

ORIGINAL ARTICLE

AIRWAY DISEASES

Three phenotypes of adult-onset asthma

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Abstract

Rationale: Adult-onset asthma differs from childhood-onset asthma in many respects. It is more heterogeneous, often severe and frequently associated with loss of lung function. To identify underlying mechanisms of adult-onset asthma and to capture predictors of disease progression, detailed characterization and phenotyping is necessary.

Objectives: To characterize adult-onset asthma and identify subphenotypes of adult-onset asthma.

Methods: A cohort of 200 patients with adult-onset (>18 year) asthma (age 54 (26–75) year) was recruited from one academic and three nonacademic pulmonary outpatient clinics in Amsterdam, the Netherlands. These patients were fully characterized with respect to clinical, functional and inflammatory markers. After data reduction, K-means nonhierarchical cluster analysis was performed to identify clusters of adult-onset asthma.

Measurements and main results: Patients with adult-onset asthma were predominately female (61%) and nonatopic (55%). Within this group of patients were identified three clusters of adult-onset asthma. Cluster 1 ($n = 69$) consisted of patients with severe eosinophilic inflammation-predominant asthma and persistent airflow limitation despite high-intensity anti-inflammatory treatment, with relatively low symptom scores. The second cluster was characterized by obese women with frequent symptoms, high healthcare utilization and low sputum eosinophils. The third cluster consisted of patients with mild-to-moderate, well-controlled asthma with normal lung function and low inflammatory markers. Repeatability accuracy was 98.2%.

Conclusions: Amongst patients with adult-onset asthma, three subphenotypes can be identified with distinct clinical and inflammatory characteristics. These subphenotypes help to understand the underlying pathobiology and provide clinicians with directions for personalized management.

Abbreviations

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; BMI, body mass index; GERD, gastro oesophageal reflux disease; HCU, Healthcare Utilization; ICS, inhaled corticosteroids; KCO, transfer coefficient expressing carbon monoxide diffusing capacity; FeNO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in one-second; FVC, forced vital capacity; NSAIDs, nonsteroidal anti-inflammatory drugs; OCS, oral corticosteroids; RV, residual volume; SD, standard deviation; SNOT-22, Sino-Nasal Outcome Test-22; TLC, total lung capacity.

Asthma is a heterogeneous disease driven by a mix of genetic and environmental factors (1, 2). Because of this heterogeneity, the term asthma is not clearly defined. Consequently, the clinical diagnosis of asthma and asthma severity are based on characteristics such as lung function and symptoms (1). However, there is increasing evidence that this approach does not reflect the multidimensional nature of the disease (2, 3). Therefore, identification of different asthma phenotypes is of increasing importance (4).

Recently, several studies have used cluster analysis in different groups of asthma patients to identify clinically well-recognized phenotypes of asthma (5–7). By using these multivariate techniques, several adult phenotypes could be identified with clinically important differences. On the one hand, they found phenotypes of asthma that started in childhood including early-onset atopic asthma (5–7), benign asthma (5–7) and symptom-predominant asthma (6). On the other hand, the cluster analysis also revealed two clusters of asthma starting mainly in adulthood: the noneosinophilic obese female cluster (5–7), and a cluster with predominantly males with eosinophilic asthma (6).

Not much is known about the mechanisms of adult-onset asthma phenotype. In 2002, Miranda and colleagues described phenotypic differences between patients with early-onset and adult-onset asthma (8), resulting in an increasing awareness that this phenotype differentiates from early-onset (childhood) asthma (9–13). Ever since, it has been shown that it is more heterogeneous, often severe (10) and frequently associated with loss of lung function (11–13). In addition, whilst childhood-onset asthma is mostly atopic in nature (14), adult-onset asthma is often associated with specific triggers such as respiratory tract infections (15), exposure to occupational agents (16), aspirin intake (17), smoking (18) and obesity (19) suggesting different underlying mechanisms.

However, to identify these mechanisms detailed characterization and subphenotyping of adult-onset asthma is necessary. In this study, we hypothesized that adult-onset asthma is heterogeneous and can be divided into various subphenotypes. Therefore, we used unbiased nonhierarchical cluster analysis techniques to identify these subphenotypes.

Methods

Subjects

A total of 200 subjects with adult-onset asthma were recruited from one academic and three nonacademic hospitals in Amsterdam, the Netherlands, between June 2009 and June 2011. Eligible subjects were adults (20–75) with a physicians' diagnosis of asthma that started after the age of 18, who were stable on asthma medication (no exacerbations or changes in asthma medication in the past 4 weeks). Asthma was defined according to GINA criteria (1). Exclusion criteria were: any respiratory symptoms or chronic lung diseases during childhood, other pulmonary diseases including, or unrelated major comorbidities (see Data S1).

Current and ex-smoking (>10py) was allowed if the patient had at least 12% improvement in FEV₁ after inhalation of 400ug salbutamol and a normal diffusion capacity at the time of inclusion (20). This study was approved by the Hospital Medical Ethics Board (MEC 08/358; NTR number 1838), and all patients provided written informed consent.

Study design

During this cross-sectional multicenter study, all patients completed the questionnaires that assessed demographic data,

medical history, medical consumption and medication use, as well as the Asthma Control Questionnaire (ACQ) (21), the Asthma Quality of Life Questionnaire (AQLQ) (22) and the Sino-Nasal Outcome Test-22 (SNOT-22) (23). Physiologic testing of lung function included pre- and postbronchodilator spirometry, carbon monoxide diffusion capacity, body plethysmography and, if possible, bronchial hyperresponsiveness to methacholine (24–26). Atopic status was assessed by total IgE and specific IgE to a panel of common aero- and food allergens by using the Pharmacia Uni-CAP System. In addition, all patients were assessed for specific IgE to *Aspergillus fumigatus*. Inflammatory status was measured by the fraction of exhaled nitric oxide (FeNO), assessment of neutrophils and eosinophils in peripheral blood and induced sputum (27) (see Data S1).

Statistical analyses

Data reduction and variable selection:

The total number of variables was reduced by elimination of data irrelevant for the current analyses or that were in written text format. With respect to lung function measurements, the clinical most relevant parameters were chosen. Demographic data were selected to cover a broad variety of routine assessments, as were data on disease severity. Eventually, a total of 35 variables were selected based on clinical relevance and avoiding redundancy (see Data S1). After initial data selection, missing data were imputed and further reduced by factor analyses with orthogonal varimax rotation. Based on the pattern of loading, 11 factors were identified with eigenvalue ≥ 1 .

Cluster analysis:

Cluster analysis was performed in a multistep approach (see Data S1). First, Ward's hierarchical cluster analysis was used for estimation of the number of likely clusters. Then, cluster quality was checked by two-step cluster analysis methods, and K-means nonhierarchical cluster analysis was performed. To ensure repeatability and stability within the model, the K-means algorithm was repeated 199 times in a leave-one-out validation model.

Other statistical methodology:

Non-normally distributed data were log-transformed before initial analysis. For comparison between clusters, chi-square tests were used for proportions and ANOVA with *post hoc* analysis for parametric variables. All analyses were performed using SPSS, version 18.0 (SPSS, Inc., Chicago, IL). *P*-values <0.05 were considered statistically significant.

Results

Subject demographics

In total, 832 patients visiting the outpatient clinic were screened for this study, of which 306 patients had a (possible) diagnosis of adult-onset asthma. However, 106 recollected childhood dyspnoea or asthma did not meet the inclusion criteria or refused to attend for various reasons. Therefore,

200 patients with adult-onset asthma were enrolled in this study, consisting of secondary and tertiary care patients, either referred by the general practitioner (70%) or by a pulmonologist (30%). Patients were aged between 26 and 75, and the majority of them were female (60.5%). The results show that patients with adult-onset asthma are mainly non-atopic (55%), and only 39% reported a positive family history for asthma. With respect to medication use and healthcare utilization, 41.5% used a high dose of inhaled corticosteroids (ICS), 23% used daily oral corticosteroids (OCS), and 26% had at least 3 or more exacerbations that required OCS in the past 12 months. Of all patients with adult-onset asthma, 38.5% met the IMI criteria of severe asthma (28). In addition, a large proportion (51%) of these patients showed elevated sputum eosinophils (median (range) 2.1 (0.2–16.4)), despite adequate treatment with inhaled corticosteroids. Baseline demographics and clinical data of the complete cohort are given in Table 1. Data on medical history, medication use and healthcare utilization are given in Table 2.

Subphenotyping adult-onset asthma

Using Ward's and K-means cluster analysis, we identified three subphenotypes of patients with adult-onset asthma separated

by gender, BMI, lung function, sputum eosinophils, sensitivity to NSAIDs or aspirin and asthma quality of life. Baseline demographics and clinical data are given in Table 1. Data on medication and healthcare use are given in Table 2.

Cluster 1: severe eosinophilic inflammation-predominant

The first group we identified consisted of 69 (34.5%) patients and described a severe eosinophilic inflammation-predominant group with persistent airflow limitation. This group was characterized by predominantly women (71%) with a postbronchodilator FEV₁/FVC percentage predicted of 85.6% (± 15.5), increased exhaled FeNO levels and increased sputum eosinophil percentages (6.3% (0.3–24.7)). These patients were treated with medium-to-high doses of ICS and in 26% of the cases combined with maintenance OCS. Twenty-nine per cent had at least three exacerbations and 13% had at least one hospitalization or emergency department visit in the past 12 months.

Cluster 2: frequent symptoms, high healthcare utilization and low sputum eosinophils

The second subphenotype ($n = 41$, 20.5%) had a higher prevalence of patients of non-Caucasian descent and was

Table 1 Baseline characteristics of the total cohort and individual clusters

	Total cohort ($n = 200$)	Cluster 1 ($n = 69$)	Cluster 2 ($n = 41$)	Cluster 3 ($n = 90$)	<i>P</i> -value
Female %	60.5	71	68.3	48.9	0.01
Age*	53.9 (10.8)	54.8 (9.7)	53 (9.7)	53.7 (12)	0.7
Age of onset*	41 (12.8)	40.6 (12.4)	41.5 (12.2)	41.1 (13.5)	0.9
Asthma duration†	10 (4–20)	10 (6.5–20)	8 (4–19.5)	8.5 (3–21)	0.09
Body Mass Index*	28 (4.9)	28.2 (5)	30.4 (5.4)	26.7 (4.3)	<0.001
Caucasian race %	85	85.5	65.9	93.3	<0.001
Pack-years†	0 (0–7.4)	1 (0–13.5)	0 (0–2.5)	0 (0–7.2)	0.15
Total IgE†	105 (33–599)	119 (47–407)	84 (51–372)	97 (26–263)	0.29
Atopy – inhaled allergens %	45	43.5	48.8	44.4	0.85
Atopy – food allergens %	8	5.8	7.3	10	0.61
Atopy – Asp. Fumigatus %	10.5	5.8	12.2	13.3	0.28
History of nasal polyposis %	37	42	31.7	35.6	0.51
Lung function					
pbFVC % pred*	106.6 (19.9)	105 (18.4)	94.4 (19.1)	113 (18.4)	<0.001
pbFEV ₁ % pred*	91.8 (20.9)	84.4 (21)	83.9 (21.1)	101.1 (16.7)	<0.001
pbFEV ₁ /FVC % pred*	91 (14.8)	85.6 (15.6)	94.3 (14.1)	93.6 (13.4)	<0.001
Change in FEV ₁ % pred†	5 (2–10)	7 (2–11)	4 (2–7.5)	5 (2–10)	0.29
KCO % pred*	101.2 (16.4)	100.7 (18.5)	103.4 (15.3)	100.5 (15.2)	0.62
pbRV/TLC % pred*	92.8 (19.4)	100.9 (19.8)	97.6 (20.6)	84.4 (15)	<0.001
PC20	2.5 (19.5)	0.6 (19.5)	2.5 (19.5)	3.9 (19.5)	0.002
FeNO.ppb†	28.7 (16.2–59.3)	34 (17–70)	20.7 (11.4–39)	28 (17–59)	0.03
Sputum eosinophils (%)†	2.1 (0.2–16.4)	6.3 (0.3–24.7)	0.9 (0.1–18)	1.4 (0.1–9.9)	0.048
Sputum neutrophils (%)†	69.7 (45–84.6)	63.1 (39.5–83.6)	74 (61–93.7)	68.7 (43.7–84.6)	0.46
Blood eosinophils (10 ⁹ /l)†	0.2 (0.1–0.34)	0.3 (0.11–0.51)	0.18 (0.1–0.28)	0.19 (0.01–0.3)	0.17
Blood neutrophils (10 ⁹ /l)†	4.3 (3.4–5.8)	4.4 (3.8–6.1)	4.3 (3.2–6.5)	4.2 (3.2–5.4)	0.29

P-value from ANOVA or chi-square analysis between the three clusters.

FeNO = fraction of exhaled nitric oxide; FEV₁ = forced expiratory flow in 1 second; FVC = forced vital capacity; KCO = transfer coefficient expressing carbon monoxide diffusing capacity; pb = postbronchodilator; RV = residual volume; TLC = total lung capacity.

Data are represented in *mean ($\pm$ SD); †median (interquartile range) or frequency (%).

Table 2 Medical history, medication use and healthcare utilization

	Total cohort (n = 200)	Cluster 1 (n = 69)	Cluster 2 (n = 41)	Cluster 3 (n = 90)	P-value
Reflux medication/symptoms %	52	50.7	75.6	42.2	<0.01
Hormonal induced symptoms % F	18.5	20.3	19.5	16.7	<0.01
NSAID sensitivity %	15	5.8	17.1	21.1	0.02
Familial asthma %	39	40	46	35	0.48
Familial atopy %	33	36.2	29.3	32.2	0.78
Occupational asthma%	5	5.8	2.4	5.6	0.69
Home environmental triggers %	0.5	1.4	4.9	4.4	0.72
Nasal corticosteroids %	57	47.8	70.7	57.8	0.06
Chronic Oral Corticosteroids %	23	26.2	31.7	16.7	0.13
Dose of Oral Corticosteroids [†]	7.5 (5–10)	10 (5–10)	7.5 (5–15)	7.5 (5–10)	0.13
Dose of ICS [†]	500 (500–1000)	1000 (500–1000)	1000 (500–1000)	500 (0–1000)	0.02
Omalizumab %	6	7.2	12.2	2.2	0.07
Exacerbations <12 months %					<0.001
0	52.5	42	29.3	71.1	
1–2	21.5	29	17.1	17.8	
>3	26	29	53.7	11.1	
Hospital of inclusion%					0.004
Secondary care	27.5	15.9	22	38.9	
Tertiary care	72.5	84.1	78	61.1	
Doctors visits <12months %					<0.001
0	8	2.9	4.9	13.3	
1–2	48	40.6	24.4	64.4	
>3	44	56.5	70.7	22.2	
ACQ score*	1.5 (1)	1.9 (0.8)	2.5 (0.8)	0.7 (0.6)	<0.001
AQLQ score*	5.2 (2.2)	5 (0.4)	3.2 (0.8)	6.2 (0.4)	<0.001
SNOT score*	1.2 (0.9)	1.4 (0.6)	1.9 (0.9)	0.9 (0.6)	<0.001

P-value from ANOVA or chi-square analysis between the three clusters.

ACQ = Asthma Control Questionnaire; AQLQ = Asthma Quality of Life Questionnaire; ICS = inhaled corticosteroids fluticasone equivalent; SNOT-22 = Sino-Nasal Outcome Test-22.

Data are represented in *mean (±SD); [†]median (interquartile range) or frequency (%).

characterized by obese women with frequent symptoms, high healthcare utilization and low sputum eosinophils. Patients in this cluster had the highest symptom scores and were most often treated for gastroesophageal reflux disease (GERD) or had complaints of GERD. Their postbronchodilator FEV₁ was reduced, but their FEV₁/VC ratio was normal. They were treated with high-dose ICS often combined with OCS or anti-IgE treatment. Despite these high treatment regimens, they had the most frequent doctors' visits (70.7%), exacerbations (53.7%) and hospitalizations or emergency department visits (31.8%). These symptoms and high healthcare utilization seemed to be out of proportion with their clinical and inflammatory markers as they showed no airways obstruction, low FeNO levels and low sputum eosinophils counts.

Cluster 3: mild-to-moderate, well-controlled asthma

The third cluster was the largest and consisted of 90 patients (45%) with a mild-to-moderate, well-controlled asthma. This group has a male preponderance of Caucasian descent and more often a history of aspirin sensitivity. Symptom scores, lung function measurements and airway inflammation were often within the normal range, and these patients were

mostly treated with an intermediate dose of ICS. In addition, patients in this cluster had the lowest number of exacerbations (29%) and hospitalizations or emergency department visits (5.6%) in the past 12 months.

Validation

To ensure repeatability and stability within the model, the k-means algorithm was repeated 199 times in a leave-one-out validation model. This showed a within repeatability accuracy of 98.2%.

Discussion

Three subphenotypes of adult-onset asthma were identified in the present study. The first cluster consisted of patients with severe eosinophilic inflammation-predominant asthma with persistent airflow limitation and few asthma symptoms. The second subphenotype was characterized by obese women with frequent asthma symptoms and high healthcare utilization, but normal lung function and low sputum eosinophils. The third cluster showed patients with mild-to-moderate, well-controlled asthma with normal lung function and low inflammatory markers.

These results reinforce the existence of two previously described clusters of mainly severe asthma patients (obese female and persistent eosinophilic) that together covered about half of the patients with adult-onset asthma, and a third cluster of patients with well-controlled, mild asthma, covering the other half. Other clinically significant clusters were not identified.

Our results confirm that the previously described clusters of adult-onset severe asthma are distinct and robust, and require a personalized management approach.

The present study is, to our knowledge, the first to thoroughly describe a large cohort of patients with adult-onset asthma with respect to clinical, functional and inflammatory markers. It shows that adult-onset asthma affects more women than men, that the majority of patients are nonatopic, and that there is a high prevalence of severe disease, which have been suggested in previous studies as well (9–13). The clusters we identified in the present study are in agreement with previous findings in heterogeneous populations of patients with asthma (5–7).

In our study, we identified a subset of mainly female patients with severe eosinophilic inflammation-predominant asthma, few asthma symptoms and persistent airflow limitation. This fits in with one of the adult-onset asthma clusters in the study by Haldar and colleagues that consisted of patients with active eosinophilic inflammation and few daily symptoms, although they found predominantly males in this cluster (6). Our results not only confirm the existence of this important adult-onset asthma phenotype, but also more sharply delineate its phenotypic characteristics in an independent population with different treatment strategies.

Remarkably, a high percentage of patients with eosinophilic inflammation showed incomplete reversibility of FEV₁, which fits in the results of several previous studies, showing that patients with adult-onset asthma are at increased risk of fixed airflow limitation (11–13). This suggests that in this specific subphenotype of asthma aggressive remodelling processes might be active. In addition, there is now convincing evidence that it is associated with increased risk of asthma exacerbations (29), persistent airflow limitation (30) and oral corticosteroid dependence (31). This implies that a symptom-guided management approach may not be effective in these patients and an inflammation-targeted management, for example with monoclonal antibodies against anti-IL-5(32), is warranted to prevent future exacerbations and lung function decline.

The second group of patients with adult-onset asthma that was identified consisted mainly of obese women with high healthcare utilization. By using the similar clustering techniques as Haldar, we could reproduce and extend these important previous results in a cohort of secondary and tertiary care patients with adult-onset asthma in the Netherlands. We also confirmed that this obese adult-onset asthma phenotype is not associated with eosinophilic airway inflammation (33) and responsiveness to inhaled corticosteroids (34) but is characterized by poor asthma control, low quality of life and high healthcare utilization. Apparently, other

mechanisms are causing asthma symptoms in these patients, including altered lung mechanics, leading to airway hyperresponsiveness and increased airway smooth muscle stiffness (35). It has also been shown that visceral adipose tissue in obese adult-onset asthma patients produces high levels of adipokines, which is associated with airway reactivity but not with airway inflammation (36). And finally, overweight patients may exhibit comorbidities that provoke or worsen asthma, in particular GERD (35). By consequence, a management strategy aimed at reducing overweight rather than reducing airways inflammation might be indicated in these patients to prevent overtreatment with corticosteroids.

Apart from these two subphenotypes of patients with severe and uncontrolled asthma, the present study identified an additional third cluster of mild-to-moderate, persistent, well-controlled adult-onset asthma. It consisted almost exclusively of Caucasians and was mainly recruited from secondary care clinics. We were not surprised that these patients with mild-to-moderate adult-onset asthma constituted the largest cluster. A prospective study investigating outcome and severity of all incident cases of adult-onset asthma in the northern part of Sweden showed that after a follow-up period of 5–10 years, 70% of the patients had developed mild-to-moderate persistent disease (10), whereas only 5% had developed severe disease. Very recently, another cluster analyses in Korean patients showed a cluster of mild adult-onset asthma (37). Our study confirms this specific adult-onset asthma subphenotype, thereby showing that adult-onset asthma is not always severe or uncontrolled, but can also have a milder course and prognosis.

Although the present study was performed in a well-characterized cohort of patients with adult-onset asthma and a validated unsupervised approach was used for the analysis, it might have some limitations. First, the age of onset and duration of asthma were based on self-report and could therefore be influenced by recall bias. Despite stringent inclusion and exclusion criteria, we cannot exclude this bias entirely, although in one study the reported year of asthma onset appeared to be rather accurate (38). Second, although every effort was made to insure as much objectivity as possible, several subjective areas needed to be addressed in the cluster analysis, such as the choice of variables used for the analysis and the optimal number of clusters. Although we used a broad selection of variables and let factor analysis decide which variables would eventually be used in the analysis, we cannot exclude the possibility that variables of greater significance were excluded by this process. However, the present study has identified clinically well-recognized subphenotypes that were identified in previous studies using similar and different clustering techniques in different populations. Therefore, we do not think that the subjective areas that needed to be addressed had a great influence on our results. Third, the persistent eosinophilia in one of our clusters might be related to nonadherence rather than being a reflection of more severe asthma (39), which we cannot exclude and warrants further investigation. Fourth, the patients in our study were recruited from secondary and tertiary outpatient clinics. As patients with mild asthma in the Netherlands are mostly

treated by the general practitioner, there might be an overestimation of patients with more severe disease in our cohort. Therefore, we cannot estimate the real proportion of the 3 clusters in the total population of patients with adult-onset asthma.

In conclusion, the present study shows that amongst patients with adult-onset asthma, three different subphenotypes can be identified with distinct clinical and inflammatory features. These results confirm and extend the phenotypic characteristics of two previously proposed clusters of severe adult-onset asthma by showing their consistency in a different population with different treatment regimens. It also shows a third subphenotype of patients with mild-to-moderate adult-onset asthma. The identification of these three subphenotypes may raise novel hypotheses on the mechanisms of adult-onset asthma and asthma severity, and may give clinicians new directions for more personalized management strategies.

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Authors' contributions

M Amelink, EJM Weersink, A ten Brinke, PJ Sterk and EH Bel conceived and designed the study; M Amelink, SB De Nijs, EJM Weersink and A ten Brinke recruited the subjects; M Amelink, PMB Tilburg, PI Van Spiegel and FH Krouwels coordinated subject recruitment per hospital;

M Amelink, SB De Nijs and JC de Groot collected the data; R Lutter was responsible for sputum processing and cell counting; M Amelink, SB de Nijs, JC de Groot and AH Zwinderman carried out the statistical analysis; M Amelink, AH Zwinderman and EH Bel analysed and interpreted the data; EH Bel was the principal investigator and had the overall responsibility. M Amelink and EH Bel drafted and revised the manuscript. All authors revised the manuscript.

Conflicts of interest

M. Amelink has received an unrestricted grant from Novartis, S.B. de Nijs has received an unrestricted grant from Glaxo Smith Klein (GSK). J.C. de Groot has received an unrestricted grant from GSK. F.H. Krouwels has received honoraria for speaking at conferences supported by GSK and Boehringer Ingelheim. A. Ten Brinke has received an unrestricted research grant from GSK, serves on an advisory committee of the Novartis National Research Advisory Board and received honoraria for speaking at conferences supported by GSK and Boehringer Ingelheim. Prof. P. Sterk received a university grant from the University of Amsterdam, from the public private programme Innovative Medicines Initiative by the EU and from GSK. Prof. E.H. Bel has consulted for Merck and Shering Plough, serves on an advisory board of Novartis and received honoraria for speaking at conferences supported by Nycomed, Novartis and GSK. She has also received unrestricted grants from Novartis, GSK and from the public private programme Innovative Medicines Initiative by the EU. For all authors, none of these funds resulted in personal financial gain. None of the remaining authors have conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Three phenotypes of adult-onset asthma.

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Three phenotypes of adult-onset asthma

Online data supplement.

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METHODS

Subjects:

For the present study a total of 200 subjects with adult-onset asthma were recruited from 1 academic and 3 non-academic hospitals between June 2009 and June 2011. Eligible 2 subjects were adults (20-75) with a physician's diagnosis of asthma onset after the age of 18 3 and stable on asthma medication for at least 4 weeks. Asthma was defined as a history of 4 episodic dyspnea and wheezing, and a documented reversibility in FEV₁ of at least 12% of 5 the predicted value or hyperresponsiveness to inhaled methacholine chloride (PC20 < 8.0 6 mg/ml)(1), in the past 5 years. Exclusion criteria were: a childhood doctors' diagnosis of 7 asthma or chronic bronchitis, a history of childhood dyspnea attacks (spontaneous or during 8 exercise), use of pulmonary medication during childhood, other pulmonary diseases, 9 including COPD, non-related major co-morbidities and pregnancy. Current and ex-smoking 10 (>10 packyears) was allowed if the patient had at least 12% improvement in FEV₁% 11 predicted after inhalation of 400 mcg of Salbutamol as well as a normal diffusion capacity at 12 the time of inclusion. This study was approved by the hospital Medical Ethics Board (MEC 13 08/358; NTR number 1838) and all patients provided written informed consent.

Study Design:

During this cross-sectional multicenter study all patients were fully assessed with respect to 1 clinical, functional and inflammatory parameters. All measurements were performed during 2 one visit. If bronchial challenge test was not performed within one year before inclusion, a 3 methacholine challenge test was performed during a second visit (1-30 days after first visit). 4 Patients fulfilled questionnaires that assessed demographic data, medical history, medical 5 consumption in the past year and medication use, as well as the Asthma Control 6 Questionnaire (ACQ)(2), the Asthma Quality of Life Questionnaire (AQLQ)(3) and the Sino- 7 Nasal Outcome Test-22 (SNOT-22)(4). For all questionnaires the authorized Dutch 8 translation was used. Physiologic testing of lung function included prebronchodilator 9 spirometry after withholding of pulmonary medication for 12hr, postbronchodilator (pb)

spirometry 15 minutes after administration of 400ug Salbutamol, carbon monoxide diffusion capacity, body plethysmography and, if possible, bronchial hyperresponsiveness to methacholine using a tidal breathing method(5). Lung function tests were performed according to standardized lung function techniques(6-8) using Masterscreenpneumo, MasterscreenPFT Pro Diffusion and Masterscreen Body; Jeager; Wurzburg, Germany. Atopic status was assessed by total IgE and specific IgE to a panel of common aeroallergens (house dust mite, grass and birch pollen, herbs, molds and cat and dog dander), a panel of common food allergens (milk, soy, cod, peanut, ovalbumin and wheat) by ImmunoCAP. Atopy was defined as a score of > 0.35 kU/L for at least one of the specific IgE. In addition all patients were assessed for specific IgE to Aspergillus Fumigatus. Inflammatory status was measured by exhaled nitric oxide, using a online device at a constant flow rate (Niox Mino; Aerocrine AB, Solna, Sweden)(9), assessment of blood neutrophils and blood eosinophils. In addition, induced sputum was collected according to a standardized protocol(10) and whole sputum samples were processed(11). Differential cell counts were expressed as percentage of non-squamous cells and all samples containing >80% squamous cells were excluded.

Variables:

Age of onset was defined as the age at which a physician's diagnosed asthma was established for the first time and asthma duration was the number of years since diagnosis.

Stable asthma was defined as no exacerbations or changes in asthma medication in the past 4 weeks. One smoking packyear was calculated as smoking 20 cigarettes a day for a whole year. COPD was defined as more then 10 packyears with an FEV₁/FVC ratio <0.7 and no documented reversibility in FEV₁ of at least 12% of the predicted value. A history of nasal polyposis was defined as nasal polyposis diagnosed by an ENT specialist by means of nasal-endoscopy and treated accordingly.

Female patients were marked as positive for hormonal asthma or hormone-induced symptoms if they had more symptoms during a specific time of the month, every month, an

increase of symptoms after menopause or a clear onset of asthma after pregnancy or spontaneous abortion. If parents or siblings had asthma or atopy, patients were classified as having familiar asthma or atopy. Occupational asthma was defined as proven onset of asthma due to occupational triggers. If the onset of asthma was possibly due to occupational triggers, although not confirmed by a physician, this was defined as possible occupational influences. Chronic use of oral corticosteroids was defined as the daily use of OCS in the previous 3 months before the study. The number of exacerbations was defined as the number of prednisone bursts needed to control increased asthma symptoms in the past 12 months.

Statistical Analyses:

Data reduction and variable selection:

The total number of 234 variables was reduced by elimination of data irrelevant for the current analyses (such as extensive medical history) or in written text format (such as names of medication). With respect to lung function measurements the clinical most relevant parameters were chosen (i.e. reversibility in FEV₁, pbFEV₁ in percentage predicted, pbFVC in percentage predicted, pbFEV₁/FVC in percentage predicted, KCO in percentage predicted, and RV%/TLC in percentage predicted).

In addition, demographic data were selected to cover a broad variety of routine assessments (e.g. gender, race), as were data on disease severity (e.g. medication use, number of exacerbations). Eventually, a total of 35 variables were selected.

After initial data selection missing data were imputed, and then further reduced by factor analyses with orthogonal varimax rotation. Based on the pattern of loading 11 factors were identified with an eigenvalue ≥ 1 .

We selected one parameter representative for each factor, based on the highest loading. Eventually the variables gender, occupational asthma, ongoing home environmental influences, familiar atopy, packyears, intolerance to NSAID's, prednisone dependency, pbFEV₁, total IgE, sputum eosinophils and AQLQ were selected for further analysis.

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107 Cluster Analyses:

108 Further subphenotyping of adult-onset asthma was performed in a multi-step approach. First,
109 by using Ward's hierarchical cluster analysis a dendogram was generated for estimation of
110 the number of likely clusters.

111 After estimation of the number of clusters, the cluster quality was checked by two-step
112 cluster analysis methods. Results of this analysis showed a "very poor" cluster quality, most
113 likely caused by the low number of patient positive for occupational asthma and/or ongoing
114 home environmental influences. Therefore, we decided to replace these variables for the
115 second highest in that factor. For occupational influences there was no second variable. The
116 second highest variable after home environmental influences was BMI. After
117 exclusion/replacement of these variables the cluster quality became "good" and was optimal
118 for 5 clusters. The number of clusters was set at 5 for the definite k-means non-hierarchical
119 cluster analysis. However, k-means analysis showed 2 subclusters of the larger clusters of 6
120 and 12 patients, respectively. These 2 subclusters were merged into the larger clusters.
121 To ensure repeatability and stability within the model, the k-means algorithm was repeated
122 199 times in a leave-one-out validation model.

123

124 Other statistical methodology:

125 Non-normally distributed data were log transformed before initial analysis. For comparison
126 between clusters of baseline variables X^2 - tests were used for proportions and ANOVA with
127 post-hoc analysis and upaired t-tests for parametric variables. All analyses were performed
128 using SPSS version 18.0 (SPSS, Inc., Chicago, IL). P-values less than 0.05 were considered
129 statistically significant.

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