. It was the only cluster with a mean ACT < 20.

Cluster 4 included 26.7% of patients that were the oldest, with the highest age of asthma onset and BMI and normal lung function, 64% of whom had atopy and the majority of whom were female. Although most patients in this cluster came from a medium-high social class in childhood, they now had a less privileged social situation and comorbidity; the cluster also had the lowest percentage of patients with school-leaving or tertiary qualifications, the most smokers, and the highest prevalence of psychiatric comorbidity and vascular risk factors.

As we can see in Figure 1, in order to explain what the clinical variables were and their cut-off points that enabled the patients to be discriminated between clusters with a high degree of accuracy, we only needed the post-bronchodilation FEV1 and the age of the patient to correctly classify 98% of the individuals included in the study. Specifically, the patients with low lung function were assigned to clusters 2 and 3 (see nodes 1-8), and the rest to clusters 1 and 4 (nodes 9-13). More nodes than clusters were observed due to the overlapping between patients of different clusters, which in turn was due to the heterogeneity. Thus, so that the algorithm had a high predictive capacity, it was necessary to construct more terminal nodes than clusters.

DISCUSSION:

Seven variables were applied to an unsupervised cluster approach, with four distinct clusters being identified. The current age of the patient and lung function were the parameters with the greatest capacity for discriminating between the clusters, although significant differences were observed regarding the age of onset, BMI and atopy. A predominance of females and high BEC (greater than 300 cells/µL) were common in all the clusters, but with no significant differences between them.

A predominance of females who were overweight was observed, and around one third were smokers or ex-smokers, similar to other Western populations (20-24).

In relation to the severity of the asthma, the majority (76% of the total) of patients had moderate-severe disease, which varied between 60% in Cluster 1 and 98% in Cluster 4, with most being atopic, usually associated with milder forms of the disease (25). This high percentage of moderate-severe patients was probably owing to the patients having been recruited from specialised asthma units, as was the case with another study carried out in Primary Care in Spain where the percentage of patients with moderate-severe asthma (37.2%) was lower (22).

Most women in adult asthma studies have been constant, which has been associated with factors such as high susceptibility to environmental contaminants, hormonal impact on airway inflammation, the differing distributions of body fat between genders and a higher remission rate of asthma in males (26-28).

Taking the BEC into account, other similar studies evaluating patients in real life conditions displayed levels greater than 292 cells/μL in all the clusters identified (25,29). In our population, this result was to be expected, given that most of our patients had moderate-severe asthma, which is associated with high counts of these cells (30,31).

Seventy-six percent of our patients were atopic, in line with most studies (20,21). Atopy prevalence differed significantly between the clusters, less so in patients with the most severe asthma and, as a result, it seems reasonable to include it so as to describe the phenotypes. In our study, 37% of the patients were smokers or ex-smokers, as was observed in asthma patients managed in Primary Care in Spain (22). These patients have often been excluded from asthma studies, but smoking is fairly common in asthmatics, and as such it more accurately reflects the real world (22,24,29). Likewise, it is known that smoking influences the course of the disease, causing more severe forms of asthma, a greater decline in lung function, more exacerbations, and a poorer response to corticosteroids (24,29,32).

Cluster 1 was similar to Cluster 1 of the Severe Asthma Research Program (SARP) "benign asthma" cohort study by Haldar et al (20,21). These patients had the best lung function and quality of life, least severity, and the lowest hospital admissions. This finding supports a low prevalence of factors associated with a poor prognosis of asthma as there were few smokers, little obesity, many tertiary qualifications and little psychiatric comorbidity (24,33-35).

Cluster 2 showed characteristics similar to Cluster 1 (early-onset atopic asthma) of the UK cohort and Cluster 2 of Moore (20,21). This group of young patients with normal lung function but with a high percentage of obesity, highlighted that they were the group with the least adherence to treatment. It is known that low severity, early age and overweight are associated with low adherence (36).

The patients of Cluster 3 were the most severe, with the least atopy and with adult-onset asthma, similar to Cluster 5 of the SARP cohort (21). In agreement with other authors (11), despite having the highest severity, they did not have the most hospital admissions or exacerbations in the past year. This could be due to a good control of the disease in severe asthma under SCSs, since the beneficial impact of these drugs for severe asthma is well known (37). The 41% of patients in this cluster had CRSwNP, a comorbidity associated with the most severe form of asthma, which backed up the results of our study (38). This relationship seems to lead to different underlying endotypes and could be useful in routine clinical practice, since patients with asthma and CRSwNP have a different type 2 inflammation and molecular signatures, and a dissimilar response to treatment with monoclonal antibodies when compared with asthmatic patients without nasal polyposis (39).

Cluster 4 was characterised by elderly onset asthma, predominance of females, normal lung function and elevated BMI, which was similar to Cluster 3 of the SARP cohort (21) and to the inflammation predominance cluster of the study by Haldar et al (20), even though in the latter, there was a predominance of males (20,21). In the study by Moore et al (21), that cluster had the most patients with arterial hypertension, as occurred in our study. Those patients had the lowest qualification levels, and the highest prevalence of smoking and comorbidities, psychiatric illnesses in particular, which were twice as high as any of the other clusters. The relationship between asthma and psychological problems as well as the high prevalence of psychiatric illnesses in individuals at low socioeconomic levels are well known (33,40).

Our work may have several limitations; firstly, as for the methodology of the cluster analysis, since the disease is a continuous spectrum, the separation into discrete clusters may not be realistic, especially in such a heterogeneous disease. Secondly, inclusion bias can occur as our patients came from specialized asthma units of hospitals, implying that the results may not be extrapolated to the general asthmatic population. Thirdly, the variables for cluster´s definition may be rather subjective as they were pre-selected by the research team even though they were chosen with relevant parameters in mind in order to classify patients with asthma and measure them in routine clinical practice, leaving aside other possible variables of major significance to define phenotypes. Fourthly, the potential impact of SCSs on eosinophilia and lung function should not be excluded either, but it would have been very difficult to avoid this effect as patients with severe asthma were included. Finally, we did not include induced sputum data since these measurements were not carried out in all the participating centres of the study, which would have also significantly reduced the size of the sample included.

This study has some strengths too; firstly, we included a large sample size with a considerable number of clinical, analytical and socioeconomic variables; secondly, the clusters identified appear to be clinically relevant since they show significant differences in aspects such as baseline severity, control of the disease, the number of exacerbations, and hospital admissions; thirdly, when making comparisons with other similar studies, our clusters coincide with those observed for other Western populations like the SARP study and the study of Leicester (20,21). Finally, the use of new clustering k-groups algorithm, which obtains more complex and general clusters than other clustering algorithms previously used to determine clinical phenotypes of asthma. The application of the k-means algorithm may be limited in many ways, for instance, due to it only detects differences in the mean between the groups established, while the hierarchical algorithms, given their heuristic nature, can give rise to suboptimal results. On the other hand, the k-groups algorithm can detect a general distributional form between established clusters, which involves differences in variance, kurtosis and other high-order moments, whereas the traditional algorithms are more likely to behave sub-optimally.

CONCLUSIONS

Four phenotypes of asthma were defined using a classification algorithm based on unsupervised cluster analysis. These clusters differ from each other as regards FEV1, age of onset, age, BMI, atopy, asthma severity, exacerbations, asthma control, social class, smoking and nasal polyps. These clusters are clinically relevant and support the heterogeneity of asthma. The different phenotypes observed suggest endotypes that may have an effect on the course of the disease.

LIST OF ABBREVIATIONS

ACT: Asthma Control Test

BEC: Blood eosinophil count

BMI: Body mass index

CRSwNP**:** Chronic rhinosinusitis with nasal polyps

DISCO: Distance components test

DLCO: The CO transfer test

FeNO: Fraction of exhaled nitric oxide

FEV_1: Forced Expiratory Volume in the first second.

GEMA**:** Spanish Guidelines for the Management of Asthma

GINA: Global Initiative for Asthma

HADS: Hospital Anxiety and Depression Scale

ICS: Inhaled corticosteroids

ICU: Intensive care unit

IgE: Immunoglobulin E

LABA: Long-acting beta-agonist

LAMA: Long-acting muscarinic antagonists

Mini- AQLQ**:** Asthma Quality of Life Questionnaire

PC20: Methacholine challenge

SARP: Severe Asthma Research Program

SCSs: Systemic corticosteroids

SNOT- 22: Sino-Nasal Outcome Test 22

SPT: Skin prick tests

REFERENCES

1. https://ginasthma.org/gina-reports/ [Retrieved 25/Jul/2021]
2. Plaza V, Blanco M, García G et al. Highlights of the Spanish Asthma Guidelines (GEMA), version 5.0. Arch Bronconeumol (Engl Ed). 2021 Jan;57(1):11-12.
3. Liu MC, Chipps B, Munoz X et al. Benefit of switching to mepolizumab from omalizumab in severe eosinophilic asthma based on patient characteristics.Respir Res. 2021 May 10;22(1):144.
4. Müllerová H, Cockle SM, Gunsoy NB et al. Clinical characteristics and burden of illness among adolescent and adult patients with severe asthma by asthma control: the IDEAL study. J Asthma. 2021 Apr;58(4):459-470.
5. Gonzalez-Barcala FJ, Calvo-Alvarez U, Garcia-Sanz MT et al. Characteristics and prognosis of near-fatal asthma exacerbations. Am J Med Sci. 2015 Aug;350(2):98-102.
6. Blanco-Aparicio M, Calvo-Alvarez U & Gonzalez-Barcala FJ. Biologics in Asthma: Don't Let the Magic Bullets Sink the Boat. Arch Bronconeumol. 2021 June;57:383-584
7. Casan-Clara P & Martinez-Gonzalez C. Biologics in the Treatment of Asthma. Arch Bronconeumol. 2020 March;56:137-138

8. Hsiao HP, Lin MC, Wu CC et al. Sex-Specific Asthma Phenotypes, Inflammatory Patterns, and Asthma Control in a Cluster Analysis. J Allergy Clin Immunol Pract. 2019 Feb;7(2):556-567.
9. Bhargava S, Holla AD, Jayaraj BS et al. Distinct asthma phenotypes with low maximal attainment of lung function on cluster analysis. J Asthma. 2021 Jan;58(1):26-37.
10. González-Barcala FJ, Muñoz-Gall X, Mariscal E et al. Cost-effectiveness analysis of anti-IL-5 therapies of severe eosinophilic asthma in Spain. J Med Econ. 2021 Jan-Dec;24(1):874-882.
11. Gonzalez Barcala FJ, La Fuente-Cid RD, Alvarez-Gil R et al. Factors associated with a higher prevalence of work disability among asthmatic patients. J Asthma. 2011 Mar;48(2):194-199.
12. Bourdin A, Molinari N, Vachier I et al. Prognostic value of cluster analysis of severe asthma phenotypes. J Allergy Clin Immunol. 2014 Nov;134(5):1043-1050.
13. Muñoz X, Álvarez-Puebla MJ, Arismendi E et al. The MEGA Project: A Study of the Mechanisms Involved in the Genesis and Disease Course of Asthma. Asthma Cohort Creation and Long-Term Follow-Up.Arch Bronconeumol. 2018 Jul;54:378-385.
14. Rial MJ, Álvarez-Puebla MJ, Arismendi E et al. Clinical and inflammatory characteristics of patients with asthma in the Spanish MEGA project cohort. Clin Transl Allergy. 2021 Mar;11(1):e12001.
15. Baptista-Serna L, Rodrigo-Muñoz JM, Mínguez P et al. Anxiety and BMI affect asthma control: data from a prospective Spanish cohort. J Allergy Clin Immunol Pract. 2021 Oct 18:S2213-2198(21)01130-2.
16. García- Río F, Calle M, Burgos F et al. Espirometría. Arch Bronconeumol. 2013;49:388-384

17. MacIntyre N, Crapo RO, Viegi G et al. Standardisation of the single- breath determination of carbon monoxide uptake in the lung. Eur Respir J. 2005;26:720- 735.
18. Crapo RO, Casaburi R, Coates AL et al. Guidelines for methacholine and exercise challenge testing—1999. Am J Respir Crit Care Med. 2000;161:309- 329
19. Franca G, Rizzo M & Vogelstein JT. Kernel k-Groups via Hartigan's Method. IEEE Trans Pattern Anal Mach Intell. 2020 May 28;PP.
20. Haldar P, Pavord ID, Shaw DE et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008 Aug 1;178(3):218-224.
21. Moore WC, Meyers DA, Wenzel SE et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program.Am J Respir Crit Care Med. 2010 Feb 15;181(4):315-323.
22. González Barcala FJ, de la Fuente-Cid R, Alvarez-Gil R et al. [Factors associated with asthma control in primary care patients: the CHAS study. Arch Bronconeumol. 2010 Jul;46(7):358-363.
23. van der Molen T, Fletcher M & Price D. Identifying Patient Attitudinal Clusters Associated with Asthma Control: The European REALISE Survey. J Allergy Clin Immunol Pract. 2018 May-Jun;6(3):962-971.
24. Tiotiu A, Ioan I, Wirth N, Romero-Fernandez R et al. The Impact of Tobacco Smoking on Adult Asthma Outcomes. Int J Environ Res Public Health. 2021 Jan 23;18(3):992.
25. Sendín-Hernández MP, Ávila-Zarza C, Sanz C et al. Cluster Analysis Identifies 3 Pheno-types within Allergic Asthma. J Allergy Clin Immunol Pract. 2018 May-Jun;6(3):955-961
26. Silveyra P, Fuentes N & Rodriguez Bauza DE. Sex and Gender Differences in Lung Dis-ease. Adv Exp Med Biol. 2021;1304:227-258.
27. Gonzalez-Barcala FJ, Takkouche B, Valdes L et al. Body composition and respiratory func-tion in healthy non-obese children. Pediatr Int. 2007 Oct;49(5):553-557.

28. Honkamäki J, Piirilä P, Hisinger-Mölkänen H et al. Asthma Remission by Age at Diagnosis and Gender in a Population-Based Study. J Allergy Clin Immunol Pract. 2021 May;9(5):1950-1959.
29. Kim TB, Jang AS, Kwon HS et al. Identification of asthma clusters in two independent Korean adult asthma cohorts. Eur Respir J. 2013 Jun;41(6):1308-1314.
30. Mallah N, Rodriguez-Segade S, Gonzalez-Barcala FJ et al. Blood eosinophil count as predictor of asthma exacerbation. A meta-analysis. Pediatr Allergy Immunol. 2021 Apr;32(3):465-478.
31. Jackson DJ, Busby J, Pfeffer PE et al. Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. Thorax. 2021 Mar;76(3):220-227.
32. Gonzalez-Barcala FJ, Pertega S, Sampedro M et al. Impact of parental smoking on childhood asthma. J Pediatr (Rio J). 2013 May-Jun;89(3):294-299.
33. Facal D, López-Lois B & Gonzalez-Barcala FJ. A Current Overview of the Psychological Aspects of Asthma in Adults. Arch Bronconeumol (Engl Ed). 2020 Aug;56(8):475-476.
34. Sastre J, Crespo A, Fernandez-Sanchez A et al. Anxiety, Depression, and Asthma Control: Changes After Standardized Treatment. J Allergy Clin Immunol Pract. 2018 Nov-Dec;6(6):1953-1959.
35. Freitas PD, Xavier RF, McDonald VM et al. Identification of asthma phenotypes based on extrapulmonary treatable traits. Eur Respir J. 2021 Jan 21;57(1):2000240.
36. Cheen MHH, Tan YZ, Oh LF et al. Prevalence of and factors associated with primary medication non-adherence in chronic disease: A systematic review and meta-analysis. Int J Clin Pract. 2019 Jun;73(6):e13350.
37. Klaustermeyer WB & Choi SH. A perspective on systemic corticosteroid therapy in severe bronchial asthma in adults. Allergy Asthma Proc. 2016 May;37(3):192-198.

38. Laidlaw TM, Mullol J, Woessner KM et al. Chronic Rhinosinusitis with Nasal Polyps and Asthma. J Allergy Clin Immunol Pract. 2021 Mar;9(3):1133-1141.

39. Lipworth BJ & Chan R. The Choice of Biologics in Patients with Severe Chronic Rhinosinusitis with Nasal Polyps. J Allergy Clin Immunol Pract. 2021 Jul 28:S2213-2198(21)00826-6.

40. Karolaakso T, Autio R, Näppilä T et al. Socioeconomic factors in disability retirement due to mental disorders in Finland. Eur J Public Health. 2020 Dec 11;30(6):1218-1224.

TABLES

Table 1.- Variables for cluster modelling. Differences between clusters						
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Total**	**p value**
Number of patients, n (%)	95 (32.6)	69 (23.6)	50 (17.1)	78 (26.7)	292	
Gender, n (%)						0.177
Male	26 (27)	28 (41)	20 (40)	22 (28)	96 (33)	
Female	69 (73)	41 (59)	30 (60)	56 (72)	196 (67)	
Current age, years, mean (SD)	42.9 (9.9)	34.3 (7.1)	55.3 (8.9)	57.9 (7.0)	47 (12.5)	<0.001
Age of onset, years, mean (SD)	24 (14.6)	15.3 (12.1)	28.6 (18.0)	34.5 (18.6)	25.5 (17.3)	<0.05
FEV1%, mean (SD)	115.7 (10.1)	89.3 (8.9)	57.6 (13.6)	92.5 (9.7)	93.3 (22.2)	<0.001
BMI, kg/m^2, mean (SD)	26.6 (5.7)	26.3 (5.4)	28.4 (5.5)	29.1 (5.8)	27.5 (5.7)	<0.01
Eosinophils, cells/µL, mean (SD)	384.4 (238.7)	381.6 (253.1)	351.4 (229.1)	357.8 (239.1)	371.0 (239.9)	0.778
Atopy, n (%)						<0.01
Positive	82 (86)	58 (84)	32 (64)	51 (65)	223 (76)	

n: number of patients; SD: standard deviation; FEV1%: Forced expiratory volume in the first second (percentage of reference value); BMI: Body mass index; Atopy positive: prick test or radioallergosorbent test positive.

Table 2.- Variables related to asthma disease. Differences between clusters

		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	p value
Number of patients, n (%)		95 (32.6)	69 (23.6)	50 (17.1)	78 (26.7)	292	
Asthma duration, years, mean (SD)		18.9 (12.8)	19.0 (12.1)	26.7 (16.3)	23.4 (17.3)	21.5 (14.8)	<0.01
Asthma severity at diagnosis, n (%)	Intermittent	23 (24)	6 (9)	1 (2)	12 (15)	42 (14)	<0.001
	Mild	26 (27)	22 (32)	5 (10)	20 (26)	73 (25)	
	Moderate	28 (29)	19 (27)	23 (46)	19 (24)	89 (31)	
	Severe	18 (19)	22 (32)	21 (42)	27 (35)	88 (30)	
Current asthma severity, n (%)	Intermittent	11 (12)	1 (2)	0	3 (4)	15 (5)	<0.001
	Mild	27 (28)	14 (20)	1 (2)	12 (15)	54 (19)	
	Moderate	37 (39)	27 (39)	17 (34)	25 (32)	106 (36)	
	Severe	20 (21)	27 (39)	32 (64)	38 (49)	117 (40)	
Asthma control, n (%)	Controlled	65 (69)	41 (59)	18 (36)	41 (53)	165 (57)	<0.001
	Not controlled	14 (15)	15 (22)	9 (18)	21 (27)	59 (20)	
	Partially controlled	15 (16)	13 (19)	23 (46)	16 (21)	67 (23)	
Severe exacerbations (last 3 years), n (%)	0	72 (76)	40 (58)	24 (48)	44 (56)	180 (62)	<0.01
	1	8 (8)	19 (28)	11 (22)	12 (15)	50 (17)	
	≥2	15 (16)	10 (14)	15 (30)	21 (29)	61 (21)	
Exacerbations last year, n (%)	0	62 (65)	41 (59)	20 (40)	42 (54)	165 (56)	NS
	1	12 (13)	14 (20)	10 (20)	8 (10)	44 (15)	
	≥2	21 (22)	14 (20)	20 (40)	28 (36)	83 (28)	
Lifetime hospital admissions due to asthma (ICU excluded), n (%)	0	78 (83)	44 (64)	26 (55)	52 (67)	200 (69)	<0.01
	1	11 (12)	12 (17)	9 (19)	10 (13)	42 (15)	
	≥2	5 (5)	13 (19)	12 (26)	16 (20)	45 (16)	
Hospital admissions due to asthma last year (ICU excluded), n (%)	0	90 (95)	64 (93)	42 (86)	67 (86)	263 (90)	NS
	1	4 (4)	4 (6)	4 (8)	8 (10)	20 (7)	
	≥2	1 (1)	1 (1)	3 (6)	3 (4)	8 (3)	
Lifetime ICU admissions due to asthma, n (%)	0	93 (99)	63 (91)	41 (84)	66 (86)	263 (91)	<0.001
	1	1 (1)	5 (7)	7 (14)	6 (8)	19 (7)	
	≥2	0	1 (1)	1 (2)	5 (6)	7 (2)	
FVC%, mean (SD)		117.6 (10.8)	98.2 (13.4)	81.8 (16.8)	100.6 (14.3)	103.0 (17.9)	<0.001
FEV1/FVC, mean (SD)		99 (0.1)	92 (0.1)	76 (0.2)	93 (0.1)	92 (0.1)	<0.001
RV%, mean (SD)		109.4 (24.8)	104.5 (30.1)	109.0 (31.5)	113.3 (17.8)	109.0 (25.4)	NS
DLCO%, mean (SD)		100.8 (16.7)	97.8 (18.5)	86.5 (17.7)	101.2 (16.8)	98.6 (17.6)	NS
FeNO, mean (SD), ppb		51.4 (49.6)	40.0 (39.2)	38.9 (27.3)	39.1 (26.0)	42.4 (36.5)	NS
ACT, mean (SD)		21.7 (4.0)	20.2 (4.2)	19.0 (4.7)	20.0 (5.4)	20.4 (4.6)	<0.001
Mini-AQLQ, mean (SD)		5.7 (1.3)	5.6 (1.0)	5.1 (1.2)	5.4 (1.3)	5.5 (1.2)	<0.05

n: number of patients; SD: standard deviation; ICU: Intensive Care Unit; FVC%: Forced Vital Capacity (percentage of reference value); RV: Residual Volume; DLCO: diffusing capacity for carbon monoxide; FeNO: Fractional exhaled **nitric oxide;** ***ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire.***

Table 3.-Comorbidities. Differences between clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	p value
Number of patients, n (%)	95 (32.6)	69 (23.6)	50 (17.1)	78 (26.7)	292	
Smokers, n (%)						
Current	7 (7)	7 (10)	2 (4)	3 (4)	19 (7)	<0.05
Former	18 (19)	19 (28)	16 (32)	33 (42)	86 (30)	
Never	62 (66)	39 (57)	27 (54)	32 (41)	160 (55)	
Passive (cohabiting)	7 (7)	3 (4)	5 (10)	10 (13)	25 (9)	
NSAID intolerance, n (%)						
Yes	16 (17)	16 (23)	11 (22)	18 (23)	61 (21)	NS
Rhinitis, n (%)						
Yes	63 (66)	46 (67)	33 (67)	48 (62)	190 (65)	NS
Nasal polyps, n (%)						
Yes	30 (32)	12 (17)	20 (41)	28 (36)	90 (31)	<0,05
Gastroesophageal reflux, n (%)						
Yes	19 (20.0)	10 (16.9)	7 (16.3)	17 (27.9)	53 (18.1)	NS
Diabetes, n (%)						
Yes	1 (1)	0 (0)	4 (8)	9 (11.5)	14 (4.8)	<0,01
Heart disease, n (%)						
Yes	2 (2.1)	1 (1.4)	2 (4.0)	4 (5.1)	9 (3.1)	NS
Hypelipidemia, n (%)						
Yes	7 (7.4)	1 (1.4)	12 (24.0)	20 (25.6)	40 (13.7)	<0,01
Obesity, n (%)						
Yes	16 (16.8)	13 (18.8)	12 (24.0)	25 (32.0)	66 (22.6)	NS
Psychiatric disease, n (%)						
Yes	7 (7.4)	6 (8.7)	8 (16.0)	22 (28.2)	43 (14.7)	<0,05
IgE, IU/mL, mean (SD)	154 (84)	140 (91)	175 (82)	148 (93)	152 (88)	NS
Depression, n (%)						
Borderline	6 (7)	4 (6)	5 (10)	9 (12)	24 (9)	NS
Normal	80 (89)	62 (91)	40 (82)	60 (81)	242 (86)	
Abnormal	4 (4)	2 (3)	4 (8)	5 (7)	15 (5)	
Anxiety, n (%)						
Borderline	17 (19)	17 (25)	14 (29)	18 (24)	66 (23)	NS
Normal	57 (63)	39 (57)	29 (59)	41 (55)	166 (59)	
Abnormal	16 (18)	12 (18)	6 (12)	15 (20)	49 (17)	
SNOT22, mean (SD)	27.1 (20.6)	28.8 (19.7)	29.0 (16.4)	30.0 (23.1)	28.6 (20.4)	NS

Non-steroidal anti-inflammatory drug (*NSAID); IU: international Units; SD: Standard deviation; SNOT: *Sino-Nasal Outcome Test.

Table 4.-Social factors. Differences between clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	p value
Number of patients, n (%)	95 (32.6)	69 (23.6)	50 (17.1)	78 (26.7)	292	
Childhood place of residence, n (%)						
Rural	38 (41)	25 (37)	19 (38)	25 (33)	107 (38)	<0,01
Urban	54 (59)	42 (63)	31 (62)	50 (67)	177 (62)	
Childhood social class, n (%)						
High	2 (2)	2 (3)	3 (6)	1 (1)	8 (3)	<0,01
Low	30 (33)	22 (33)	11 (22)	7 (9)	70 (25)	
Medium	60 (65)	43 (64)	36 (72)	67 (89)	206 (73)	
Childhood expsoure to farm animals, n (%)						
No	39 (42)	23 (34)	25 (50)	42 (56)	129 (45)	NS
Yes	53 (58)	44 (66)	25 (50)	33 (44)	155 (55)	
Current place of residence, n (%)						
Rural	34 (36)	25 (37)	10 (20)	19 (25)	88 (30)	NS
Urban	61 (64)	43 (63)	40 (80)	58 (75)	202 (70)	
Educational level, n (%)						
No studies	0	2 (3)	4 (8)	0	6 (2)	<0,001
Primary	18 (19)	13 (19)	11 (22)	26 (34)	68 (24)	
Secondary	36 (38)	22 (33)	20 (40)	39 (51)	117 (40)	
University	41 (43)	30 (45)	15 (30)	12 (16)	98 (34)	
Current social class, n (%)						
Class I	16 (17)	8 (12)	3 (6)	0	27 (9)	<0,05
Class II	6 (6)	4 (6)	7 (14)	11 (14)	28 (10)	
Class III	26 (28)	15 (22)	13 (26)	11 (14)	65 (23)	
Class IVa	26 (28)	23 (34)	9 (18)	24 (32)	82 (29)	
Class IVb	7 (7)	7 (10)	5 (10)	16 (21)	35 (12)	
Class V	13 (14)	10 (15)	13 (26)	14 (18)	50 (17)	
Current exposure to farm animals, n (%)						
No	56 (59)	28 (41)	30 (60)	45 (58)	159 (55)	NS
Yes	39 (41)	41 (59)	20 (40)	32 (42)	132 (45)	

Table 5.-Treatment. Differences between clusters

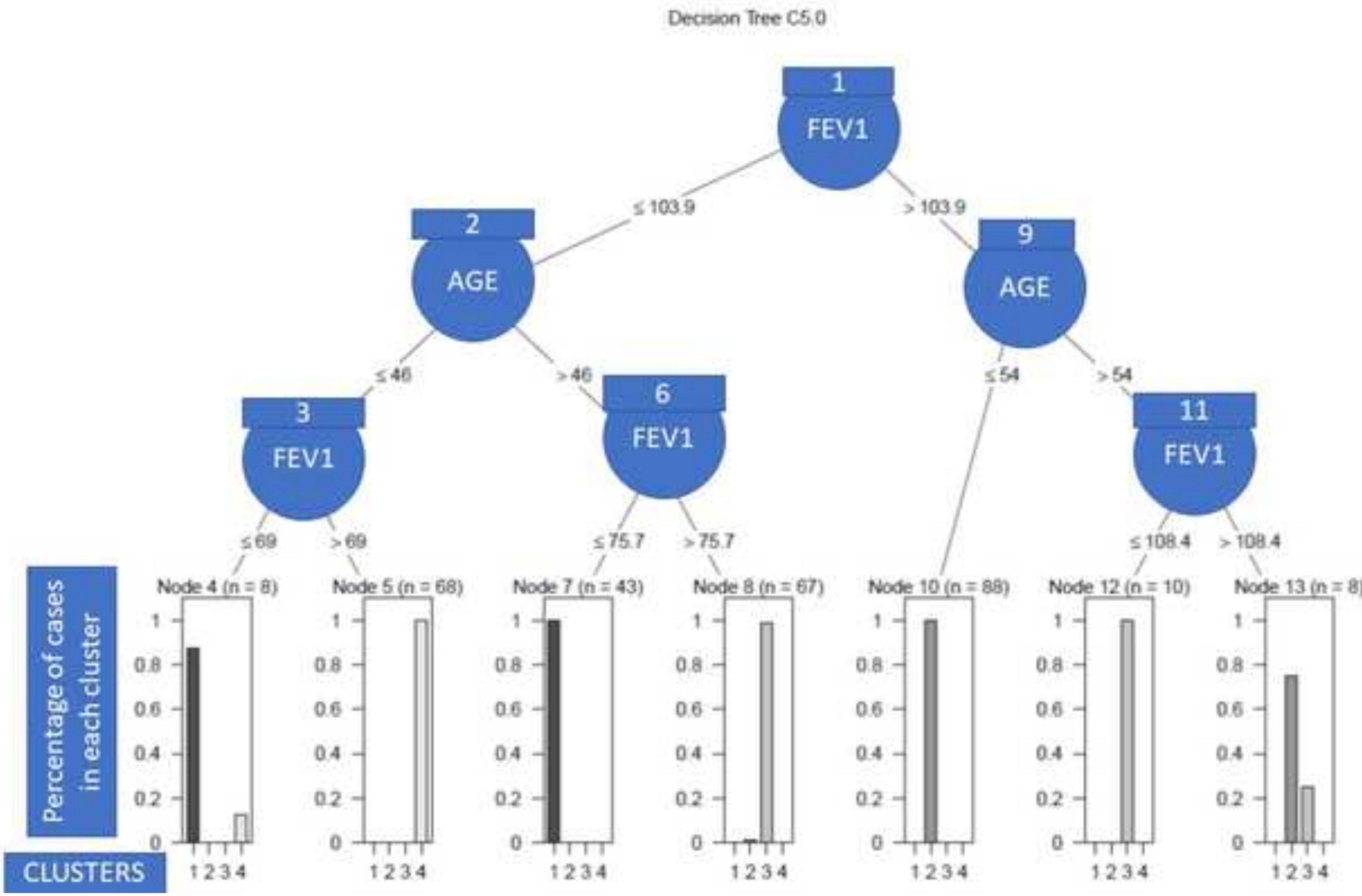
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total	p value
Number of patients, n (%)	95 (32.6)	69 (23.6)	50 (17.1)	78 (26.7)	292	
Inhaled steroids, mcg/day, mean (SD)	566 (458)	607 (452)	858 (601)	718 (498)	673 (506)	<0,01
Systemic steroids, n (%)						
No	88 (94)	66 (96)	41 (82)	72 (92)	267 (92)	<0,05
Yes	6 (6)	3 (4)	9 (18)	6 (8)	24 (8)	
LAMA, n (%)						
No	80 (85)	59 (86)	31 (62)	61 (78)	231 (79)	<0,01
Yes	14 (15)	10 (14)	19 (38)	17 (22)	60 (21)	
ICS-LABA combination, n (%)						
No	25 (26)	11 (16)	2 (4)	15 (19)	53 (18)	<0,01
Yes	70 (74)	58 (84)	48 (96)	63 (81)	239 (82)	
Treatment adherence, n (%)						
No	33 (37)	31 (46)	11 (22)	24 (31)	99 (35)	<0,05
Yes	57 (63)	36 (54)	39 (78)	53 (69)	185 (65)	

n: number of patients; SD: standard deviation; LAMA: Long-acting muscarinic antagonist; ICS: Inhaled corticosteroids; LABA: Long acting beta agonist.

FIGURES

Figure 1. Classification tree of cluster construction. Using two variables, post-bronchodilation FEV1 and the age of the patient, subjects were classified in four clusters.

Figure


Decision Tree C5.0
1
FEV1
≤ 103.9
> 103.9
2
AGE
9
AGE
≤ 46
> 46
≤ 54
> 54
3
FEV1
6
FEV1
11
FEV1
≤ 69
> 69
≤ 75.7
> 75.7
≤ 108.4
> 108.4
Node 4 (n = 8)
Node 5 (n = 68)
Node 7 (n = 43)
Node 8 (n = 67)
Node 10 (n = 88)
Node 12 (n = 10)
Node 13 (n = 8)
Percentage of cases in each cluster
CLUSTERS
1 2 3 4