Clinical and inflammatory markers in asthma and COPD phenotyping

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Chapter 4

EARLY PHENOTYPES OF NEWLY DIAGNOSED ASTHMA IN ADULTS

Submitted

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Abstract

Background: Several reports have shown that adult-onset asthma is more severe at onset, is more likely to persist and shows a faster decline in FEV₁ as compared to childhood-onset asthma. To capture the complexity of this heterogeneous disease, a first requirement is to define distinct phenotypes at an early disease stage.

Objective: We hypothesized that in a population of newly diagnosed adult-onset asthma distinct asthma phenotypes can be identified.

Methods: 202 patients with newly diagnosed (<1 yr) adult-onset asthma (57% female; age (range) 20-82 yr; 45% atopic) participated in the study. Thirty-two clinical, functional and inflammatory characteristics were assessed. Factor analysis was performed followed by cluster analysis using Wards hierarchical and K-means non-hierarchical cluster analysis.

Results: The largest cluster (n=114) consisted of patients with mild asthma. The second cluster (n=45) consisted of patients with high symptom scores, high medication requirement, poor quality of life, low exhaled nitric oxide levels and high numbers of blood neutrophils. Patients in the third cluster (n=43) had lower postbronchodilator FEV₁, more air trapping and more severe airway hyperresponsiveness than the other clusters. Of these, 62% had chronic rhinosinusitis and 29% had nasal polyps. In clusters 1 and 3 asthma onset was associated with a history of respiratory infection in one-third of the patients.

Conclusion: In newly diagnosed adult-onset asthma three phenotypes of different severities can be identified, characterized by different triggers of asthma onset, and different types of airway inflammation. These phenotypes can be used to unravel disease mechanisms and predict clinical outcome.
**Introduction**

Asthma is a complex and heterogeneous disorder that cannot be described adequately by symptoms, variable airflow limitation and airway hyperresponsiveness alone (1;2). Recently, different asthma phenotypes have been identified by cluster analysis, either based on severity of symptoms and type of airway inflammation (eosinophilic or non-eosinophilic) (3) or based on lung function and onset of asthma symptoms (4).

So far, adult-onset asthma has not systemically been investigated. Several reports have proposed that adult onset asthma is more severe at onset (5), is more likely to persist (6) and shows a faster decline in FEV₁ (7) as compared to childhood onset asthma. Adult-onset asthma also has risk factors that are distinct from those observed in childhood onset asthma. Whilst childhood onset asthma is largely associated with atopy and male gender (4) asthma in adulthood is rather associated with occupational exposures, respiratory infections, obesity, hormonal influences, psychological stress, smoking, and acetaminophen intake (8). Identification of clinically relevant asthma phenotypes in this population at an early stage may increase our understanding of underlying pathobiological processes and help to identify risk factors for the development of severe disease.

In the present study we postulated that unsupervised non-hierarchical cluster analysis in a population of patients with recently diagnosed adult-onset asthma would reveal distinct adult-onset asthma phenotypes. To that end we fully characterized 200 adults with recently-diagnosed asthma and performed cluster analysis on clinical, functional and inflammatory characteristics.

**Methods**

**Patients**

Three-hundred fifty-eight adult patients who were visiting the outpatient clinics of one academic and two non-academic pulmonary outpatient clinics and were recently (within one year) diagnosed with asthma were asked to participate in the study. The diagnosis of asthma was based on symptoms of dyspnea, cough and wheeze, and confirmed by reversibility in FEV₁ ≥ 12% of the predicted value and/or hyperresponsiveness to inhaled methacholine (PC₂₀ <8mg/mL).

Patients were excluded if they had a history of childhood asthma or “chronic bronchitis” or had used bronchodilator or other asthma medication in childhood, or recalled to have frequently missed days at school due to respiratory symptoms.
Smokers or ex-smokers with a smoking history > 10 packyears were included only if they showed reversibility in FEV$_1$, $\geq 12\%$ of the predicted value, with normal post-bronchodilator FEV$_1$/FVC > 0.7 and normal diffusion capacity.

The study was approved by the Hospital Medical Ethics Committee and was registered in the Netherlands Trial Register under NTR 1846. All patients gave their written informed consent.

**Study Design**

The study had a cross-sectional design comprising one or two hospital visits. During the first visit questionnaires were taken, lung function was measured, sputum was induced and venous blood was taken. Then sinus CT-scanning and nasal endoscopy were performed. If bronchial challenge test was not performed at time of asthma diagnosis, methacholine challenge test was performed during a second visit (1-30 days after first visit).

**Measurements**

**Questionnaires**

Patients completed questionnaires that assessed demographic data, medical history and medication use, as well the Asthma Control Questionnaire (ACQ) (9), the Asthma Quality of Life Questionnaire (AQLQ) (10) and the sino-nasal outcome test-22 (SNOT) (11). Self reported trigger factors for the onset of asthma were identified by the answer to the following question: “What do you consider to be the inciting trigger for the onset of your asthma”.

**Assessment of Chronic rhinosinusitis and nasal polyps**

Sinus CT-scans were scored by a radiologist according to the validated Lund-MacKay scoring system (12). Nasal polyps were detected by nasal endoscopy and were scored as absent or present. Chronic rhinosinusitis (CRS) was defined according to the European Position Paper on Rhinosinusitis and Nasal polyps (EP3OS) (13).

**Pulmonary function measurements and allergy testing**

Pre- and postbronchodilator spirometry was performed, according to the European Respiratory Society recommendations (14). Diffusion capacity of the lung for carbon monoxide was measured and corrected for hemoglobin (15). Static lung volumes were measured using body plethysmography (16). Methacholine challenge test was performed using the tidal breathing methods, according ERS standardization (17), with adequate withholding time of lung-medication.

Atopic status was assessed by total and specific IgE to a panel of common aeroallergens (house dust mite, grass and birch pollen, herbs, aspergillus fumigatus, moulds
and cat and dog dander) and 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’s.

Markers of airway inflammation
Sputum was induced by inhalation of NaCl 4.5% during 3x5 min intervals using a high
output nebulizer [KLAVAmed, Bielefeld, Germany] (18). Whole sputum samples were
processed as previously described according to international recommendations (19).
Sputum samples containing > 80% squamous cells were excluded from analysis. Differ-
etial cell counts were expressed as the percentage of non-squamous cells. Differential
cell counts were also assessed in venous blood.
Exhaled nitric oxide (FeNO) was measured with a portable rapid-response chemolumi-
nescent analyser (flow rate 50mL/s; NIOX System, Aerocrine, Sweden) (20).

Statistical analysis
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, postbronchodilator FEV₁ in
percentage predicted, postbronchodilator FVC in percentage predicted, postbroncho-
dilator FEV₁/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)
and data about lower airway disease (e.g. sinus CT-scan score, nasal endoscopy score,
presence of nasal polyps). Eventually, a total of 32 variables were selected.
After initial data selection missing data were imputed (21), and then further reduced
by factor analyses with orthogonal varimax rotation. Based on the pattern of loading
9 factors, based on the highest loading, were identified with an eigenvalue ≥1. The fol-
lowing nine variables were selected to perform the cluster analysis: 1) Asthma Quality
of Life Questionnaire score (10); 2) sinus CT-scan scores, expressed according Lund en
Mackay score (12); 3) postbronchodilator change in FEV₁, expressed as percentage from
predicted; 4) serum neutrophilic concentration; 5) natural logarithm of the serum IgE
concentration; 6) natural logarithm of PC₂₀ methacholine; 7) RV/TLC ratio, expressed as
percentage of the predicted value; 8) TLC, expressed as the percentage of the predicted
value; 9) cumulative tobacco cigarette consumption (in packyears). Of these variables, missing data made up a small proportion (< 8%).

Cluster analysis
Further phenotyping of adult-onset asthma was performed in a multi-step approach. Ward’s hierarchical cluster analysis was used and a dendogram was generated for estimation of the number of likely clusters. After estimation of the number of clusters, the cluster quality was checked by two-step cluster analysis methods and k-means non-hierarchical cluster analysis was performed.

Using Wards hierarchical cluster analysis, three clusters were identified. Two step cluster analysis confirmed that the best cluster quality was found using a cut-off point of three clusters. Finally, the number of clusters was set at 3 for the definite k-means non-hierarchical cluster analyses, which resulted in a cluster of 114, 45 and 43 patients.

Validation of the clusters
To ensure repeatability and stability within the model, the k-means algorithm was repeated 201 times in a leave-one-out validation model. The percentage of correct placement of patients in cluster 1, 2 and 3 was, 97, 94 and 88%, respectively. This resulted in a within a repeatability accuracy of 93%.

Other statistical methodology
For comparison between the indentified clusters Chi², Student’s t-test and one-way analysis of variance (ANOVA) were used. The least-significant difference (LSD) pairwise comparison test was used for post hoc multiple comparisons. All analyses were performed using SPSS version 18.0 (SPSS, Inc., Chicago, IL). P-values less than 0.05 were considered statistical significant.

Results
Patient characteristics
Of 358 adult patients who were asked to participate in the study, 202 agreed. There were no differences between participants and non-participants with respect to age (p=0.23), atopic status (p=0.5), bronchial hyperresponsivess to methacholine (p=0.43) or bronchoconstriction based on postbronchodilator FEV₁ (p=0.75). The included patients were significantly more often males than females (p=0.02) and had more often a Caucasian ethnic origin (p<0.001) as compared to the non-participants. The final population included 202 adults with newly diagnosed adult-onset asthma. Demographics and clinical data are presented in Tables 1-3.
Chapter 4

Adult-onset asthma phenotypes

Ward’s hierarchical cluster analysis identified 3 clusters and two-step cluster analysis confirmed that the best cluster quality was found by this number of clusters. The distance between the three clusters of 114, 45 and 43 patients, respectively, was calculated using K-means non-hierarchical cluster analysis.

Cluster 1

Cluster 1 was the largest cluster and consisted of 114 patients with mild asthma with the least medication requirement, the lowest asthma symptom scores and the best

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**Table 1. Demographics and clinical features**

<table>
<thead>
<tr>
<th></th>
<th>All $n=202$</th>
<th>Cluster 1 $n=114$</th>
<th>Cluster 2 $n=45$</th>
<th>Cluster 3 $n=43$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48 ±14.8</td>
<td>47 ±15.1</td>
<td>46 ±14.6</td>
<td>51 ±14.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>57</td>
<td>51</td>
<td>64</td>
<td>65</td>
<td>0.14</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 ± 5.3</td>
<td>27 ± 5.3</td>
<td>28 ± 5.4</td>
<td>28 ± 5.2</td>
<td>0.67</td>
</tr>
<tr>
<td>Body mass index &gt; 30, %</td>
<td>30</td>
<td>25</td>
<td>33</td>
<td>37</td>
<td>0.24</td>
</tr>
<tr>
<td>Caucasian race, %</td>
<td>84</td>
<td>87</td>
<td>80</td>
<td>79</td>
<td>0.38</td>
</tr>
<tr>
<td>Packyears*</td>
<td>2 (0-14)</td>
<td>1 (0-13)</td>
<td>1 (0-12)</td>
<td>0 (0-23)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Atopic status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- positive RAST panel, %</td>
<td>45</td>
<td>47</td>
<td>44</td>
<td>40</td>
<td>0.74</td>
</tr>
<tr>
<td>- positive food allergens, %</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>0.34</td>
</tr>
<tr>
<td>- positive A fumigatus, %</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>0.47</td>
</tr>
<tr>
<td>Total IgE *, kU/L</td>
<td>61 (26-209)</td>
<td>67 (26-221)</td>
<td>53 (26-156)</td>
<td>51 (26-317)</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Medication use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ICS, %</td>
<td>79</td>
<td>75</td>
<td>84</td>
<td>84</td>
<td>0.32</td>
</tr>
<tr>
<td>- Dose ICS*, µg¹</td>
<td>250 (250-500)</td>
<td>250 (100-500)</td>
<td>500 (250-1000)</td>
<td>500 (250-500)</td>
<td>0.001</td>
</tr>
<tr>
<td>- Nasal corticosteroids, %</td>
<td>28</td>
<td>30</td>
<td>33</td>
<td>16</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>CO-morbidities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CRS,%</td>
<td>51</td>
<td>41</td>
<td>67</td>
<td>62</td>
<td>0.04</td>
</tr>
<tr>
<td>- Nasal polyps, %⁴</td>
<td>18</td>
<td>19</td>
<td>11</td>
<td>24</td>
<td>0.32</td>
</tr>
<tr>
<td>- History of atopic dermatitis, %</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>0.80</td>
</tr>
<tr>
<td>- History of GERD, %</td>
<td>39</td>
<td>36</td>
<td>49</td>
<td>37</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ACQ-score*</td>
<td>1.2 (0.5-1.8)</td>
<td>0.7 (0.3-1.2)</td>
<td>2.3 (1.8-2.8)</td>
<td>1.5 (1.0-1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- AQLQ-score*</td>
<td>5.7 (4.9-6.3)</td>
<td>6.2 (5.8-6.6)</td>
<td>4.6 (3.9-4.9)</td>
<td>5.2 (4.9-5.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data expressed as mean/standard deviation; * median/Interquartile range

¹ fluticasone equivalent; ² based on EP3OS criteria; ³ detected by nasal endoscopy or history of surgery

**Definition of abbreviations:** ACQ= Asthma Control Questionnaire; AQLQ= Asthma Quality of Life Questionnaire; CRS= Chronic rhinosinusitis; ICS= inhaled corticosteroids; GERD= Gastro Oesophageal Reflux disease
asthma related quality of life. Lung function measurements and airway inflammation were within the normal range. In this cluster asthma onset was associated with a history of viral and/or bacterial respiratory infections in 38% of the cases.

Table 2. Lung function variables and markers of inflammation

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=202</td>
<td>n=114</td>
<td>n=45</td>
<td>n=43</td>
<td></td>
</tr>
<tr>
<td><strong>Spirometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- FVC, % pred</td>
<td>100 ±17.2</td>
<td>102 ±15.1</td>
<td>106 ±17.4</td>
<td>88 ±17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- FVC/FVC, % pred</td>
<td>95 ±11.0</td>
<td>97 ±10.1</td>
<td>95 ±10.9</td>
<td>93 ±13.0</td>
<td>0.16</td>
</tr>
<tr>
<td>- reversibility in FVC, %</td>
<td>5.4 ±5.3</td>
<td>5.2 ±4.7</td>
<td>5.8 ±5.2</td>
<td>5.8 ±6.6</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Lung volumes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- TLC, % pred</td>
<td>99 ±13.8</td>
<td>99 ±13.7</td>
<td>102 ±14.4</td>
<td>97 ±13.2</td>
<td>0.20</td>
</tr>
<tr>
<td>- RV, % pred</td>
<td>92 ±25.4</td>
<td>86 ±21.5</td>
<td>87 ±19.7</td>
<td>113 ±28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- RV/TLC, % pred</td>
<td>88 ±19.3</td>
<td>82 ±14.7</td>
<td>82 ±13.4</td>
<td>113 ±16.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- KCO, % pred</td>
<td>98 ±15.4</td>
<td>98 ±15.1</td>
<td>96 ±16.8</td>
<td>98 ±14.9</td>
<td>0.66</td>
</tr>
<tr>
<td>- PC_{20} methacholine**, mg/mL</td>
<td>1.8 ±2.8</td>
<td>2.7 ±2.4</td>
<td>1.8 ±2.7</td>
<td>0.7 ±3.4</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Markers of inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- FeNO*, ppb¹</td>
<td>34 (13-42)</td>
<td>37 (15-48)</td>
<td>23 (12-32)</td>
<td>38 (12-52)</td>
<td>0.02</td>
</tr>
<tr>
<td>- Sputum eosinophils*, %</td>
<td>0.6 (0.1-3.6)</td>
<td>0.7 (0.2-3.5)</td>
<td>0.5 (0.1-4.0)</td>
<td>0.6 (0.1-4.1)</td>
<td>0.62</td>
</tr>
<tr>
<td>- Blood eosinophils*, 10⁹/L</td>
<td>0.16 (0.09-0.3)</td>
<td>0.16 (0.1-0.3)</td>
<td>0.15 (0.09-0.3)</td>
<td>0.18 (0.08-0.3)</td>
<td>0.89</td>
</tr>
<tr>
<td>- Sputum neutrophils*, %</td>
<td>71 (50-84)</td>
<td>74 (53-84)</td>
<td>70 (55-82)</td>
<td>69 (47-85)</td>
<td>0.67</td>
</tr>
<tr>
<td>- Blood neutrophils*, 10⁹/L</td>
<td>3.6 (2.9-4.6)</td>
<td>3.4 (2.8-4.4)</td>
<td>4.0 (3.3-5.1)</td>
<td>3.8 (3.0-4.8)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data expressed as mean/standard deviation; * median/Interquartile range; ** Geometric mean ± SD in doubling doses; ¹ corrected for current smoking

Definition of abbreviations: FeNO = Fraction exhaled nitric oxide; FVC = Forced Expiratory volume in 1 second; FVC = Forced Vital Capacity; KCO = Transfer coefficient expressing carbon monoxide diffusing capacity; pb=postbronchodilator; ppb= parts per billion; RV= Residual volume; TLC= Total lung capacity

Table 3 Self-reported factors for asthma onset

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=202</td>
<td>n=114</td>
<td>n=45</td>
<td>n=43</td>
<td></td>
</tr>
<tr>
<td><strong>Self-reported factor asthma onset¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- History of viral or bacterial respiratory infection, %</td>
<td>32</td>
<td>38</td>
<td>16</td>
<td>35</td>
<td>0.03</td>
</tr>
<tr>
<td>- History of environmental allergies, %</td>
<td>14</td>
<td>13</td>
<td>16</td>
<td>16</td>
<td>0.89</td>
</tr>
<tr>
<td>- History of (non) allergic occupational exposure, %</td>
<td>9</td>
<td>8</td>
<td>18</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>- History of stressful life event, %</td>
<td>7</td>
<td>5</td>
<td>13</td>
<td>7</td>
<td>0.22</td>
</tr>
<tr>
<td>- No specific trigger, %</td>
<td>28</td>
<td>26</td>
<td>29</td>
<td>30</td>
<td>0.87</td>
</tr>
<tr>
<td>- Other, %</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>0.87</td>
</tr>
</tbody>
</table>

¹ Due to rounding, the sum may not exactly 100%
Cluster 2
Cluster 2 (n=45) consisted of patients with high symptom scores, high medication requirement and poor quality of life. This cluster included the highest percentage of patients with a history of occupational exposure (18%) and a high frequency of patients with upper airway diseases (67% chronic rhinosinusitis and 11% nasal polyps). This cluster showed the lowest level of exhaled nitric oxide (median/IQR: 23/12-32) and high numbers of sputum and serum neutrophils (interquartile range 3.3-5.1).

Cluster 3
Patients in cluster 3 had a lower postbronchodilator FEV₁ (mean/SD: 88 ±17.3 % pred), more air trapping as defined by RV/TLC ratio (RV/TLC ratio (mean/SD): 113 ± 16.5 % pred), and more severe airway hyperresponsiveness (PC₂₀ methacholine (geometric mean/doubling doses): 0.7 mg, ± 3.4 doubling doses), as compared to the other clusters. Sixty-two percent of the patients had chronic rhinosinusitis, and 29% had nasal polyps. The most important self-reported factor of asthma onset in this cluster was a history of a viral or bacterial airway infection (35%).

Validation
The k-means algorithm that was tested in a leave-one-out validation model showed a within repeatability accuracy of 93%.

Discussion
In our population of adults with recent onset asthma three different phenotypes could be identified. One phenotype of patients with mild, well-controlled asthma that was associated with respiratory infection as the inciting trigger, and two phenotypes of patients with moderate-severe asthma: one associated with high symptoms scores but normal lung function and low FeNO level and one with more prominent airway hyperresponsiveness, decreased postbronchodilator FEV₁, signs of air trapping, and nasal polyps. These results show that already in the earliest phases of adult-onset asthma specific clinical characteristics and triggers of asthma onset are associated with asthma severity and control.

The present study is the first to identify phenotypes among adults with recently diagnosed asthma using cluster analysis. Previous cluster analysis studies were performed in adults with either long-standing or severe asthma. These studies indentified several phenotypes of patients with adult-onset asthma (3;4;22), including a phenotype consisting of obese females (3;4), a phenotype with “benign” asthma (3), a cluster with
active eosinophilic inflammation but few daily symptoms (3) and a phenotype with severe asthma and fixed airflow limitation (4;22). Although the phenotypes in our study were different from the above mentioned, it can be speculated that the mildest asthma phenotype in our study corresponds with “benign” adult-onset asthma, and that the most severe one represents the early phase of the severe asthma phenotypes of these previous studies.

The identification of distinct phenotypes shortly after the onset of asthma generates several hypotheses on the aetiology of this disease in adults. First, the role of respiratory infections. In about one third of the patients in our study, mainly in clusters 1 and 3, asthma onset was triggered by a clinically significant viral or bacterial respiratory infection. This fits in with the results from a recent study showing that respiratory infections are a strong determinant of adult-onset asthma (23). Apparently, patients in clusters 1 and 3 differed with respect to the severity and extent of the disease after resolution of the respiratory infection. Cluster 1 had mild, well controlled asthma with low medication requirement and normal lung function, whereas cluster 3 had more severe airway hyperresponsiveness, relatively low FEV1, higher medication requirement and higher incidence of CRS with or without nasal polyps.

This suggest that respiratory infections in adults can either lead to mild post infectious wheeze or to more extensive and persistent inflammatory disease of upper and lower airways.

There are plausible mechanisms by which infectious agents could contribute to induction of these different asthma phenotypes (24;25). Respiratory infections cause airway epithelial damage and airway inflammation. It can be speculated that in cluster 1 normal repair mechanisms led to restoration of epithelial integrity and (near) resolution of inflammation, whereas in the other phenotype, cluster 3, repair mechanisms have failed or negative feedback mechanisms have been insufficient, thereby perpetuating airway inflammation in upper and lower airways, leading to bronchial hyperresponsiveness and asthma symptoms. Indeed, this has been described in experimental animals, showing virus-induced persistent activation of pro-inflammatory innate immune responses within the airways (24). Such ongoing inflammation may not only lead to chronic rhinosinusitis with polyp formation in the paranasal sinuses but also to peripheral airways involvement as reflected by increased residual volume. Since chronic rhinosinusitis is strongly associated with severe asthma, and air trapping has been identified as a distinct physiological characteristic of severe asthma (26;27) our cluster 3 may very well represent the early stage of a developing severe asthma phenotype.
The mechanism of asthma onset in cluster 2 seems to be related to sensitizing agents leading to upper and lower airway inflammation. Eighteen percent of the patients in this cluster had a history of exposure to occupational agents. The role of work-related sensitizing agents in the onset of asthma has long been recognized and extensively studied (28-31). For the non-exposed patients in the same cluster it might be speculated that environmental pollutants, such as traffic generated gases and particles had contributed to the onset of asthma by similar mechanism (32). It has been shown that such emissions may induce an airway inflammatory response by oxidant injury. Also is known that diesel exhaust particles emitted by heavy-duty vehicles have been shown to be cytotoxic as well as to enhance allergic inflammatory responses in sensitized individuals (33-35).

The strength of our study is that all patients were recruited within one year after diagnosis of asthma, and extensively characterized using clinical, functional and inflammatory characteristics. Second, patients were derived from the outpatient clinics of one academic and two non-academic hospitals, which strengthens the applicability of our findings. One of the participating non-academic hospitals had an agreement with the general practitioners in the area to refer all adults with recent symptoms suggestive for asthma. We also analyzed whether non-response to participating in the study biased our results, which was not the case. Finally, for the cluster analysis, we performed factor analysis on 32 clinical, functional and inflammatory parameters to objectively determine the number of clusters as well as the variables to be used in the cluster analysis.

Our study has some limitations as well. First, the time of asthma-onset was defined by time of doctors’ diagnosis, which might not have been fully accurate in some patients with poor perception of dyspnea. Second, some patients might have had mixed asthma/COPD since (ex)smokers were allowed to participate in the study, provided that they had normal diffusion capacity and at least ≥12% reversibility. However, excluding all patients with a smoking history would have reduced the relevance of our findings since smoking is an important risk factor for the development of asthma (36).

Our results may have important clinical and research implications. We identified three phenotypes that represent the earliest stages of adult-onset asthma. Two of the 3 phenotypes were associated with a respiratory infection as the inciting trigger, one of which was associated with features consistent with those of severe asthma. This implies that an adult who develops asthma for the first time following a respiratory infection, especially when the asthma is associated with chronic rhinosinusitis and peripheral airways disease, may be at risk of developing severe refractory asthma in the years to follow. This type of asthma should be intensely studied for underlying mechanisms
and patients with these characteristics should be monitored carefully to prevent poor asthma outcome.

In conclusion, the present study shows that amongst adults with newly diagnosed adult-onset three phenotypes of different severity could be identified, characterized by different triggers of asthma onset and different functional abnormalities. Whether these phenotypes are stable over time and whether membership of a certain cluster predicts a more severe course of the disease with worse long-term prognosis needs to be confirmed in prospective long-term follow-up studies.
References


(20) ATS/ERS recommendations for standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide. 912-930. 2005.


