

The effect of smoking cessation on airway inflammation in young asthma patients

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Summary

Background Smoking has been shown to have several detrimental effects on asthma, including poor symptom control, attenuated treatment response and accelerated decline in lung function. In spite of this, smoking is at least as common among asthma patients as in the rest of the population. The aggravations of smoking on asthma may be caused by effects on airway inflammation, which has been found to be changed in asthmatic smokers. It is not known whether these smoking-induced airway inflammation changes are reversible after smoking cessation.

Objective The aim of this study was to assess airway changes in asthmatic smokers before and during smoking cessation.

Methods Forty-six smokers with asthma, all steroid-free (age range: 19–40), were recruited. All participants attempted smoking cessation over a period of 3 months. Visits were performed at weeks 0, 6 and 12 and included induced sputum, FeNO, methacholine challenge, lung function, Asthma Control Questionnaire (ACQ6) and exhaled CO.

Results Twenty-six of 46 patients succeeded in quitting smoking. In the quitters, improvements in methacholine AHR (77% before and 52% after smoking cessation, respectively, $P = 0.016$) and ACQ6 score (1.7–0.7, $P = 0.034$) and FeNO (8.7–14.8 p.p.b., $P = 0.002$) were observed, whereas no significant changes were found regarding eosinophils or lung function. A small but significant decrease in neutrophils (54.1–52%, $P = 0.003$) was present in quitters compared with the non-quitters. Non-quitters experienced no changes in any parameters.

Conclusion Smoking cessation improved asthma control, but the changes were not related to change in eosinophilic inflammation, and the reduction in neutrophils was small. Thus, airway inflammation with eosinophils and neutrophils may be less important drivers of asthma control in smokers than other factors.

Clinical Relevance Smoking cessation may improve clinically important disease parameters such as AHR and symptom score, but likely unrelated to changes in airway inflammation.

Keywords airway hyperresponsiveness, airway inflammation, asthma, smoking cessation

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Introduction

Asthma is a common chronic condition caused by airway wall inflammation and affecting the lower respiratory tract. Smoking among asthma patients is at least as frequent as in the rest of society in spite of the fact that asthma patients who smoke experience more pronounced symptoms and higher exacerbation rates. Hospitalization due to asthma attacks is more common in this group of patients, and the mortality rate due to asthma is higher [1]. Furthermore, smoking significantly

attenuates the treatment response to inhaled corticosteroids (ICS) [2–4], which constitutes a substantial clinical challenge in that ICS in non-smokers are highly effective and therefore play a crucial part in the treatment of persisting asthma. Long-term aggravating effects of smoking on asthma in terms of a highly accelerated decline in FEV₁ also pose a significant problem [5].

These detrimental effects on asthma of exposure to tobacco smoke may in part be attributed to alterations in the airway inflammation, which has been shown to be of a different character than that of non-smoking

asthmatics, in terms of a higher degree of neutrophilic inflammation with enhanced IL-8 response [2], thus resembling what is found in COPD patients. A chronic state of neutrophilic inflammation is likely to lead to irreversible tissue damage even in non-asthmatics [6], probably due to increased activity of enzymes such as neutrophilic elastase. Smoking may also suppress eosinophilic inflammation in asthma through inhibition of eotaxins [7], but results of studies investigating this are not entirely consistent. As ICS treatment efficacy is associated with the presence of eosinophilic and not neutrophilic airway inflammation [8–10], the altered inflammatory balance observed in smokers may contribute to the known reduction in their steroid responsiveness.

Although of considerable clinical interest, it is not known whether these smoking-induced inflammatory changes are potentially reversible in both young and old smokers after smoking cessation. It is also unknown whether duration of smoking in years, daily consumption and pack year are of importance. One previous study found no changes in eosinophils after smoking cessation, whereas neutrophilic inflammation was reduced in patients who quit smoking [11]. This may be explained by the fact that IL-8 and other chemoattractant cytokines for neutrophils are produced by macrophages activated by cigarette smoke [12]. Hence, attenuated neutrophilia would be expected as the stimulus of smoke is eliminated. However, the results of that study may well have been biased by treatment with steroids, both inhaled and oral, during the study period, and, furthermore, the sample size of the study was limited to very few patients. In patients with COPD, surprisingly, an increase in airway neutrophil count was demonstrated in patients who quit smoking [13].

Equivalently, little is known about the effect of smoking and smoking cessation on AHR to methacholine in asthma patients. In a study of a population primarily consisting of non-asthmatic patients with rhinitis, an improvement in AHR to methacholine was demonstrated 1 year after smoking cessation, whereas no change was observed after 6 months [14], indicating a long-lasting effect of smoking on AHR. A similar finding of improvement of methacholine AHR 1 year after smoking cessation was made in COPD patients [13]. Furthermore, in a study using histamine provocation, significant improvement in PD₂₀ after 4 months of smoking cessation in asthma patients was found [15].

There is little information regarding asthma symptoms after smoking cessation in the literature. In earlier studies, improvements in asthma symptoms score such as Asthma Control Questionnaire (ACQ) score [11] and asthma quality of life (AQLQ) score [15] have been demonstrated, in accordance with the fact that smokers

have been shown to express higher ACQ score despite similar lung function [16].

To the knowledge of the present authors, no studies have investigated airway responsiveness to methacholine and changes in asthma symptom scores, and their relation to changes in airway inflammation, in asthmatic patients during smoking cessation.

The aim of the present study was to assess alterations in airway inflammation, airway hyperresponsiveness to methacholine and other disease parameters during 6 and 12 weeks of smoking cessation in young asthma patients not treated with steroid at baseline, hypothesizing that improvement in asthma control and AHR relates to changes in the inflammatory profile.

Material and methods

Design

This was a 12-week follow-up study of changes in airway inflammation, AHR, lung function and symptom score in young asthma patients during smoking cessation. The study was part of a clinical trial investigating the effect of varenicline on smoking cessation in asthma patients. All participants received a smoking cessation course, and participants were randomized 1 : 1 to receive either varenicline or placebo tablets as smoking cessation support treatment. However, the focus of the present analysis was the effect of smoking cessation per se on airway inflammation and hyperresponsiveness. Therefore, data were analysed in relation to whether smoking cessation succeeded or not.

Material

The patients were recruited consecutively, primarily through newspaper advertisement or through the primary healthcare system. The inclusion criteria were as follows: (i) 18–40 years of age, (ii) current smoking with a daily consumption of at least 10 cigarettes per day within the last year, and a total consumption of at least 10 packyears, (iii) current asthma symptoms within the last year, confirmed through interview with study physician and (iv) at least one positive objective diagnostic test suggestive of asthma: positive methacholine test (PD₂₀ < 8 µmol), positive mannitol test (PD₁₅ < 635 mg), FEV₁ reversibility to β₂-agonist of ≥ 12% and 250 mL or a 2-week day-to-day FEV₁ variation of ≥ 20%. The exclusion criteria were as follows: (i) treatment with ICS, oral steroids, leukotriene antagonist (LTRA), long-acting β₂-agonists or long-acting anticholinergics within 3 months prior to inclusion, (ii) lower respiratory tract infections within the last 3 months, (iii) a medical history of other chronic lung disease, including COPD and (iv) pregnancy.

Methods

After participants signed informed consent, a screening visit was performed to confirm asthma diagnosis. This visit included a clinical interview with asthma classification, tobacco history, spirometry with reversibility, methacholine challenge, blood samples and skin prick test. Furthermore, 2-week home measurements of FEV₁ were commenced. Later, on a separate day, the mannitol test was performed. After inclusion and within 2 weeks before randomization, all patients completed a 2-h smoking cessation course, conducted by the study physician, who was trained in teaching smoking cessation.

Subsequently, three visits (week 0, week 6 and week 12) were performed, including the following: fractional exhaled nitric oxide (FeNO), CO measurement, spirometry with reversibility, methacholine challenge, ACQ questionnaires and induced sputum. ACQ6 was filled out at home by the participants through an Internet-based program (EasyTrial, Virum, Denmark) in accordance with the visit date. The Fagerström score was assessed at week 0 as a marker of nicotine dependence.

At the visit at week 0 (visit 1), varenicline or placebo tablets were handed out to the participants, who were asked to commence treatment the next morning. The attempt to quit smoking was then commenced from day 8 of treatment. Smoking status was recorded at all visits through interviews and verified by CO breath test (cut-off value 10 ppm).

The study was approved by the local ethical committee (No. H-2-2010-140). All participants signed an informed consent form: Eudract No.: 2010-024208-81. Clinicaltrial.gov ID: Young smoking asthmatics.

Descriptions of the tests used

- Spirometry (EasyOne™; ndd Medizintechnik AG, Zürich, Switzerland), best of three measurements of FEV₁, FVC and FEV₁/FVC ratio in accordance with ATS recommendations [17]. Reference values according to fixed data of the spirometer. Reversibility test 20 min after inhalation with terbutalinesulfate (Bricanyl Turbuhaler) 1.5 mg.
- Home FEV₁ measurements: Piko-6 (Ferraris, UK) used twice daily for 2 weeks. Day-to-day FEV₁ variation was calculated as follows: $[(\text{highest measured FEV}_1 - \text{lowest measured FEV}_1) / \text{highest measured FEV}_1] \times 100$.
- FeNO (NIOX; Intra Medic, Gentofte, Denmark) average of 3 measurements (flow rate 50 ml) was measured in accordance with the ATS/ERS guidelines [18].
- Mannitol provocation test (Osmohale™ Mannitol, Pharmaxis, Burnham, UK): inhalation of ascending dosages 0, 5, 10, 20, 40, 80, 160, 160, 160 mg [19].

A positive test was defined as a $\geq 15\%$ fall in FEV₁ and resulted in termination of the test. RDR was calculated as follows: $[100 \times (\text{initial FEV}_1 - \text{last dose FEV}_1) / \text{initial FEV}_1] / \text{Cumulated dose of mannitol in mg}$. If FEV₁ was $< 60\%$ of expected, mannitol challenge was not performed.

- Methacholine provocation test with the Yan method (Nebichex device, nebulized fluid: methacholine bromide): inhalation of ascending dosages, cumulated: 0, 0.03, 0.06, 0.12, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 μmol [20]. Measurements of FEV₁ exactly 1 min af inhalation. A $\geq 20\%$ fall in FEV₁ was defined as a positive result and terminated the test.
- Induced sputum: sputum preparations were collected in accordance with published guidelines [21]. Samples were fixed with methanol and dyed with Eosin Y 2% (BioStain), Methylene Blue $< 2\%$ and Azur I $< 2\%$ (BioStain). Dyed slides were counted by the study physician with a Zeiss Axio microscope, Jena, Germany. Four hundred non-squamous cells (eosinophils, neutrophils, macrophages, lymphocytes and cylindrical epithelial cells) were counted for each patient to determine cell distribution. Inflammatory phenotyping was performed in accordance with earlier studies [22], with cut-off at 1% eosinophils and 61% neutrophils. However, in order to equalize numbers between groups to allow for parametric statistics, a cut-off at 55% was chosen in smoking cessation analysis.
- Skin prick test. A standard panel of common allergens was used: cat, dog, horse, alternaria, cladosporium, birch, mugwort, grass, Derm.pter. and Derm.far. Atopy was defined as at least one positive response with a weal diameter of at least 3 mm.
- CO breath test: Bedfont Micro⁺ Smokerlyzer or CO monitor; Bedfont Scientific Ltd., Harrietsham, UK. Cut-off value was 10 ppm.
- Questionnaires: ACQ [23]. ACQ6 (including all questions except for lung function) was used for symptom score. The Fagerström nicotine dependence questionnaire was filled out at baseline, while all participants were still smoking. Some patients filled out retrospectively after study completion.

Statistics

All statistical analyses were performed with IBM SPSS Statistics ver. 20 (SPSS Inc., Armonk, NY, USA). Chi-squared analysis was used for unpaired crosstabs, McNemar's test for paired crosstabs. For parametric independent two-sample test, unpaired *t*-test was used. If possible and if necessary, logarithmic transformation was performed before *t*-test analysis. Mann-Whitney *U*-test was used for nonparametric, unpaired two-sample

test, Wilcoxon signed rank test for paired tests. Pearson's r was used for correlation analysis. Using this method for correlation analysis between FeNO and sputum eosinophils, one outlier with extreme eosinophilia (59.5%) (quitter) was excluded at visits 1 and 3. For repeated measurements (3 or more), Friedman's and linear mixed model statistics were used. Analysis for interaction between time interval and smoking cessation was carried out in mixed model statistics. Due to negative RDR values of mannitol and methacholine, values of 0.008 and 0.5 were added to all mannitol and methacholine RDR values, respectively, to allow for logarithmic transformation. Subsequently, the same values were subtracted after calculating means and CI. Significance level was $P \leq 0.05$. Missing data from two patients (of which one quitter) not attending specific visits were carried forward from last observation. One patient quitting between week 6 and 12 was excluded from mixed model analysis.

Pre-study sample size calculation: With a power of 90%, an α of 0.05 and an anticipated quit rate of 50%, 44 subjects were required to show a change in cellular distribution after smoking cessation. The calculation was based on change in eosinophil-%.

Statistical assistance was provided by a statistician at the University of Copenhagen.

Results

The study population

Seventy-six persons signed informed consent forms and participated in the screening procedure, 20 of whom dropped out between screening and randomization, due to unwillingness to quit smoking, despite having agreed to participate. In addition, three subjects did not have positive asthma test and were therefore excluded prior to randomization, and one proved to be a screening failure (steroid treatment). Thus, 52 subjects were eligible and were included. Six of these subjects completed only the first visit and dropped out subsequently. Of the 46 remaining subjects, one did not complete the second visit at week 6, and one did not complete all procedures of the visit at week 12.

Baseline characteristics

Baseline characteristics of the subjects are summarized in Table 1.

At baseline, no significant differences were observed when comparing different variables of the quitters and non-quitters (Table 1). The patients were about 32 years old in each group, but already had more than 15 pack-years on average. The mean number of years since asthma diagnosis was around 15. Thirteen patients had

Table 1. Baseline characteristics of quitters and non-quitters at week 12, expressed as mean $\pm$ SD

	Quitter group (<i>N</i> = 26)	Non-quitter group (<i>N</i> = 20)	<i>P</i>
Females	42%	30%	0.391
Age (years)	31.6 $\pm$ 6.0	31.9 $\pm$ 5.9	0.873
Years of duration since asthma diagnosis	16.2 $\pm$ 12.9	14.8 $\pm$ 12.6	0.727
No. of packyears	15.6 $\pm$ 4.7	15.5 $\pm$ 4.3	0.955
Fagerström score	5.2 $\pm$ 2.2	5.6 $\pm$ 1.7	0.525
Atopy	68%	45%	0.121
Body mass index (kg/m ²)	24.9 $\pm$ 4.1	25.9 $\pm$ 3.8	0.412
FEV ₁ % of predicted	83.5 $\pm$ 17.3	83.0 $\pm$ 15.4	0.913
FVC% of predicted	96.5 $\pm$ 14.6	97.8 $\pm$ 13.5	0.768
FEV ₁ /FVC%	73.4 $\pm$ 9.7	71.5 $\pm$ 10.6	0.516
FEV ₁ % increase after terbutaline, median (min–max)	2.9 (–6.8 to 32.4)	–0.2 (–10.5 to 15.4)	0.144
FEV ₁ home measurements% variation, median (min–max)	24.0 (11.2–66.3)	26.9 (16.3–63.1)	0.480
% of methacholine positive subjects	77	70	0.596
Methacholine RDR (% FEV ₁ fall per μ mol) [GM (CI)]	7.9 (3.8–15.9)	8.6 (4.2–17.1)	0.872
% of mannitol positive subjects	64	72	0.624
Mannitol RDR (% FEV ₁ fall per mg) median (min–max)	0.036 (–0.003 to 0.242)	0.037 (–0.002 to 0.540)	0.514
ACQ6 score	1.7 $\pm$ 0.8	1.6 $\pm$ 0.9	0.730
FeNO (p.p.b.) [GM (CI)]	8.7 (6.0–12.8)	6.4 (6.0–12.8)	0.245
Sputum eosinophil-% median (min–max)	1.25 (0.00–59.50)	1.63 (0.00–14.00)	0.519
Sputum neutrophil-%	52.8 $\pm$ 22.5	56.1 $\pm$ 17.1	0.740
Sputum pollution% [GM (CI)]	14.1 (9.4–21.2)	9.7 (6.6–14.2)	0.178

ACQ, Asthma Control Questionnaire.

not previously been diagnosed with asthma, before inclusion in the study. The FEV₁ of all of the patients was slightly lower than expected, but the FVC was normal, as would be expected in a group of asthma patients primarily presenting intermittent, mild or moderate degrees of asthma. Furthermore, in both groups, a low degree of airway reversibility to β_2 -agonist was found. Hyperresponsiveness to both methacholine and mannitol was found frequently (77% and 64%, respectively). In total, more than half expressed allergic sensitization. Most had significant asthma symptoms with a mean ACQ score of more than 1.5 in both groups. Importantly, at baseline, no significant differences in

Table 2. Distribution of inflammatory phenotypes in all patients at baseline

Eosinophilic	15/52 (29%)
Mixed granulocytic	13/52 (25%)
Neutrophilic	9/52 (17%)
Paucigranulocytic	15/52 (29%)

inflammation were found between the two groups. The nicotine dependence, assessed with the Fagerström questionnaire, did not differ between quitters and non-quitters.

Inflammatory phenotypes

At baseline, most patients (54%) expressed eosinophilic phenotype with about equal proportions of eosinophilic and mixed granulocytic phenotype (Table 2). Neutrophilic asthma was found in 17% of the smokers, which meant that, overall, neutrophilia was found in 42% of the patients. Twenty-nine percent showed no increase in inflammatory cells in the sputum (paucigranulocytic phenotype). There were no differences in the distribution of these inflammatory phenotypes between the two groups (not shown).

Quit rates

The criterion for successful smoking cessation was defined as a CO breath test value of < 10 ppm along with anamnestic confirmation. In total, 26 patients succeeded in quitting during the study period, while 20 did not; of these, exhaled CO was significantly reduced at week 6 compared with baseline (27 ppm vs. 20 ppm, $P = 0.026$), but at week 12, the reduction was not significant (27 ppm vs. 23 ppm, $P = 0.143$) (not shown).

Furthermore, some non-quitting patients quit for a period of a few weeks, followed by relapse during the rest of the study. One patient quit between week 6 and 12.

Airway inflammation during smoking cessation

No significant changes in airway eosinophils were observed, neither when comparing median% values or when classifying patients according to cut-off values (1% eosinophils) (Table 3). In the mixed model, a small but significant reduction in neutrophils was observed in the quitter group as opposed to the continuing smokers, most pronouncedly at week 6, less so at week 12 (Table 3). When analysing the variables as Δ and dichotomous, no significant changes were found (not shown), although the change in neutrophil-% at week 6 was close to reach significance (quitters: -7.0% , non-quitters $+5.1\%$, $P = 0.062$).

FeNO during tobacco cessation period

As expected, a significant increase in FeNO in the quitters was observed at weeks 6 and 12 (Table 3) as opposed to the non-quitters. No significant differences were found in Δ FeNO values.

Airway responsiveness to methacholine during tobacco cessation period

When patients were classified according to hyperresponsiveness ($PD_{20} < 8 \mu\text{mol}$), a significant improvement was observed in the quitter group, at both weeks 6 and 12. This was not found in the continuing smokers (Table 4). In both quitters and non-quitters, a non-significant decrease in methacholine RDR was observed.

Table 3. Sputum eosinophils, neutrophils and FeNO during tobacco cessation period

	Week 0	Week 6	Week 12	P
Sputum eosinophil-% median (range)				
Quitters	1.25 (0.00–59.50)	0.75 (0.00–31.00)	1.50 (0.00–26.00)	NS
Non-quitters	1.63 (0.00–14.00)	1.25 (0.00–11.00)	0.75 (0.00–8.00)	
Sputum eosinophilia (> 1%)				
Quitters	11/26 (42%)	11/25 (44%)	14/26 (54%)	NS
Non-quitters	10/20 (50%)	12/21 (57%)	7/20 (35%)	
Sputum neutrophil-% mean $\pm$ SD				
Quitters	52.8 $\pm$ 22.5	45.9 $\pm$ 22.1	50.3 $\pm$ 19.1	0.002
Non-quitters	56.1 $\pm$ 17.1	63.0 $\pm$ 11.8	61.5 $\pm$ 13.0	
Sputum neutrophilia (> 55%)				
Quitters	11/26 (42%)	7/25 (28%)	8/26 (31%)	NS
Non-quitters	12/20 (60%)	15/21 (71%)	13/20 (65%)	
FeNO p.p.b. [GM (CI)]				
Quitters	8.7 (5.7–13.1)	13.7 (9.2–20.4)	14.8 (10.4–20.8.)	0.002
Non-quitters	6.4 (4.5–9.3)	7.9 (5.0–12.4)	7.1 (4.6–10.9)	

Table 4. Methacholine AHR, RDR, lung function and Asthma Control Questionnaire (ACQ) score during tobacco cessation period

	Week 0	Week 6	Week 12	P
Methacholine AHR (PD ₂₀ < 8 µmol)				
Quitters	20/26 (77%)* [†]	12/25 (48%)*	13/26 (50%) [†]	0.016*
Non-quitters	14/20 (70%)	14/21 (67%)	12/20 (60%)	0.016 [†]
Methacholine RDR GM (CI)				NS
Quitters	7.9 (3.8–15.9)	6.0 (2.6–13.2)	6.1 (3.0–11.9)	NS
Non-quitters	8.6 (4.2–17.1)	7.4 (3.4–15.7)	6.8 (2.7–16.1)	
FEV ₁ % expected				
Quitters	83.5 ± 17.3	84.4 ± 15.7	85.3 ± 15.4	NS
Non-quitters	83.0 ± 15.4	82.4 ± 15.8	83.0 ± 16.2	
ACQ6 scores				
Quitters	1.7 ± 0.8	0.7 ± 0.7	0.7 ± 0.7	0.034
Non-quitters	1.4 ± 0.8	1.3 ± 0.8	1.2 ± 0.8	

*Represents week 0 vs. week 6.

[†]Represents week 0 vs. week 12. Both with a *P*-value of 0.016.

No significant differences between quitters and continued smokers were found in Δ methacholine RDR values.

Lung function during tobacco cessation period

A trend towards a small but not significant increase in pre- β_2 FEV₁-% was observed in the quitters. No change was found in the continuing smokers (Table 4).

Symptom score during tobacco cessation period

In the quitter group, a significant improvement in symptom score was observed, from 1.7 to 0.7, whereas in the continuing smokers, the change was small and insignificant (Table 4). Only 12 of the non-quitters completed the questionnaire at all visits, whereas all but one of the quitters did. At week 6, the median Δ values between quitters and non-quitters were – 1.0 and – 0.3, respectively (*P* = 0.010) and at weeks 12 – 1.0 and – 0.3 (*P* = 0.005) (not shown).

Discussion

The present study is, to our knowledge, the first to assess airway inflammation and airway hyperresponsiveness during smoking cessation in a group of young steroid-free asthmatic smokers.

Our main findings were as follows:

- During smoking cessation, methacholine AHR, a key feature of asthma, improved significantly among quitters and not among continuing smokers. This was observed already at week 6 and was sustained at week 12. An earlier study of asthma patients showed improvement in AHR to histamine, another direct bronchial challenge 4 months after smoking cessation [15]. However, the present study is the first to dem-

onstrate an improvement in AHR after only a short period of abstinence from smoking, indicating an immediate effect of smoking cessation in asthma patients which could potentially be superior to what is found in other patient groups [13] and healthy individuals with rhinitis [14]. However, there are insufficient data regarding the short-term effects of smoking cessation in these groups. On the other hand, in the present study, when we analysed methacholine responsiveness as a scale variable (methacholine RDR), no significant change was found among either quitters or non-quitters, indicating that patients expressing mild AHR, with a PD₂₀ close to 8 µmol had a beneficial effect from smoking cessation, reverting to negative test, whereas continuing smokers with mild AHR had not. This interpretation was confirmed in the data when we analysed the degree of improvement in AHR in relation to baseline AHR severity in quitters and non-quitters (not shown).

- Asthma symptom scores, assessed with ACQ6, improved considerably among quitters at mean values that would clinically correspond to changing from uncontrolled (1.7) to well-controlled (0.7) asthma already after 6 weeks and sustained at 12 weeks, confirming what has been found earlier [11]. In another study of 27 asthma patients who quit smoking, using the asthma quality of life score as symptom assessment, improvements after smoking cessation have also been demonstrated [15].
- Airway inflammation, assessed with eosinophils, did not significantly change after a smoking cessation period of 12 weeks. Regarding the neutrophils, a slight reduction neutrophilia in the quitter group was observed, using mixed model statistics, which included the small increase found in the non-quitters.

This finding is in correspondence with earlier findings [11], although the decrease in neutrophils after smoking cessation in the present study is markedly smaller than earlier observed.

- At week 12, an increase in FeNO was observed among the quitters, but not among the non-quitters. A rapid increase in FeNO after smoking cessation has been demonstrated earlier [16], perhaps owing to elimination of tobacco-induced increased arginase expression, which reduces the amounts of NO synthase substrate [24]. Thus, the reduced FeNO observed in the quitters of the present study was expected, as smoking cessation induces an increase in FeNO as a physiological response independent of asthma phenotype, in addition to the FeNO produced in some patients as a result of eosinophilic inflammation [25]. Sputum eosinophils did not increase after smoking cessation, indicating that the increase in NO was mainly related to the physiological effect of smoking cessation.

Previous studies of non-smokers have shown that FeNO and sputum eosinophils are correlated [26], which was also the case in the present study (data not shown). Interestingly, however, the correlation coefficients between eosinophils and FeNO remained constant before and after smoking cessation (data not shown), suggesting that although NO is suppressed by smoking, it remains a marker of airway eosinophilia in asthma.

The present study also investigated the effect of smoking cessation on FEV₁, which did not significantly improve, as opposed to the results of previous studies [11, 27]. This lack of increase in FEV₁ may be a matter of sample size, in that an insignificant increase in FEV₁ was found among quitters as opposed to continuing smokers.

To sum up these findings, during smoking cessation, several important asthma parameters were improved (methacholine AHR and symptom score), without corresponding changes in airway inflammation with eosinophils. Regarding the neutrophils, the observed decrease was relatively small after 12 weeks of smoking cessation and likely also of less importance in relation to the observed improvement in asthma control parameters. These findings could indicate that asthma symptoms and hyperresponsiveness towards methacholine are driven by smoking and other factors to a higher degree than airway inflammation with eosinophils and neutrophils.

Certain limitations to the interpretation of the results of the present study should be noted. First, it could be speculated that a longer tobacco cessation period may be required to fully elucidate what happens with both the eosinophils and the neutrophils during smoking cessation. However, the patients were steroid-free although expressing an asthma severity which needed ICS treat-

ment. Thus, a period of 12 weeks should be sufficient according to earlier follow-up studies [11]. In previous cross-sectional studies, results regarding neutrophilic inflammation in currently asthmatic smokers and ex-smokers are not consistent [4, 7–28], rendering long-term effects of smoking cessation on airway neutrophilia a future topic of interest. With regard to eosinophils, the present study is in full correspondence with earlier studies [11]. Cigarette smoking has been shown to down-regulate IL-10 production from macrophages, resulting in a less pronounced Th2-response with attenuated eosinophil recruitment [12]. However, as the results of the present and earlier studies indicate, this process does not seem to revert after smoking cessation over a short period. This finding is somewhat surprising, considering that in the present study, more than 2/3 of the quitters were atopic individuals, in whom a high proportion of eosinophilic phenotype is expected.

Secondly, the lack of blinding of the intervention (smoking cessation) may have biased the improvement of symptoms in the quitter group and thus overestimated the effect of smoking cessation on ACQ score. This bias factor seems less significant with regard to other end-points, in that these are objective measures.

Thirdly, airway inflammation in asthma is affected by many other factors than tobacco smoke. Bacterial and viral infections can induce inflammation with eosinophils and neutrophils. Ongoing subclinical courses of infections may have had an influence on the degree of airway neutrophilia. Furthermore, seasonal, allergic asthma and rhinitis could potentially have played a role on the degree of airway eosinophilia and FeNO. This may be especially important given the differences in atopy and FeNO between the groups. As the majority of the study participants were resident and working in urban areas, heavy air pollution [29] may also have contributed to the absence of reduction in airway neutrophilia after smoking cessation. Furthermore, obesity has also been shown to relate to neutrophilic asthma [30]. However, only five of 46 patients could be classified as obese (BMI > 30), and at baseline, no correlation between BMI and neutrophilia was present (not shown). Neutrophilic inflammation has also been associated with occupational exposures [31]. However, the included participants were young individuals, who had presumably not been exposed to significant quantities of occupational agents.

Fourth, although the ACQ6 scores of our patients were high at baseline, the study population consisted mainly of patients with mild and moderate degree of disease, when considering the relatively high average lung function and the very modest degree of airway reversibility. Different results may have been obtained

in a selected group of severe asthmatics, not least when lung function improvement after smoking cessation is considered.

Fifth, in the non-quitter group, the amount of continued smoking may not have been entirely consistent. The daily cigarette consumption was not formally registered at follow-up visits, but a substantial part of the non-quitters did quit smoking for a period of a few weeks or may have reduced their daily consumption, which may be reflected in the trend towards reduction in exhaled CO. This is possibly one explanation for the observed tendencies towards improvement of methacholine responsiveness and in part ACQ score found in the non-quitter group.

Sixth, three patients in the quitter group smoked cannabis on occasion (all < 1 joint per day), which may also have inhibited the positive effects of smoking cessation in these patients and may in some patients also have inhibited the urge for tobacco.

In conclusion, the results of the present study indicate that several beneficial effects can be quickly gained by asthma patients after quitting smoking in terms of improvement in airway hyperresponsiveness to methacholine and symptom scores. The participants in the present study were not treated with inhaled steroids at any time during the study period, and hence, these beneficial effects may be ascribed solely to the effect of

smoking cessation. However, our hypothesis of change in the type of airway inflammation after smoking cessation was not entirely confirmed, suggesting that in young asthmatic smokers, having markedly fewer pack years than participants in previous studies [11], airway inflammation with leucocytes may be less important drivers of AHR and asthma symptoms than the impact of tobacco smoke, as opposed to what is the case in non-smokers with asthma. Thus, the present study supports the presumption that the benefits of quitting smoking are evident in asthma patients, and although much remains to be elucidated in regard to distinguishing between which disease parameters are influenced mainly by asthmatic inflammation and which by cigarette smoke alone, asthma patients who smoke should be encouraged to quit.

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Conflict of interests

The authors declare no conflict of interest.

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