Clinical and inflammatory markers in asthma and COPD phenotyping

de Nijs, S.B.

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Chapter 5

CHRONIC RHINOSINUSITIS: PREDICTOR OF THE EOSINOPHILIC PHENOTYPE IN ADULT-ONSET ASTHMA

Submitted

SB de Nijs¹, M Amelink¹, EJM Weersink¹, C Georgalas², J van de Berg³, R Lutter¹², PJ Sterk MD¹, WJ Fokkens², EH Bel¹

¹ Dept. of Respiratory Medicine,
² Dept. of Otorhinolaryngology,
³ Dept. of Radiology,
⁴ Dept. of Experimental Immunology, Academic Medical Centre and University of Amsterdam
Abstract

Background: Adults with severe eosinophilic asthma have a high prevalence of chronic rhinosinusitis. Whether a diagnosis of chronic rhinosinusitis at asthma onset predicts the eosinophilic asthma phenotype is unknown.

Objective: To investigate whether chronic rhinosinusitis with or without nasal polyposis in adults with a recent (<1 year) diagnosis of asthma is associated with eosinophilic inflammation in the lower airways.

Methods: 200 patients with newly diagnosed adult-onset asthma were assessed. Chronic rhinosinusitis was diagnosed according to EP3OS criteria (symptoms and sinus CT-scanning or nasal endoscopy). Lower airway inflammation was assessed by eosinophils in induced sputum and nitric oxide in exhaled air.

Results: At asthma diagnosis 54% of patients had CRS, of which 36% had nasal polyposis. Both sinus CT-scan score and nasal endoscopy score correlated with sputum eosinophil percentages (r=0.57, p<0.001 and r=0.21, p=0.04, resp.) and exhaled nitric oxide levels (r=0.50, p<0.001 and r=0.30, p=0.004, resp.). Sinus CT-scan score was independently associated with sputum eosinophil percentage (p<0.001) and exhaled nitric oxide level (p<0.001). Patients with nasal polyposis had the highest percentages of sputum eosinophils and exhaled nitric oxide levels.

Conclusion: Chronic rhinosinusitis and nasal polyposis are highly prevalent in newly diagnosed asthma in adults and might be an early sign of the severe eosinophilic asthma phenotype.
Introduction

Asthma is a complex disorder that includes distinct subphenotypes, potentially with
different aetiologies, natural histories and responses to treatment (1-3). The classical
type of asthma is associated with atopy, starts early in childhood and typically runs in
families (4;5). This type of asthma has been extensively studied in in vivo and in vitro
asthma models, and can be reasonably well controlled by current anti-asthma medica-
tions (6). In contrast, asthma that starts in adulthood is often more severe (7-9) and less
responsive to therapy (10), in particular if associated with persistent eosinophilic airway
inflammation (2;3;7). Studies have shown that this type of asthma is often associated
with airway remodelling (11) and loss of lung function (12). Therefore, it might be im-
portant for the practicing clinician to identify patients with this phenotype at an early
stage of the disease in order to apply adequate therapeutic interventions. It would be
particularly helpful if this phenotype could be identified on the basis of clinical features.
Patients with persistent eosinophilic airway inflammation often have chronic rhinosi-
nusitis (13-15). Therefore, we hypothesized that the presence of chronic rhinosinusitis
at asthma diagnosis is predictive of the severe eosinophilic adult asthma phenotype.

The aim of the present study was to assess sinus disease by nasal endoscopy and
CT-scanning in adults with a recent asthma diagnosis, and to investigate whether the
severity of chronic rhinosinusitis (with or without nasal polyposis) was associated with
active eosinophilic inflammation in the lower airways.

Methods

Patients

Adults with newly (<1 year) diagnosed asthma were recruited from the pulmonary
outpatient clinics of one academic and two non-academic hospitals. The diagnosis of
asthma was confirmed by bronchodilator reversibility in FEV1 ≥ 12% of the predicted
value, or hyperresponsiveness to inhaled methacholine (PC20 < 8mg/ml) (16). Patients
were excluded if they had a previous doctor’s diagnosis of “asthma” or “bronchitis” or
ever use of asthma medication in childhood. Smokers or ex-smokers were included
only if postbronchodilator FEV1 and diffusion capacity were normal or they showed a
bronchodilator reversibility in FEV1 ≥ 12% of the predicted value with normal diffusion
capacity. At study entry, patients had had to be stable and free of respiratory infection
for at least 4 weeks. The study was approved by the local Hospital Medical Ethics Com-
mittee and was registered in the Netherlands trial register under NTR 1846. All patients
gave their written informed consent.
Design
The study had a cross-sectional design. All assessments and measurements were performed during one hospital visit. These included a history and physical examination, allergy assessment, spirometry, exhaled nitric oxide, induced sputum cells, nasal endoscopy and CT-scanning of the sinuses.

Measurements

Spirometry
Spirometry (MasterscreenPneumo; Jaeger; Würzburg, Germany) was performed according to the latest recommendations (17). Reversibility in FEV$_1$ was measured as change in FEV$_1$, expressed as percentage of the predicted value, 10 min after inhalation of 400µg inhaled salbutamol.

Sputum induction and processing
Sputum induction, processing and analysis were performed according to previously validated methods (18). Prior to sputum induction, patients inhaled 400µg salbutamol. Sputum was induced by inhalation of NaCl 4.5% during 3x5 min intervals using a high output nebulizer [KLAVAmed, Bielefeld, Germany]. Whole sputum samples were processed according to international recommendations (18). Sputum samples containing > 80% squamous cells were labelled as not-interpretable and excluded from analysis. Differential cell counts were expressed as the percentage of non-squamous cells. Sputum counting was performed by one experienced and qualified technician blinded to the clinical details.

Fraction of exhaled nitric oxide (FeNO)
FeNO level was measured with a portable rapid-response chemoluminescent analyser (flow rate 50mL/s; NIOX System, Aerocrine, Sweden) according to recent guidelines (19). Before FeNO measurement, patients were asked to refrain from smoking and eating for > 2 hours and withhold short and long-acting bronchodilators, anti-cholinergics and inhaled corticosteroids for > 8 hours.

Assessment of CRS
CRS was defined according to the European Position Paper on Rhinosinusitis and Nasal polyps (EP3OS) based on symptoms of CRS with either sinus CT-scan abnormalities OR endoscopic signs of CRS/nasal polyps (20). Symptoms of CRS were scored using the Sino-Nasal Outcome test-22 (SNOT-22) (21). Sinus CT-scans were scored by a radiologist according to the validated Lund-MacKay scoring system (22) with scores ranging from 0 to 24. Nasal endoscopy was performed by a rhinologist using 2.7 mm rigid 30° endo-
scopes and scored according to a standardised scorings system as described previously (23).

**Statistical analysis**

Non-normally distributed data were log-transformed for further analysis. Variables were summarized by descriptive statistics. For comparisons of proportions between groups, chi-square test was used. Student’s t-test and one-way analysis of variance (ANOVA) was used when normality and variance equality were confirmed and with Wilcoxon and Kruskal-Wallis test otherwise. Univariate analyses using Pearson’s correlation tests and multiple linear regression tests were performed to indentify factors associated with the percentage of eosinophils in sputum or FeNO level.

**Results**

Two-hundred adult patients with recently diagnosed asthma participated in the study. The characteristics of the patients included in the study are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
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<tr>
<td>n=200</td>
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<table>
<thead>
<tr>
<th>Gender (% female)</th>
<th>56</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>48 ± 14.9</td>
</tr>
<tr>
<td>BMI</td>
<td>28 ± 5.3</td>
</tr>
<tr>
<td>Caucasian ethnicity, %</td>
<td>83</td>
</tr>
<tr>
<td>Smoking history (py)</td>
<td>3 (0-14)</td>
</tr>
<tr>
<td>Inhaled corticosteroids, %</td>
<td>80</td>
</tr>
<tr>
<td>Dose ICS, µg/day</td>
<td>250 (250-500)</td>
</tr>
<tr>
<td>Antihistamines, %</td>
<td>11</td>
</tr>
<tr>
<td>Leukotriene receptor agonists, %</td>
<td>5</td>
</tr>
<tr>
<td>Atopy (% positive RAST)</td>
<td>44</td>
</tr>
<tr>
<td>Nasal corticosteroids, %</td>
<td>29</td>
</tr>
<tr>
<td>symptoms of CRS, %¹</td>
<td>60</td>
</tr>
<tr>
<td>CRS according to EP3OS criteria, %¹</td>
<td>54</td>
</tr>
<tr>
<td>nasal polyposis, %¹</td>
<td>20</td>
</tr>
</tbody>
</table>

* mean/SD; # median (interquartile range); ¹ Defined by EP3OS criteria (20).
ICS= inhaled corticosteroids; Dose ICS= fluticasone equivalent; py= pack years.
Pulmonary secondary care referral outpatient clinics
- Area 600,000 people living
- Inclusion period: june 2008 - may 2011

358 cases of new adult-onset asthma

158 non-participants
  - 38 unable to speak Dutch
  - 61 lack of time
  - 18 incorrect address information
  - 41 unknown/other reasons

200 patients included

16 patients excluded
  - Incomplete data to diagnose CRS

184 patients complete data for primary analyses

110 induced sputum samples
  - 64 samples > 80% squamous cells
  - 26 patients unable to produce sputum

11 patients CT-scan data missing
21 patients nasal endoscopy data missing

Complete data:
99 patients: sputum + CT-scanning
89 patients: sputum + nasal endoscopy

Figure 1. Flow chart
**Frequency of CRS and nasal polyposis in newly diagnosed asthma in adults**

In 16 patients not enough data were available for a reliable diagnosis of sinus disease. A certain diagnosis of CRS was made in 100 (54%) patients, and 36 (20%) had CRS with nasal polyposis.

**Association between CRS and lower airways inflammation**

Adequate sputum samples could be obtained in 110 patients. In 99 of these patients sinus-CT-scanning was performed and in 89 patients nasal endoscopy [Figure 1].

Patients with nasal polyps had higher percentages of sputum eosinophils as compared to patients without polyps (median: 2.2% vs 0.4%; p<0.05), or patients without CRS (median: 2.2% vs 0.5%; p=0.02), (Figure 2). Similarly, patients with polyps had a higher FeNO level, as compared to patients without polyps (median: 40 ppb vs 22 ppb; p=0.02) or those without CRS (median: 40 ppb vs 20 ppb; p=0.001). No relationship was

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**Figure 2.** Histogram showing group comparison for percentage of sputum eosinophils (left) and FeNO level (right)

**Figure 3.** Correlation between the sinus CT scan score and the percentage of sputum eosinophils (left) and FeNO level (right) in patients with recently diagnosed adult-onset asthma
found between nasal symptoms according to the SNOT-22 score and percentage of sputum eosinophils or FeNO-level (p=0.57 and p=0.07). There was a significant correlation between sinus CT-scan-score and the percentage of eosinophils in sputum (r=0.57, p< 0.001) and the level of FeNO (r=0.50, p<0.001) (Figure 3). Also the nasal endoscopy score for CRS correlated significantly with the percentage of sputum eosinophils (r=0.21, p=0.04) and level of FeNO (r=0.30, p=0.004).

**Multivariate analysis**

Multivariate regression analysis including sinus CT-scan score, nasal endoscopy score, the presence of nasal polyps, as well as age, gender and atopic status showed that only the sinus CT-scan score (p<0.001) was independently associated with sputum eosinophilia (correlation r=0.58, r²=34%; p<0.001; Table 2). Repeating the analysis with FeNO level as dependent variable showed that the sinus CT-scan score (p<0.001), male gender (p=0.04) and atopy (p<0.001) (correlation r=0.62, r²=39 %; p<0.001) were independent determinants of increased FeNO level. In addition, multivariate regression analysis with CRS, defined by EPOS criteria, as well as age, gender and atopic status showed that atopy was significant (p=0.04) and CRS was borderline significant (p=0.05) associated with sputum eosinophilia. Repeating the analysis with FeNO level as dependent variable showed that CRS, defined by EPOS criteria (p=0.002) and atopy (p<0.001) were independent determinants of increased FeNO level.

**Table 2**  Multiple regression analysis. Factors independently associated with the percentage of sputum eosinophils (n=110).

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>p value</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>0.08</td>
<td>0.01</td>
<td>0.43</td>
<td>-0.015 – 0.035</td>
</tr>
<tr>
<td>Male gender</td>
<td>-0.02</td>
<td>0.35</td>
<td>0.82</td>
<td>-0.787 – 0.622</td>
</tr>
<tr>
<td>Positive atopic status</td>
<td>0.09</td>
<td>0.34</td>
<td>0.36</td>
<td>-0.366 – 0.988</td>
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<tr>
<td>Sinus CT-scan score</td>
<td>0.48</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>0.098 – 0.244</td>
</tr>
<tr>
<td>Nasal endoscopy score</td>
<td>0.03</td>
<td>0.04</td>
<td>0.76</td>
<td>-0.073 – 0.099</td>
</tr>
<tr>
<td>Nasal polyps</td>
<td>0.10</td>
<td>0.45</td>
<td>0.35</td>
<td>-0.472 – 1.322</td>
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</tbody>
</table>

B= β coefficient; SE= standard error; CI= confidence interval

**Discussion**

Our study shows that chronic rhinosinusitis and nasal polyposis are highly prevalent in adults with newly diagnosed asthma, and that chronic rhinosinusitis, in particular if accompanied by nasal polyposis is associated with active eosinophilic inflammation in the lower airways. This suggests that chronic rhinosinusitis in adults may be an early clinical indicator of a developing severe eosinophilic asthma phenotype.
The present study identifies for the first time that chronic rhinosinusitis with or without nasal polyposis is an important presenting characteristic of patients with newly diagnosed adult-onset asthma. Previous studies have shown an association between allergic and non-allergic rhinitis and onset of asthma in adulthood (24-26) whereas others found that asthma patients with CRS reported a later onset of disease as compared to those without CRS. Our study not only shows that the prevalence of CRS (54%) and of nasal polyposis (20%) is much higher in patients with recent onset asthma than in the normal adult population (10.9% and 2%, respectively) (27;28) but also that these patients have more severe eosinophilic airway inflammation. This indicates that adult onset asthma, CRS and lower airway eosinophilic inflammation, are elements of a distinct asthma phenotype that is apparent already from the start.

The mechanisms underlying the association between sinus disease, adult-onset asthma and lower airway inflammation are not completely understood (29;30). From a pathophysiological point of view there are many similarities between CRS and asthma. Cytokine patterns in sinus mucosa from patients with CRS resemble those in bronchial mucosa from patients with asthma (31). Eosinophils have been shown to be the dominant cell type in both nasal and broncho-alveolar lavages in patients with CRS and co-morbid asthma (32). One factor that might have contributed to the onset of asthma is sensitization to enterotoxin of Staphylococcal aureus, a pathogen that often colonizes the sinuses in patients with CRS (33;34). This might lead to an "intrinsic" IgE response, with subsequent eosinophilic inflammation of the airway mucosa, as has been suggested recently (35). Another explanation might be related to the expression of transforming growth factor-beta 1 (TGF-ß1) in both upper and lower airways (36;37). TGF-ß1 has been demonstrated to represent a master switch in inflammation and remodelling processes and might provide a key for understanding the onset and persistence of mucosal inflammation and remodelling in paranasal sinuses and bronchi (37). The key question is whether inflammation of the upper and lower airways in patients with adult onset asthma are causally related. The observation that CRS is highly prevalent in the earliest stages of adult onset asthma and that the extent of upper airway inflammation is associated with the intensity of inflammation in the lower airways does suggest such a causal link. If so, eosinophilic CRS with nasal polyposis might be regarded as an early predictor of eosinophilic asthma (38).

Our study may have some limitations. First, we could not obtain adequate sputum in all patients. However, no significant differences were found with respect to severity of chronic rhinosinusitis between the patients who successfully produced sputum and those who did not (data not shown). Therefore, we do not believe that the results of
our study are biased by these limitations. Second, we were not informed about aspirin sensitivity, which has been shown to be associated with CRS, nasal polyposis and eosinophilic mucosal inflammation in previous studies (39;40). The reason is, first, that in The Netherlands patients do not often use aspirin as a pain killer but rather prefer acetaminophen, and second that asthma patients are advised by their treating physician and the pharmacists not to use non-steroidal anti-inflammatory drugs (NSAIDs) in order to prevent exacerbations. We cannot exclude that many of our patients might have had aspirin sensitivity as well.

The present study has clinical implications. Adult onset asthma is an underexposed and often misdiagnosed respiratory condition (41). The presence of CRS, in particular if associated with nasal polyposis should be a warning sign for physicians that eosinophilic adult-onset asthma might be present or at least imminent. Since eosinophilic asthma in adults often progresses to more severe disease and loss of lung function, patients with CRS and nasal polyposis at asthma diagnosis should be followed intensively, and treated with targeted treatments if standard asthma therapy fails. Alternatively, patients with a recent diagnosis of adult-onset asthma should be screened for CRS and nasal polyposis. This is important, because in our study, 34% of the patients with CRS were not previously diagnosed with this condition and required treatment. Moreover, it has been shown that medical or surgical treatment of CRS and nasal polyposis may improve asthma control (42).

In conclusion, this study shows that CRS and nasal polyps are highly prevalent in newly diagnosed adult-onset asthma and that the severity of CRS in these patients correlates with the intensity of eosinophilic inflammation in the lower airways. This suggests a pathogenetic link between CRS and newly developed eosinophilic asthma in adulthood, and might indicate that CRS, in particular if accompanied by nasal polyposis is a predictor of the eosinophilic asthma phenotype. Severe polypos CR at the time of asthma diagnosis suggests extensive lower airway inflammation, and should urge clinicians to closely monitor patients with this clinical condition and provide targeted treatments if necessary.
References


(19) 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.


