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TWO NOVEL SEVERE ASTHMA PHENOTYPES IDENTIFIED DURING CHILDHOOD USING A CLUSTERING APPROACH

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Two novel severe asthma phenotypes in children

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Abstract

Background: Unsupervised cluster analysis has already been used to identify severe phenotypes of childhood asthma, but without taking into account inflammatory markers. The aim of this study was to define independent homogeneous phenotypic clusters of severe asthma in a cohort of asthmatic children.

Methods: Cluster analysis was applied to 19 variables from 315 children enrolled in the Trousseau Asthma Program in Paris.

Results: Three independent clusters of asthma were identified. Children in Cluster 1, "*asthma with severe exacerbations and multiple allergies*" (n=103), had more sensitizations to inhaled and food allergens, more blood eosinophils and basophils, more uncontrolled asthma despite high doses of inhaled corticosteroid and more hospitalizations for exacerbation. Children in cluster 2, "*severe asthma with bronchial obstruction*" (n=72), were significantly older, had the highest BMI, a lower FEV_1, more pronounced blood neutrophils and significantly higher levels of all classes of immunoglobulin (except IgE). Children in cluster 3, "mild asthma" (n=140), did not show statistically significant features.

Conclusion: These results could lead to improved management of severe asthma in children by optimizing treatment strategies i.e. antiallergic drugs such as anti-IgE for children with the allergic phenotype, and anti-neutrophil drugs such as macrolides for those with the obstructive phenotype.

INTRODUCTION

Asthma constitutes a growing public health problem due to the increasing prevalence over the past decades, particularly among young children and in emerging countries[1]. In addition, severe asthma is the leading cause of hospitalization among preschool children and this explains, in part, that the cost associated with this disease is largely confined to the most severe forms by the indirect costs it generates[2].

Asthma is no longer considered as just one disease but rather as a complex of multiple and variously associated syndromes expressing different phenotypes[3]. In children, asthma is characterized by a different prognosis according to the age of onset[4]. The Tucson birth cohort[5] identified a phenotype of early-onset asthma which persists in childhood. Children presenting this phenotype have impaired lung function by this time whereas their lung function in early childhood was comparable to that of children who never wheeze. This phenotype has a strong familial component, is predominantly allergic[6] and is often associated with a more severe disease[7]. For preschool children, other severe asthma phenotypes have been described[8] such as “intermittent severe wheezing” often associated with allergic manifestations, and “multiple trigger wheezing” with a poorer long-term prognosis than the others. The clustering approach has been used in cases of severe asthma to identify novel phenotypes. In this respect, most studies to date have been conducted on adult patients though a recent study identified four phenotypes of asthma in school age children through cluster analysis according to the severity of obstructive airway disease[9].

To sum up, phenotyping of childhood asthma has rarely been based on cluster analysis and features other than atopic status and lung function level in spite of the clinical characteristics of the asthmatic condition in children. However, asthma is also characterized by inflammation of the air passages resulting in the temporary narrowing of the airways. The purpose of the present study was to apply an unsupervised analytical approach to distinguish complex

asthma phenotypes without *a priori* definitions of the condition on the hypothesis that in school age children, asthma is divided into phenotypes depending on severity and related not only to atopic status and airways obstruction but also to the underlying inflammatory mechanisms.

METHODS

Patients were part of the 9-year (1998-2009) Trousseau Asthma Program of the "Centre de l'Asthme" at the Hôpital Trousseau in Paris which included children aged 6 to 12 years suffering from severe asthma. Two third of the children were outpatients from Paris and the surrounding area and the rest of them were from regions throughout France and were referred to the centre by a primary care physician due to persistent asthma. The children's data were collected in a computerized database using questionnaires and clinical exams following a standardized protocol for all the children.

The population analyzed in the present study consists of all the children meeting the following inclusion criteria: i. aged between 6 and 12 years at the time of exploration, ii. having a history of at least a 12% change in FEV1 after bronchodilator administration, iii. meeting the criteria for persistent asthma[10], iv. for whom any other chronic obstructive pulmonary disease (congenital or acquired origin) than asthma had been ruled out and, v. who had undergone the clinical exams outside of episodes of exacerbation or acute respiratory illness.

The parameters included in the cluster analysis were:

(1) Age, Body Mass Index (BMI) as defined by weight/heightxheight (kg/m^2) measured during the medical visit.

(2) Asthma duration in two classes: less than or more than 5 years

(3) Maternal or paternal asthma

(4) Allergic sensitization to aeroallergens and trophoallergens as defined by a positive skin prick test (SPT) (wheal allergen ≥ 3 mm in the absence of a positive reaction to the negative control), and confirmed by positive specific immunoglobulin E (IgE) (≥ 0.35 kU/L) (ImmunoCAP®, Phadia, France). The following standardized inhaled and food allergens were

administered: house dust mites, cat dander and dog dander, grass and birch pollen, *Alternaria*, cow's milk, egg, peanut, wheat and fish.

(5) Total immunoglobulin E (IgE) level (kU/L).

(6) Inflammatory markers measured in peripheral blood through lymphocyte, neutrophil, eosinophil, basophil, and monocyte counts expressed in absolute rate (cell counting by automated Sysmex ®, Roche Diagnostics, France) and serum immunoglobulin G, A and M (IgG, A, M) levels (g/L) (Immunoturbidimetry technique, PLC Modular ®, Roche Diagnostics, France).

(7) Lung function measured by spirometry according to the ATS / ERS recommendations[11] (SpiroDyn'R ®, Muret, France). Baseline FEV_1 was expressed as a percentage of the predicted value with respect to sex, size and weight.

(8) Severity (i. mild persistent, ii. moderate persistent and, iii. severe persistent) and control (controlled or partially and uncontrolled) were assessed according to GINA[12]. All enrolled children were on a stable dose of inhaled corticosteroids (ICS) for at least 6 months and were compliant with their prescribed asthma treatment. Thresholds for high-doses of ICS were defined as ≥ 500 μg fluticasone equivalents per day [10]. Asthma control was assessed in four classes: controlled with or without high dose ICS or uncontrolled (including partially controlled) with or without high dose of ICS. Lastly, severe exacerbation defined as at least one hospitalization for asthma exacerbations, was also recorded.

Variable reduction and data transformation

The initial dataset provided almost 40 variables selected from a large spectrum of routine assessments of asthmatic children. The following *a priori* strategies were used to reduce the number of variables before performing the cluster analysis. Missing variables were excluded.

The number of variables that were clinically redundant was reduced by selecting variables reflecting major physiologic parameters (for example for pre- and post bronchodilator FEV_1, basal FEV_1 was only included in cluster analysis), data from the questionnaires were excluded if the data were presented in the text (such as the name of the inhaled corticosteroids), or if the information would have been irrelevant for the current analysis (such as the type of dwelling or parental race). A spectrum of responses variables were transformed into "composite variables" (for example, positive allergen SPTs were transformed into number of sensitizations to food allergens or inhaled allergens). In addition, correlated variables correlated each other were mutually excluded from the model to avoid bias (for example FEV_1 and forced expiratory flow between 25% and 75% of forced vital capacity).

The principal component analysis was then used to select the final variables to be included in the model according to statistical significance. Nineteen variables resulted from this process. Finally, subjects were required to have all of the following 19 variables: demographic data (age, BMI); additional variables previously reported to have an effect on disease severity (asthma duration); elements of current classification schemes (medication use) or risk (hospitalization for asthma exacerbation); parameters with important physiologic measures (lung function, atopy) were included in the cluster analysis.

In spite of the fact that our cluster analysis was unsupervised, the stability of the clusters was tested using 1) data resampling techniques by randomly drawing the variables from the model and 2) by altering the sample or subsetting [13].

Statistical analysis

All available variables were included in the statistical analysis. Data standardization was performed before beginning analyses. The k-means method (PROC FASTCLUST in SAS 9.2) was used as a preliminary analysis to produce a large number of disjoint clusters of observations. Hierarchical cluster analysis was then performed to hierarchically cluster these preliminary clusters using Ward's minimum-variance method (PROC CLUSTER). Categorical variables between the different groups, of asthma severity and clusters respectively, were compared with Chi^2 test or Fisher's exact test when the required conditions were not respected. In the case of continuous variables, groups were compared with the Student's t-test and one-way analysis of variance (ANOVA) when the hypotheses of normality and variance equality were confirmed and with Wilcoxon and Kruskal-Wallis test otherwise. Tukey and Dunn's tests were used for *post hoc* multiple comparisons[14]. The 95% confidence interval of the mean rather than the standard deviation was introduced to better see whether there was an overlapping of the variable values among the clusters. All analyses were performed with SAS version 9.2 software. For each bilateral test, type I error was taken to be 5%.

Ethics

Parents of each child provided written informed consent before the questionnaire was distributed. Since all procedures reflect a common patient care at the study center, the protocol was endorsed by the Institutional Review Board of the Medical Ethics Committee on Research of the Hospital Saint Antoine by the direct procedure. With respect to the confidentiality of patient records, data handling for the study was authorized by the '*Commission Nationale d'Informatique et Libertés.*'

RESULTS

Description of the population

The study initially included 351 consecutive children consulting as outpatients for asthma management and who met the inclusion criteria. Of these, 36 were excluded from analysis as one or more of the cluster analysis parameters were missing. The final study population thus included 315 children, 203 boys (64.4%). The features of excluded children did not differ from those of the final sample (data not shown). The characteristics of the final population according to GINA classification are described in Table I.

Asthma phenotypes according to the cluster analysis

Using the cluster approach, a dendogram was generated and revealed three clusters of children with shared phenotypic characteristics (Figure 1). These clusters were distinguished on the basis of age, BMI, FEV_1 level, the degree of sensitization to food or inhaled allergens, and systemic eosinophilia, neutrophilia, lymphocytosis (as well as monocytosis although at a borderline level), elevation of the different immunoglobulin classes (IgG, IgA, IgM, IgE) in the peripheral blood, and lastly asthma control under ICS treatment and hospitalization for asthma exacerbation (Table II). The clusters were shown to be stable as the results persisted when variables were excluded even though the statistical significance of other variables decreased slightly. In addition, the results persisted after altering the sample or subsetting (either for the sample including the 36 patients with missing data for the other variables or by drawing randomly 150 individuals).

Cluster 1

One hundred and three children were classed in this cluster (Table II). They were more atopic with the highest average number of positive SPTs to both inhaled and food allergens, the

highest total IgE level, combined with higher eosinophil values (mean value 734/mm^3, range 650-817) and higher basophil values (mean value 42/mm^3, range 34-50) and had more maternal or paternal asthma. Duration of asthma was longer than in other clusters (asthma duration more than 5 years in 88% of the children). The FEV_1 was slightly lower in this cluster compared with cluster 3. Asthma was more frequently uncontrolled despite high-doses of ICS (14% of this population) in this group and more severe exacerbation requiring at least one hospitalization (65 %) were reported. This asthma phenotype can be termed *"asthma with severe exacerbations and multiple allergies"*.

Cluster 2

Seventy two children were grouped in this cluster that we called "*severe asthma with bronchial obstruction*" (Table II). The children in this group were significantly older (mean age 10 years) and had the highest BMI (mean value 20, range 19-21). Children in this cluster had a significantly lower baseline pulmonary function in terms of FEV_1 (mean value 82% of predicted value (PV), range 78-86). This phenotype was associated with the most pronounced "neutrophil inflammation" with a non-significant increase in monocyte values, (550 range 511-588) (p=0.08), and a significant increase in absolute neutrophil values (3423 range 3082-3765) as well as a significant increase in all classes of immunoglobulin (average value of IgG in grams per liter 11.7 (range 11.2-12.3), an average value of IgA in grams per liter of 1.8 (range 1.6-1.9) and an average value of IgM in grams per liter of 1.3 (range 1.1-1.4)).

Cluster 3

Cluster 3 was the largest group with 140 children (Table II). This cluster was characterized by the highest average value of FEV_1 (mean value 97 % of PV, range 95-100), less sensitization to inhaled or food allergens, no elevation of either blood cells or immunoglobulins. In this population, asthma was more often controlled with low doses of ICS than in the other clusters (26% compared to 9% and 3% in cluster 1 and 2, respectively). This phenotype can be

summarized as *"mild asthma"*.

Asthma phenotypes and asthma severity

Each GINA class was heterogeneously distributed in each cluster without predominance of severity in any cluster. (Figure 2).

DISCUSSION

Our study identified two novel severe phenotypes in childhood asthma as compared to a mild asthma phenotype. These two novel severe asthma phenotypes are:*"asthma with severe exacerbations and multiple allergies"* and *"severe asthma with bronchial obstruction*".

Similarly to the previous report[9], in our study all the clusters of childhood asthma presented atopic features, but the magnitude and the nature of allergic sensitization differed among clusters. Moreover, the nature of "systemic inflammation" – "eosinophil-basophil-type", 'neutrophil-type" or "no inflammation" – was different amongst the groups and this difference in asthmatic children has not been previously described

"*Asthma with severe exacerbations and multiple allergies*".

This type of severe asthma is associated with an inflammation predominantly of "allergic type" (with eosinophil and basophil cells) in combination with multiple allergic sensitizations and elevated total IgE. An earlier study by our team in asthmatic children showed the link between intra-alveolar eosinophilia and atopy[15]. Moreover, it is known that blood eosinophilia is closely correlated to eosinophil inflammation in the deep lung tissues[16]. This phenotype is associated with severe exacerbations requiring hospitalisations and uncontrolled asthma despite high doses of ICS. Many studies have confirmed that asthma at risk of severe exacerbations or difficult to control is associated with allergic asthma mainly in children[17, 18]. Severe acute asthma requiring intensive care was more frequently found in cases of food allergy[19, 20]. Roberts *et al.*[19] comparing 19 asthmatic children ventilated for severe exacerbation and 38 mild asthmatic children showed that the two independent risk factors were the severity of asthma (OR 5.89 [1.06 to 32.61] and food allergy (OR 9.85 [1.04 to 93.27]).

Lastly, in the phenotype with a higher duration of asthma (frequently more than 5 years)

FEV_1 values were slightly lower compared with the FEV_1 values observed in the mild asthma cluster. This was also found in the SARP study[9] in which the most allergic children had severe exacerbations despite a high level of therapy but did not have severely decreased FEV_1 values. Indeed, severe exacerbations could be responsible for the more rapid decline in lung function[21].

"Severe asthma with bronchial obstruction"

This phenotype of severe asthma is characterized in our study by a significant decrease in FEV_1 (mean value 82 % of PV, range 78%-86%) even if it remains within the accepted limits of normal, as in other studies performed in severe asthmatic children[22, 23]. This finding is probably due to the fact that the normal value of FEV_1 in children at school age is more than 90%, like in our cluster 3*"mild asthma"* in which the mean value of FEV_1 is 97 % of PV (range 95-100).

The cluster was also characterized by a significant elevation in neutrophils in peripheral blood in comparison with the other clusters. Many studies have shown an association between the severity of asthma and neutrophilic inflammation detected by induced sputum in particular[24, 25]. Furthermore, our study supports the findings of Siroux *et al*[26] who showed that the phenotype of "active treated allergic childhood onset asthma" is associated with blood eosinophilia, while "active treated adult-onset asthma" is associated to blood neutrophils. Systemic neutrophilic inflammation is mainly described in inflammation of infectious type. In the same way, elevation of immunoglobulins has been related to an inflammation in asthma originating from a bacterial colonization. It is well known that this inflammation type induces uncontrolled asthma[27] and corticosteroid resistance[28] and therefore probably increases the risk of tissue remodeling, which would explain the lower FEV_1 values in this phenotype.

This phenotype was also associated with a higher BMI. The association between obesity and

severe asthma has been found by many authors[29-31]. Halder *et al.*[30] showed that a distinct cluster of asthmatic subjects with high BMI was associated with symptomatic asthma without eosinophilic inflammation. The study by Moore *et al.*[31] also found a cluster of asthma associated with a moderate reduction in FEV_1 described more in women who were overweight with delayed onset. Lastly, our results are consistent with the SARP study, including the fact that asthma severity is not related to a markedly low FEV_1 or to the GINA severity score[9].

The main limitation to our study lies in the fact that all the patients were recruited in one center, and this of course could represent a geographical bias. Nevertheless, while two thirds of the asthmatic children were from Paris and the surrounding area (more than 10 million people), the remaining one third lives in regions from all over France, which limits this potential bias. However, our result cannot be generalized in another population especially with greater ethnic diversity. Moreover, our sample is the largest among those used in previous similar works and our results fit the clinical context. An important strength of our study is that the unsupervised cluster analysis identified the clusters without any *a priori* assignment and the stability of the clusters was tested. This has rarely been done before for children. Furthermore, our investigation is unlike previous cluster analyses of asthma as we included "blood inflammatory markers" and demonstrate that this constitutes an important feature of asthma.

In conclusion, this large study of asthmatic children differentiates two novel severe asthma phenotypes. We would suggest that these phenotypes be included in recommendations on the management of severe asthma in children to optimize treatment strategies i.e. antiallergic drugs such as anti-IgE or anti Il-5 for children with the severe allergic phenotype, and anti-neutrophil drugs such as macrolides for those with the obstructive phenotype, and thus potentially improve long-term outcome. Further studies investigating inflammation in deep

lung tissues are required to confirm these results.

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Table I: Features of the studied population (n=315)

	Mild asthma (n=101)	Moderate asthma (n=140)	Severe asthma (n=74)	*p* value*
Age (y),	8.7 (8.4;9.0)	9.1 (8.8;9.3)	9.0 (8.6;9.4)	0.26
BMI (Kg/m^2)	17.3 (16.7;17.9)	17.9 (17.3;18.4)	17.5 (16.8;18.1)	0.25
Maternal asthma (%)	15 (17.2%)	25 (21.4%)	13 (18.8%)	0.76
Paternal asthma (%)	19 (21.8%)	16 (13.7%)	12 (17.4%)	0.31
Number of sensitizations to food allergens median (range)	0 (0;2)	0 (0;2)	0 (0;3)	0.18
Number of sensitizations to inhaled allergens, median (range)	2 (0;7)	2 (0;8)	1.9 (0;8)	0.10
Total IgE (kU/L) , median (range)	349 (5;4022)	374 (2;5117)	674 (4;4351)	0.38
IgG (g/L), median (range)	9.8 (0.0;15.5)	9.8 (0.0;20.3)	9.8 (0.0;14.6)	0.68
IgA (g/L), median (range)	1.3 (0.0;3.9)	1.3 (0.0;4.0)	1.3 (0;3.0)	0.36
IgM (g/L), median (range)	1.0 (0.0;2.8)	1.0 (0.0;2.8)	1.1 (0.0;2.5)	0.24
Blood eosinophils ($/mm^3$)	554 (464;645)	542 (479;604)	602 (510;694)	0.43
Blood neutrophils ($/mm^3$)	2970 (2756;3184)	3203 (2964;3441	3210 (2834;3585)	0.57
Blood lymphocytes ($/mm^3$)	2929 (2778;3079)	2853 (2722;2985)	2879 (2693;3064)	0.76
Blood monocytes ($/mm^3$)	514 (480;548)	519 (490;547)	523 (486;561)	0.94
Blood basophils ($/mm^3$)	27 (20;34)	24 (18; 30)	30 (20;39)	0.66
Baseline FEV_1 (% predicted)	99 (97;101)	88 (85;90)	86 (81;90)	<.0001
Asthma duration ≥ 5y (%)	71 (70.3%)	99 (70.7%)	63 (85.1%)	0.04
≥ 1 hospitalization for asthma exacerbation (%)	28 (27.7%)	32 (22.9%)	34 (46.0%)	0.002
Uncontrolled with high-dose ICS (%)	3 (3.0%)	7 (5.0%)	24 (32.4%)	<.0001
Controlled with low doses of ICS (%)	15 (14.9%)	29 (20.7%)	3 (4.1%)	<.0001

Severe asthma was defined according to GINA criteria.

Data represent mean (CI 95%) unless otherwise specified

*One-way analysis of variance (ANOVA) or Chi2 test when conditions were respected Kruskal-Wallis or Fisher's exact test otherwise

BMI: Body Mass Index

Table II: Features of children according to cluster analysis in the entire population (n=315)

	Cluster 1 *"Asthma with severe exacerbations and multiple allergies"* (n=103)	Cluster 2 *"Severe asthma with bronchial obstruction"* (n=72)	Cluster 3 *"Mild asthma"* (n=140)	*p* value*
Age (y)	8.8 (8.5;9.2)	10.3 (10.0;10.6)	8.3 (8.0;8.5)	<.0001
BMI (Kg/m^2)	17.1 (16.6;17.5)	20.0 (19.1;21.0)	16.7 (16.3;17.0)	<.0001
Maternal asthma (%)	25 (33)	8 (13)	16 (13)	<.001
Paternal asthma (%)	25 (29)	11 (18)	11 (9)	.001
Number of sensitizations to food allergens, median (range)	0.3 (0.2;0.5)	0.0 (0.0; 0.0)	0.1 (0.0;0.1)	<.0001
Number of sensitizations to inhaled allergens, median (range)	3.0 (2.6;3.5)	1.9 (1.5;2.3)	1.2 (1.0;1.5)	<.0001
Total IgE (kU/L), median (range)	805 (657;952)	485 (365;605)	450 (323;577)	<.0001
IgG (g/L), median (range)	9.9 (9.6;10.4)	11.7 (11.2;12.3)	8.6 (8.1;9.0)	<.0001
IgA (g/L), median (range)	1.3 (1.2;1.4)	1.8 (1.6;1.9)	1.1 (1.0;1.2)	<.0001
IgM (g/L), median (range)	1.1 (1.0;1.2)	1.3 (1.1;1.4)	0.9 (0.9;1.0)	0001
Blood eosinophils ($/mm^3$)	734 (650;817)	514 (421;607)	454 (395;515)	<.0001
Blood basophils ($/mm^3$)	42 (34;50)	3 (4;14)	24 (18;23)	<.0001
Blood lymphocytes ($/mm^3$)	3036 (2889;3182)	3030 (2852;3208)	2691 (2561;2820)	<.001
Blood neutrophils ($/mm^3$)	2767 (2540;2993)	3423 (3082;3765)	3250 (3009;3492)	.001
Blood monocytes ($/mm^3$)	505 (474;536)	550 (511;588)	515 (483;541)	.08
Baseline FEV_1 (% predicted)	89 (86;92)	82 (78;86)	97 (95;100)	<.0001
Asthma duration ≥ 5y (%)	91 (88)	49 (68)	93 (66)	<.001
≥ 1 hospitalization for asthma exacerbation (%)	67 (65)	7 (10)	20 (14)	<.0001
Uncontrolled with high-dose ICS (%)	15 (15)	6 (8)	13 (9)	<.0001
Controlled with low doses of ICS (%)	9 (9)	2 (3)	36 (26)	<.0001

Data represent mean (CI 95%) unless otherwise specified

* One-way analysis of variance (ANOVA) or Chi2 test when conditions were respected, Kruskal-Wallis or Fisher's exact test otherwise

BMI: Body Mass Index

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Figure 1

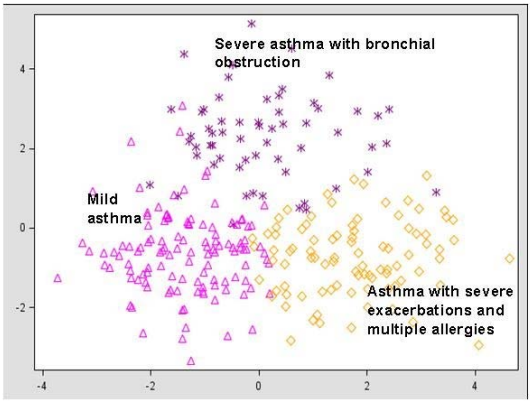

Figure 1: Scatterplot for clusters using Ward's method in the entire population (N=315)
Each point represents a single subject. The plot depicts clustering and clear separation of children with "Asthma with severe exacerbations and multiple allergies" (n=103) *(diamonds)*, "Severe asthma with bronchial obstruction" (n=72) *(star)*, and "Mild asthma "(n=140) (*triangles*).

Figure 2

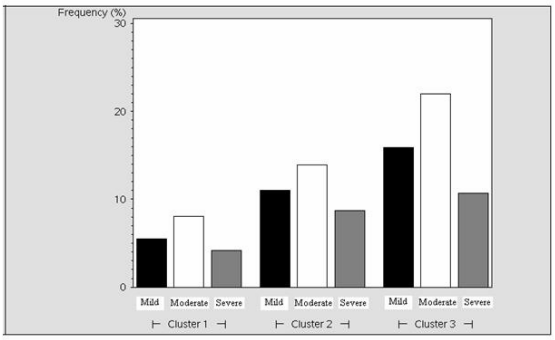

Figure 2: Frequency of children with mild, moderate and severe asthma defined by GINA guidelines
The sum of the nine bars (divided by GINA classification in each cluster) is equal to 100%.
Each GINA class was heterogeneously distributed in each cluster without predominance of severity in any cluster.