Inflammation and corticosteroid responsiveness in ex-, current- and never-smoking asthmatics

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Abstract

Background: It has been suggested that smoking asthmatics benefit less from corticosteroid treatment than never-smoking asthmatics. We investigated differences in blood and sputum inflammatory profiles between ex-, current-, and never-smokers and assessed their ICS treatment response after 2-week and 1-year treatment.

Methods: We analyzed FEV1, PC20 methacholine and PC20 AMP, (differential) cell counts in sputum and blood in ex-, current- and never-smokers at baseline (n=114), after 2-week treatment with fluticasone 500 or 2000 μg/day (n=76) and after 1-year treatment with fluticasone 500 μg/day or a variable dose of fluticasone based on a self-management plan (n=64).

Results: A total of 114 patients were included (29 ex-, 30 current- and 55 never-smokers. At baseline, ex- and current-smokers had less eosinophils in sputum and blood than never-smokers. Blood neutrophil counts were higher in current- than in never-smokers. A higher number of cigarettes smoked daily was associated with lower blood and sputum eosinophils. After 2-week ICS treatment, FEV1 %predicted improved less in current-smokers than never-smokers (2.4% versus 8.1%, p=0.010) and ex-smokers tended to improve less than never-smokers (4.1%, p=0.067). In contrast, no differences in ICS treatment response in lung function or inflammatory cells were found between the three groups after 1 year.

Conclusions: Ex- and current-smokers have less eosinophils and more neutrophils in their sputum and blood than never-smokers. Although ex- and current-smokers have a reduced short-term corticosteroid treatment response, we did not find a difference in their long-term treatment response.

Keywords: Asthma, Smoking, Corticosteroid responsiveness, Lung function

Background

Asthma is a chronic inflammatory airway disease in which a variety of inflammatory cells and mediators play a role. Inhaled corticosteroids (ICS) are the cornerstone of treatment, since they exert broad anti-inflammatory effects. They have been shown to improve symptoms and lung function as well as bronchial hyperresponsiveness and markers of airway inflammation in blood, induced sputum and bronchial biopsies [1]. In addition, the use of ICS reduces the number of asthma exacerbations [2].

About 20-30% of asthma patients smoke and another 20-40% are ex-smokers [3-6]. Current-smokers appear to have a different airway inflammatory profile than never-smokers, with less eosinophilic and more neutrophilic inflammation [7-12]. Thus far, very little is known about the inflammatory profile of ex-smokers.

The few studies investigating the effects of smoking on the short-term efficacy of oral or inhaled corticosteroid treatment in asthma, demonstrate that the forced expiratory volume in one second (FEV1) improves significantly in never-smokers, but not in current-smokers [7,13-15]. However, none of these studies found statistically significant differences in improvement in FEV1 when directly comparing never- and current-smokers. The only study that included ex-smokers, showed no improvement in FEV1 or asthma control after 2-week oral corticosteroid treatment in ex- and current-smokers [15]. We aimed to investigate whether ex-, current- and never-smokers with asthma have different inflammatory profiles and if current number of cigarettes or packyears...
smoked affect this. Furthermore, we assessed whether the short- and long-term responsiveness to corticoste-
roids after 2-week and 1-year treatment is different be-
tween ex-, current- and never-smoking asthmatics. We
have analyzed this in a relatively large group of 114 well-
characterized patients with allergic, mild to moderately
severe asthma [16].

Methods
Patients
Patients with a diagnosis of asthma, 18–65 years old,
were included if they met the following criteria: provoca-
tive concentration of methacholine inducing a 20% fall
of FEV\textsubscript{1} (PC\textsubscript{20} methacholine) ≤8 mg/mL, at least one
positive skin-prick test out of 17 common aero-allergens,
reversibility to salbutamol 200 μg ≥9% of the predicted
FEV\textsubscript{1} and the ability to expectorate sputum after hyper-
tonic saline inhalation. This study was conducted in ac-
cordance with the amended declaration of Helsinki and
the study was approved by the medical ethics committee
of the University Medical Center Groningen and all par-
ticipants gave their written informed consent.

Study design
Figure 1 shows the outline of the study. ICS were tapered
before enrollment in the study, as described in the original
manuscript [16]. After discontinuation of ICS completely
for three weeks, or earlier, if they experienced symptoms
of an asthma exacerbation, patients were randomized to 3
treatment arms, with minimization according to smoking
status, age, previous dose of ICS, FEV\textsubscript{1} %predicted, revers-
ibility after 200 μg of salbutamol, PC\textsubscript{20} methacholine, and
serum IgE. Patients were first treated for 2 weeks with
either prednisolone 30 mg/day, fluticasone 500 μg/day or
fluticasone 2000 μg/day via Diskhaler, followed by another
50 weeks of treatment as follows.

The prednisolone 30 mg/day group was treated
according to a self-management plan. They first received
fluticasone 200 μg/day and were instructed to change the
dose according to a self-management plan (see Additional
file 1: Table S1). The fluticasone 500 μg/day group contin-
ued with the same dose for another 50 weeks. The
fluticasone 2000 μg/day arm followed a program with
step-down and eventually complete discontinuation of
corticosteroids. The latter is not in agreement with the
current guidelines and therefore this arm was removed
from our long-term analyses. During the first 2 weeks, the
study had a double-blind, double-dummy design, followed
by 50 weeks open label treatment. Rescue medication
consisted of salbutamol 400 μg via Diskhaler. No other
concomitant pulmonary medication was allowed.

Patients with an exacerbation were treated with a stan-
dardized 7-day course of oral prednisolone. Patients
were withdrawn if they required >1 hospitalization, >4
courses of oral prednisolone or >2 courses within 3
months. Requirement of >2000 μg fluticasone in the
self-management group additionally led to withdrawal.

Lung function and bronchial hyperresponsiveness
FEV\textsubscript{1} was measured with a calibrated, water-sealed spi-
rometer according to standardized guidelines before and
20 minutes after 200 μg of salbutamol [17]. Provocation
tests were performed using a 2-minute tidal breathing
method, adapted from Cockcroft and coworkers [18].
After an initial nebulized saline challenge, subjects in-
haled doubling concentrations of the provocative agent

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Figure 1 Flow chart of the study. FEV\textsubscript{1} = forced expiratory volume in one second, PC\textsubscript{20} = provocative concentration causing a 20% fall in FEV\textsubscript{1}.
AMP = adenosine-5′-monophosphate.
Chemiluminescence analyzer (CLD 700 AL, ECO physics, Sweden) was measured by tidal breathing method using a fluoroenzyme assay (ImmunoCAP Eosinophilic cationic protein (ECP) in serum and sputum intervals. All calculations of PC20 were performed with a base-2 logarithm, reflecting doubling concentrations and normalizing the distribution.

Sputum induction and processing
Sputum was induced by inhalation of hypertonic saline as previously described [16]. Fifteen minutes after salbutamol (200 μg) inhalation, hypertonic saline (3%, 4%, and 5%) was nebulized for each concentration during 7 minutes. Whole samples were processed according to the method of Fahy et al. with some modifications [19].

Cell counts in blood were performed by flow cytometry. Eosinophilic cationic protein (ECP) in serum and sputum were measured with a fluoroenzyme assay (ImmunoCAP ECP, Pharmacia, Uppsala, Sweden). Exhaled nitric oxide (NO) was measured by tidal breathing method using a chemiluminescence analyzer (CLD 700 AL, ECO physics, Switzerland) as described previously [16].

Statistical methods
In case of non-normal distribution, log-transformation was performed to obtain normally distributed variables. Baseline differences between ex-, current- and never-smokers were tested by analysis of variance (ANOVA), Kruskal-Wallis or Chi-square test. If a significant difference between the three groups was found, we performed post-hoc tests with Holm’s Bonferroni correction for multiple testing. Short-term treatment effects were analyzed only in the two groups using ICS (i.e. fluticasone 2000 μg/day or 500 μg/day). To test for changes after treatment within a group (i.e. ex-, current- or never-smokers), we performed paired t-tests. To test for differences in corticosteroid treatment responsiveness between groups, we performed linear regression analyses with change from baseline of each variable as outcome variable and smoking status as the predictor variable and age, gender and type of treatment as covariates. In addition, we adjusted for the baseline value of each variable, since this has been shown to be one of the major predictors of treatment response [20]. To test the effect of current and cumulative smoke exposure on baseline differences and treatment response, we performed linear regression analyses with either the number of cigarettes/day or packyears as predictor variables. We added age as a covariate in these analyses. The reported correlation coefficient (b) signifies the change in an outcome variable (e.g. FEV1) for every unit increase of the predictor variable (e.g. cigarettes/day). In all regression analyses with absolute FEV1 we corrected for age, gender and height.

Results
Patient characteristics
114 patients were included, 29 ex-smokers, 30 current-smokers and 55 never-smokers. Their baseline characteristics, after tapering of ICS (visit 2), are presented in Table 1. During the ICS tapering period, 16 patients returned to the hospital earlier due to symptoms compatible with an asthma exacerbation. From these 16 patients, 6 still used ICS at the start of the treatment period (2 ex-smokers, 2 current-smokers and 2 never-smokers) with a median beclomethasone equivalent dose of 450 μg/day (range 400 – 800 μg/day); the remaining 10 patients had discontinued ICS completely for a median period of 12 days (range 2 – 21 days). Ex-smokers had a median smoking cessation period of 7 years (inter-quartile range (IQR) 1.5 -15.5 years) and had smoked a median of 6.9 packyears (IQR 3.5 – 20.8). Current-smokers had smoked 7.4 packyears (IQR 2.5 – 14.1) and smoked a median of 8.0 cigarettes/day (IQR 4.6 – 15.0).

Baseline differences between ex-, current- and never-smokers
Ex-smoking asthmatics were significantly older than current- and never-smokers (median 38 versus 27 and 25 years, respectively; p=0.001). Sputum eosinophil percentages and blood eosinophil counts were significantly lower in ex- and current-smokers than in never-smokers. Serum ECP, a marker of eosinophil activation, was significantly lower in ex- than never-smokers. Blood neutrophil counts were higher in current- than in never-smokers. Blood neutrophil counts of ex-smokers were between those of never- and current-smokers, but not significantly different from either group. FEV1, reversibility to salbutamol, bronchial hyperresponsiveness to methacholine or AMP and exhaled NO were comparable between ex-, current- and never-smokers.

Association between current and cumulative smoke exposure and baseline clinical and inflammatory parameters
In current-smokers, a higher number of cigarettes smoked daily was associated with lower sputum eosinophil percentages, blood eosinophil counts and serum ECP (Table 2). Furthermore, it was associated with less severe bronchial hyperresponsiveness to both methacholine and AMP (0.1 doubling dose per cigarette/day for methacholine and 0.2 doubling concentrations per cigarette/day for AMP). In ex- and current-smokers, a higher number of packyears was associated with a lower FEV1 %predicted (p=0.034).

Short-term efficacy of ICS treatment in ex-, current- and never-smokers
76 patients were treated with fluticasone 2000 μg/day or 500 μg/day. After 2-week treatment, FEV1 %predicted
levels improved significantly in never-smokers (8.1%, p<0.001, Table 3), but not in ex- or current-smokers (4.1%, p=0.073 and 2.4%, p=0.172 respectively). The magnitude of improvement in FEV1 %predicted was significantly lower in current- than in never-smokers (p=0.010, Figure 2A) and tended to be lower in ex- than in never-smokers (p=0.067). Sputum eosinophil percentages and ECP concentrations improved less in current- than never-smokers and tended to improve less in ex- than never-smokers.

No significant differences in short-term ICS-induced improvements in bronchial hyperresponsiveness and exhaled NO were observed between the three groups. A higher number of packyears smoked was associated with less improvement in FEV1 %predicted (−0.55% per packyear, p=0.025, Additional file 1: Table S1). The number of cigarettes smoked daily was not associated with the short-term ICS response in current-smokers.

Long-term efficacy of ICS treatment in ex-, current- and never-smokers

Data from 64 patients treated for 1-year with fluticasone 500 μg/day or a variable dose of fluticasone according to the self-management plan were available (Table 4). In the self-management group, the median daily dose of

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### Table 1 Differences in clinical and inflammatory variables between ex-, current- and never-smokers at baseline

|                          | Ex smokers (n=29) | Current-smokers (n=30) | Never-smokers (n=55) | p-value |
|--------------------------|-------------------|------------------------|----------------------|---------|
| Age (years)              | 38 (28, 41)       | 27* (25, 37)           | 25* (25, 35)         | 0.001   |
| Gender (male/female)*    | 13 / 16           | 10 / 20                | 16 / 39              | 0.349   |
| Daily number of cigarettes | -                 | 8.0 (46, 150)          | -                    |         |
| Packyears (number)       | 6.9 (3.5, 20.8)   | 7.4 (2.5, 14.1)        | -                    |         |
| Duration of smoking cessation (years) | 7.0 (1.5, 15.9) | -                      | -                    |         |
| Still using ICS after tapering (yes/no)* | 2 / 27        | 2 / 28                 | 2 / 53               | 0.645   |
| Treatment* (prednisolone/FP500/FP2000) | 10 / 11 / 8     | 11 / 6 / 13            | 17 / 20 / 18         | 0.508   |
| FEV1 (L)                 | 2.9 (2.3, 3.4)    | 2.8 (2.4, 3.4)         | 3.0 (2.3, 3.4)       | 0.911   |
| FEV1 (%predicted)        | 79 (68, 89)       | 78 (70, 91)            | 82 (62, 94)          | 0.957   |
| Reversibility (%predicted) | -                 | -                      | -                    | 0.057   |
| PC20 methacholine (mg/ml)* | 0.7 (0.06, 7.9) | 0.8 (0.03, 7.3)        | 0.4 (0.02, 7.8)      | 0.123   |
| PC20 AMP (mg/ml)§         | 10.3 (0.2, 640)   | 7.2 (0.2, 640)         | 3.6 (0.02, 640)      | 0.179   |
| Sputum eosinophils (%)   | 2.8* (1.1, 6.0)   | 4.7* (0.8, 10.7)       | 7.7 (3.8, 14.3)      | 0.015   |
| Blood eosinophils (109/L)* | 0.27* (0.14, 0.43)| 0.26* (0.15, 0.41)    | 0.44 (0.34, 0.61)    | 0.001   |
| Sputum ECP (μg/L)        | 33 (19, 124)      | 67 (16, 126)           | 49 (17, 163)         | 0.790   |
| Serum ECP (μg/L)         | 10* (8, 17)       | 14 (9, 23)             | 22 (12, 29)          | 0.001   |
| Sputum neutrophils (%)   | 39 (22, 53)       | 42 (26, 65)            | 29 (20, 50)          | 0.175   |
| Blood neutrophils (109/L)* | 3.9 (3.0, 4.6) | 4.1* (3.4, 5.3)        | 3.1 (2.4, 6.1)       | 0.003   |
| Exhaled NO (ppb)         | 15 (11, 21)       | 12 (6, 17)             | 16 (12, 21)          | 0.058   |

Values are presented as medians with interquartile ranges, unless stated otherwise, *= number, § geometric mean (range), prednisolone = prednisolone 30 mg once daily, FP500 fluticasone propionate 500 μg/day, FP2000 fluticasone propionate 2000 μg/day, FEV1 forced expiratory volume in one second, PC20 provocative concentration causing a 20% fall in FEV1, AMP adenosine-5′-monophosphate, ECP eosinophilic cationic protein, NO nitric oxide, ppb parts per billion, * = p<0.05 compared to never-smokers with Holm’s Bonferroni correction, ‡ = p<0.05 compared to ex-smokers with Holm’s Bonferroni correction.

### Table 2 Association between the amount of smoke exposure, as reflected by the number of cigarettes smoked daily and number of packyears and clinical and inflammatory variables at baseline

| Cigarettes/day | Packyears |
|----------------|-----------|
|                | b         | p-value | b         | p-value |
| FEV1 (L)       | 0.13      | 0.508   | -0.01     | 0.450   |
| FEV1 (%predicted) | 0.06   | 0.897   | -0.31     | 0.034   |
| Reversibility (%predicted) | -0.22 | 0.162   | -0.00     | 0.968   |
| PC20 methacholine (doubling concentrations) | 0.11 | 0.050   | 0.04      | 0.123   |
| PC20 AMP (doubling concentrations) | 0.19 | 0.031   | 0.02      | 0.593   |
| Sputum eosinophils (%)* | -0.06 | 0.024   | -0.01     | 0.496   |
| Blood eosinophils (109/L)* | -0.02 | 0.021   | 0.00      | 0.645   |
| Sputum ECP (μg/L)* | -0.04 | 0.313   | -0.00     | 0.839   |
| Serum ECP (μg/L)* | -0.04 | 0.025   | 0.00      | 0.829   |
| Sputum neutrophils (%)* | -0.00   | 0.779   | 0.00      | 0.713   |
| Blood neutrophils (109/L)* | 0.00 | 0.673   | -0.00     | 0.338   |
| Exhaled NO (ppb) | -0.09    | 0.708   | 0.10      | 0.254   |

b = unstandardized regression coefficient, FEV1 forced expiratory volume in one second, PC20 provocative concentration causing a 20% fall in FEV1, AMP adenosine-5′-monophosphate, ECP eosinophilic cationic protein, NO nitric oxide, ppb parts per billion, * variable log-transformed.
fluticasone over the 50 week period was 275 μg/day (range 200–1375 μg/day), which was significantly lower than the 500 μg/day used by the fixed-dose group. The level of FEV₁ %predicted improved significantly in ex- and never-smokers, (5.1%, p=0.011 and 10.2%, p<0.001 respectively) and tended to improve in current-smokers (3.1%, p=0.058). There was no significant difference in the magnitude of improvement in FEV₁ between the three groups (Figure 2B). The treatment-induced changes in PC<sub>20</sub> methacholine and numbers and percentages of inflammatory cells in blood and sputum did also not differ significantly between ex-, current- and never-smokers. A higher number of packyears was associated with less improvement in FEV₁ %predicted (p=0.032, Additional file 1: Table S2). In addition, the severity of PC<sub>20</sub> methacholine improved less with a higher number of packyears smoked (p=0.043). The number of cigarettes smoked daily was not associated with the magnitude of improvement in FEV₁ or PC<sub>20</sub> methacholine.

Effect of inflammation on improvement in lung function

To investigate if the baseline type and level of inflammation was associated with the corticosteroid treatment response, we analyzed the independent associations between the improvement in FEV₁ %predicted after 2-week and 1-year ICS treatment and eosinophils in sputum and blood and smoking status. Sputum: higher percentages of sputum eosinophils were significantly associated with a greater improvement in FEV₁ %predicted both after 2-week and 1-year treatment (b = 0.252, p= 0.005 and b=0.232, p=0.002 respectively, Additional file 1: Table S3), whereas sputum neutrophils were not independently associated with improvement in FEV₁ %predicted. Blood: higher levels of blood eosinophil and lower levels of blood neutrophils were independently associated with a higher improvement in FEV₁ %predicted after 2-week ICS treatment (b=0.529, p=0.022 and b=−0.343, p=0.049 respectively, Additional file 1: Table S4). After 1 year ICS treatment blood eosinophil levels were still significantly associated with improvement in FEV₁ %predicted. Smoking status was not significantly associated with improvement in FEV₁ %predicted, when inflammation was taken into account (Additional file 1: Tables S3 and S4).

Discussion

Our study shows that current-smokers with asthma have a different type of inflammation, i.e. they have less eosinophils and more neutrophils in their sputum and blood than never-smokers, even though the severity of airflow obstruction and bronchial hyperresponsiveness is comparable. Moreover, a higher number of cigarettes smoked daily was associated with a lower percentage of eosinophils in sputum, suggesting that the type of airway inflammation may be influenced by the amount of smoke exposure. Interestingly, the inflammatory profile of a group of asthmatics with a median smoking cessation of 7 years was more similar to that of the current-smoking than that of the never-smokers, suggesting that effects of
smoking may persist for a long time after smoking cessation in asthmatics. Additionally, we show that current-smokers have a blunted short-term corticosteroid treatment response. Again, ex-smokers are more similar to current-smokers than to never-smokers, with a trend for a blunted response. However, we found no evidence for a blunted response in both ex- and current-smokers on the long-term.
After short-term treatment with ICS, current-smokers had less improvement in FEV₁ than never-smokers, as reported earlier [7,13,15]. We extend these findings by showing that ex-smokers also tend to respond less to corticosteroid treatment than never-smokers on the short-term. Thus far, the efficacy of corticosteroid treatment in ex-smokers has only been investigated in one study with 15 asthmatic ex-smokers [15]. Comparable to our findings, they observed that the short-term improvement in FEV₁ after 2-week treatment with oral corticosteroids in ex-smokers was intermediate between current- and never-smokers.

Interestingly, we found that the long-term effects of 1-year ICS treatment were not significantly different in ex- and current-smokers compared to never-smokers. This observation is in line with a study in 492 current- and 2,432 never-smokers, showing that 400 µg/day budesonide or placebo for 3 years was equally effective in current- and never-smokers [21]. Furthermore, in a large, real-life study in 619 asthmatics, the level of improvement in FEV₁ and asthma control was similar in ex-, current- and never-smokers after 1-year treatment with small particle budesonide/formoterol formulation [22]. Taken together, these findings suggest that ex- and current-smokers with asthma have a lower corticosteroid treatment response on the short-term than never-smoker, whereas the long-term response is similar between the three groups. We extend these observations by showing that 1-year ICS treatment response is not driven by smoking per se. Rather the underlying inflammatory process present drives the ICS response over 1 year, i.e. a better response with higher sputum and blood eosinophils, independent of smoking. In this context, the findings of Tomlinson and colleagues are of interest [14]. They found a reduced short-term response to inhaled beclometasone in current-smokers with asthma, which could be overcome by increasing the dose of beclometasone from 400 µg/day to 2000 µg/day. It is tempting to speculate that the blunted corticosteroid treatment response in ex- and current-smokers can also be overcome by prolonged treatment, although this remains to be formally demonstrated in future prospective studies.

We did not find any differences in the level of lung function or severity of bronchial hyperresponsiveness between ex-, current- and never-smokers at baseline. However, we did observe a lower level of eosinophilic inflammation in blood and sputum and higher blood neutrophil counts in current-smokers than in never-smokers. These findings are consistent with earlier studies [7-12]. Additionally, we demonstrated that the level of eosinophilic inflammation was also lower in ex-smokers and very similar to that seen in current-smokers. To date, only one other study, also from our research group, reported on the inflammatory profile in ex-smoking asthmatics [23]. This study demonstrated that ex-smoking asthmatics have lower percentages of eosinophils in airway wall biopsies than never-smokers and that the percentage of sputum neutrophils is significantly higher in ex- than in never-smokers. The above findings suggest that smoking does not only have an acute effect on airway inflammation, but also a chronic effect that may persist for years after smoking cessation.

More severe neutrophilic inflammation in asthma has been associated with a reduced corticosteroid treatment

| Table 4 Treatment differences between ex-, current- and never-smokers after 1-year ICS treatment |
| --- |
| Ex-smokers (n=16) | p-value | Current-smokers (n=16) | p-value | Never-smokers (n=32) | p-value |
| Age (years) | 37 (27, 40) | 0.100 | 29 (25, 36) | 0.052 | 25 (25, 34) | <0.001 |
| Gender (male/female) | 9 / 12 | 0.17 | 7 / 10 | 0.002 | 10 / 27 | 0.001 |
| Treatment (FP500/self management) | 11 / 10 | 0.005 | 6 / 11 | 20 / 17 | <0.001 |
| ΔFEV₁ (L) | 0.15 (0.00, 0.60) | 0.010 | 0.17 (-0.07, 0.82) | 0.052 | 0.35 (0.22, 0.71) | <0.001 |
| ΔFEV₁ (%predicted) | 5.1 (0.4, 13.9) | 0.011 | 3.1 (-17, 21.5) | 0.058 | 10.2 (6.4, 20.1) | <0.001 |
| ΔPC₂₀ methacholine (doubling concentrations) | 2.7 (1.5, 5.7) | 0.002 | 2.3 (14, 3.1) | <0.001 | 4.4 (2.1, 5.5) | <0.001 |
| ΔSputum eosinophils (%) | -2.7 (-4.5, -0.3) | 0.005 | -2.0 (-14.3, -0.1) | 0.029 | -7.0 (11.9, -1.3) | <0.001 |
| ΔBlood eosinophils (×10⁹/L) | -0.04 (-0.05, 0.02) | <0.001 | -0.04 (-0.08, 0.05) | <0.001 | -0.16 (-0.26, -0.04) | <0.001 |
| ΔSputum ECP (µg/L) | 11 (-5, 33) | 0.194 | -11 (-165, 6) | 0.096 | -19 (-73, 3) | 0.023 |
| ΔSerum ECP (µg/L) | 0.1 (-3.1, 2.3) | 0.576 | -1.8 (-128, 29) | 0.268 | -9.2 (-193, 4) | <0.001 |
| ΔSputum neutrophils (10⁹/L) | 8.5 (-18.2, 23.3) | 0.126 | 4.8 (-22.2, 24.3) | 0.641 | 6.9 (-5.1, 24.6) | 0.077 |
| ΔBlood neutrophils (10⁹/L) | 0.32 (0.51, 0.70) | <0.001 | -0.15 (-0.87, 0.21) | <0.001 | -0.40 (-0.78, 0.039) | <0.001 |
| ΔExhaled NO (ppb) | -4.9 (-7.1, 2.1) | 0.182 | -6.1 (-9.3, 2.9) | 0.033 | -4.4 (-7.9, -1.4) | 0.002 |

Values are presented as median change from baseline with interquartile range. ICS inhaled corticosteroids, FP500 fluticasone propionate 500 µg/day, FEV₁ forced expiratory volume in one second, PC₂₀ provocative concentration causing a 20% fall in FEV₁, AMP adenosine-5'-monophosphate, ECP eosinophilic cationic protein, NO nitric oxide, ppb parts per billion.
In conclusion, ex- and current-smokers have a different type of inflammation with less eosinophils and more neutrophils in their blood and sputum. These differences in the type of inflammation were present even several years after smoking cessation. Although we agree with the literature that ex- and current-smokers have a blunted short-term response to ICS, we did not find a difference in their long-term treatment response. Therefore, they should not be withheld from ICS treatment.

Additional file

Additional file 1: Table S1. Association between the amount of smoke exposure, as reflected by the number of cigarettes smoked daily and number of packyears and improvement in clinical and inflammatory variables after 2-week ICS treatment. Table S2. Association between the amount of smoke exposure, as reflected by the number of cigarettes smoked daily and number of packyears and clinical and inflammatory variables after 1-year ICS treatment. Table S3. Independent associations between improvement in FEV\(_1\) % predicted after 2-week or 1-year ICS treatment and smoking status and sputum eosinophil and neutrophil percentages. Table S4. Independent associations between improvement in FEV\(_1\) % predicted after 2-week or 1-year ICS treatment and smoking status and blood eosinophil and neutrophil levels. Table S5. Differences in clinical and inflammatory variables between ex-, current- and never-smokers at baseline.

Competing interests

E.D. Telenga has no conflicts to declare. The University of Groningen received funding for research by Prof. H.A.M. Kerstjens from the following manufacturers of inhaled corticosteroids: GlaxoSmithKline, the manufacturer of beclometasone and fluticasone; AstraZeneca, the manufacturer of budesonide; and Nycomed, the manufacturer of ciclesonide. The University of Groningen received funding for research by Dr. N. H. T. ten Hacken from Boehringer Ingelheim, GSK, AstraZeneca, Nycomed and Chiesi. He has been consultant to Chiesi. The University of Groningen received funding for research by Prof. D. S. Postma from AstraZeneca, GSK, Nycomed. Travel to ERS or ATS has been partially funded by AstraZeneca, GSK, Chiesi, Nycomed. She has been consultant to AstraZeneca, Boehringer Ingelheim, Chiesi, GSK, Nycomed, TEVA. The University of Groningen received a research grant for research by dr. M. van den Berge from GlaxoSmithKline and Chiesi.

Authors’ contributions

EDT Performed analysis, wrote the manuscript and approved the final version of the manuscript. HMK. Supervised the original study, critically revised the manuscript and approved the final version of the manuscript. NHTH Critically revised the manuscript and approved the final version of the manuscript. DSP Supervised the original study, critically revised the manuscript and approved the final version of the manuscript. MB Supervised the original study, critically revised the manuscript and approved the final version of the manuscript. EDT Performed analysis, co-authored the manuscript and approved the final version of the manuscript.

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