Clinical and inflammatory markers in asthma and COPD phenotyping

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Chapter 3

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. In order to identify underlying mechanisms of adult-onset asthma and to capture predictors of disease progression, detailed characterisation 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 (>18yr) asthma (age 54 (26-75) yr) was recruited from one academic and three non-academic 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 non-hierarchical cluster analysis was performed in order to identify clusters of adult-onset asthma.

**Measurements and main results:** Patients with adult-onset asthma were predominantly female (61%) and non-atopic (55%). Within this group of patients identified three clusters of adult-onset asthma. Patients in 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 females with frequent symptoms, high health care utilisation 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.
**Introduction**

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 symptom (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 characterisation and subphenotyping of adult-onset asthma is necessary. In the present study, we hypothesized that adult-onset asthma is heterogeneous and can be divided into various subphenotypes. Therefore we used unbiased non-hierarchical cluster analysis techniques to identify these subphenotypes.

**Methods**

**Subjects**

A total of 200 subjects with adult-onset asthma were recruited from 1 academic and 3 non-academic 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 COPD, or non-related major co-morbidities (see online supplement).

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 fulfilled 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).

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 redundance. 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 Eigen value $\geq$1.

Cluster Analysis

Cluster analysis was performed in a multi-step approach. 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 non-hierarchical 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 $X^2$-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 less than 0.05 were considered statistically significant.

**Results**

**Subject demographics**

In total 832 patients visiting the outpatient clinic were screened for the present study, of which 306 patients had a (possible) diagnosis of adult-onset asthma. However, 106 recollected childhood dyspnea 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 the present study, consisting of secondary and tertiary care patients, either referred by the general practitioner (70%) or by a pulmonologist (30%). Patients were aged between 26-75 and the majority of them being 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 health care utilisation, 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 health care utilisation are given in Table 2.

**Subphenotyping adult-onset asthma**

Using Wards and K-means cluster analysis we identified 3 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 health care use is given in Table 2.
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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 females (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, in 26% of the cases combined with maintenance OCS. Twenty-nine percent had at least three exacerbations and 13% had at least one hospitalisation or emergency department visit in the past 12 months.

Table 1. Baseline characteristics of the total cohort and individual clusters

<table>
<thead>
<tr>
<th></th>
<th>Total Cohort (n=200)</th>
<th>Cluster 1 (n=69)</th>
<th>Cluster 2 (n=41)</th>
<th>Cluster 3 (n=90)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female %</td>
<td>61</td>
<td>71</td>
<td>68</td>
<td>49</td>
<td>0.01</td>
</tr>
<tr>
<td>Age</td>
<td>54 (10.8)</td>
<td>55 (9.7)</td>
<td>53 (9.7)</td>
<td>54 (12)</td>
<td>0.7</td>
</tr>
<tr>
<td>Age of onset†</td>
<td>41 (12.8)</td>
<td>41 (12.4)</td>
<td>42 (12.2)</td>
<td>41 (13.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>Asthma duration†</td>
<td>10 (4-20)</td>
<td>10 (7-20)</td>
<td>8 (4-20)</td>
<td>9 (3-21)</td>
<td>0.09</td>
</tr>
<tr>
<td>Body Mass Index†</td>
<td>28 (4.9)</td>
<td>28 (5.0)</td>
<td>30 (5.4)</td>
<td>27 (4.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasian Race %</td>
<td>85</td>
<td>86</td>
<td>66</td>
<td>93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Packyears‡</td>
<td>0 (0-7)</td>
<td>1 (0-14)</td>
<td>0 (0-3)</td>
<td>0 (0-7)</td>
<td>0.15</td>
</tr>
<tr>
<td>Total IgE†</td>
<td>105 (33-599)</td>
<td>119 (47-407)</td>
<td>84 (51-372)</td>
<td>97 (26-263)</td>
<td>0.29</td>
</tr>
<tr>
<td>Atopy – inh. allergens%</td>
<td>45</td>
<td>44</td>
<td>49</td>
<td>44</td>
<td>0.85</td>
</tr>
<tr>
<td>Atopy – food allergens%</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>0.61</td>
</tr>
<tr>
<td>Atopy – Asp. Fumigatus%</td>
<td>11</td>
<td>6</td>
<td>12</td>
<td>13</td>
<td>0.28</td>
</tr>
<tr>
<td>History of Nasal Polyposis%</td>
<td>37</td>
<td>42</td>
<td>32</td>
<td>36</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Lung function:

- pbFVC %pred†: 107 (19.9), 105 (18.4), 94 (19.1), 113 (18.4), <0.001
- pbFEV1 %pred†: 92 (20.9), 84 (21.0), 84 (21.1), 101 (16.7), <0.001
- pbFEV1/FVC %pred†: 91 (14.8), 86 (15.6), 94 (14.1), 94 (13.4), <0.001
- Change in FEV1 %pred†: 5 (2-10), 7 (2-11), 4 (2-8), 5 (2-10), 0.29
- KCO %pred†: 101 (16.4), 101 (18.5), 103 (15.3), 101 (15.2), 0.62
- pbRV/TLC %pred†: 93 (19.4), 101 (19.8), 98 (20.6), 84 (15.0), <0.001

FeNO.ppb†: 29 (16-59), 34 (17-70), 21 (11-39), 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.10⁹/l: 0.2 (0.1-0.34), 0.3 (0.1-0.51), 0.18 (0.1-0.28), 0.19 (0.01-0.3), 0.17

Blood neutrophils.10⁹/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 X² analysis between the three clusters.

Data is represented in *mean(±SD); †Median (interquartile range) or frequency (%).

Definition of abbreviations: FVC = Forced Vital Capacity; FEV1 = Forced expiratory flow in 1 second; KCO = Transfer coefficient expressing carbon monoxide diffusing capacity; RV = Residual volume; TLC = Total lung capacity; pb = post bronchodilator; FeNO = fraction of exhaled nitric oxide
Cluster 2: frequent symptoms, high health care utilisation and low sputum eosinophils

The second subphenotype (n = 41, 20.5%) had a higher prevalence of patients of non-
Caucasian descent and was characterized by obese females with frequent symptoms,
high health care utilisation and low sputum eosinophils. Patients in this cluster had the
highest symptom scores and were the 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 hospitalisations or emergency department visits (31.8%). These symptoms and high health care utilisation 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 hospitalisations 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 females with frequent asthma symptoms and high health care utilisation, 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 non-atopic and that there is a high prevalence of severe disease, which has 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 e.g. 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 females with high healthcare utilisation. 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 health care 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.
And finally, overweight patients may exhibit co-morbidities that provoke or worsen asthma, in particular gastroesophageal reflux (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-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-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 non-adherence 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. Since patients with mild asthma in The Netherlands are mostly treated by the general practitioner there might be an over-estimation 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-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.
Chapter 3

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Phenotypes of longstanding adult-onset asthma


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