

Influence of cigarette smoking on airway inflammation and inhaled corticosteroid treatment in patients with asthma

Terufumi Shimoda, M.D., Ph.D.,¹ Yasushi Obase, M.D., Ph.D.,² Reiko Kishikawa, M.D., Ph.D.,¹ and Tomoaki Iwanaga, M.D., Ph.D.¹

ABSTRACT

Background: Cigarette smoking induces neutrophilic airway inflammation and relative resistance to inhaled corticosteroids (ICS).

Objective: We evaluated the influence of cigarette smoking on airway inflammation in patients with asthma and also compared the effect of ICS between smoking and nonsmoking in patients with asthma.

Methods: Smokers with asthma ($n = 81$) and nonsmokers with asthma ($n = 52$) were recruited for the study. We examined lung function, fractional exhaled nitric oxide (FeNO) concentration, induced sputum, and acetylcholine inhalation before and 6 months after inhaling budesonide at 800 $\mu\text{g}/\text{day}$. Thirty-four healthy volunteers were included as controls.

Results: Smokers with asthma showed a lower forced expiratory volume in 1 second (FEV_1) to forced volume capacity (FVC) ratio ($p < 0.05$), a lower FeNO ($p < 0.01$), a lower eosinophil proportion ($p < 0.05$), and a higher neutrophil proportion ($p < 0.05$) in induced sputum than nonsmokers with asthma. Bronchial hyperresponsiveness (the provocative concentration of acetylcholine [Ach] that produced a 20% fall in FEV_1 [$\text{PC}_{20}\text{-Ach}$]) was increased in smokers with asthma compared with nonsmokers with asthma ($p < 0.05$). Both smokers with asthma and nonsmokers with asthma exhibited more prominent airway obstruction, a higher FeNO, and a higher percentage of sputum eosinophils than the controls ($p < 0.05$ to $p < 0.001$, each). After 6 months of treatment with inhaled budesonide at 800 $\mu\text{g}/\text{day}$, the improvement in lung function (FEV_1 to FVC ratio, flow at 50% forced vital capacity [V50% predicted] and flow at 25% forced vital capacity [V25% predicted]), the eosinophil proportion in induced sputum and $\text{PC}_{20}\text{-Ach}$ were lower in smokers with asthma than nonsmokers with asthma ($p < 0.05$).

Conclusion: Smokers with asthma showed neutrophilic airway inflammation in addition to eosinophilic inflammation, and cigarette smoking impaired the efficacy of ICS treatment in mild-to-moderate asthma. These findings have important implications for the management of patients with asthma and who smoke.

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Guidelines for the control and treatment of asthma recommend inhaled corticosteroids (ICS) as the first-line drugs for patients with mild-to-severe persistent asthma.¹ Patients with asthma have irreversible airflow obstruction as a result of chronic airway inflammation, which can be prevented by early introduction to ICS therapy.² ICS are also known to reduce the annual deterioration of pulmonary function.³ Steroids suppress the release of cytokines from cells that line the airway, including airway epithelial cells, smooth-muscle cells, vascular endothelial cells, and fibroblasts.^{4–7} Smoking is one of the most important factors that reduces the anti-inflammatory effects of steroids.^{8,9} ICS control asthma symptoms by suppressing eosinophilic inflammation in the airway, whereas smoking is

thought to worsen respiratory function and symptoms in patients with asthma by inducing neutrophilic inflammation in the airway, thus reducing the efficacy of the ICS.¹⁰ In the present study, we compared the effect of smoking on the airways of patients with asthma with those of healthy control subjects by examining respiratory function, bronchial hyperresponsiveness, and airway inflammation. We also evaluated the effect of smoking status on the anti-inflammatory effect of ICS by comparing patients with asthma and with and without a smoking habit.

METHODS

Subjects

We enrolled 214 patients with newly diagnosed mild-to-moderate asthma who visited Fukuoka National Hospital. No patient had a history of ICS treatment or complications associated with chronic obstructive pulmonary disease. Among 214 patients, 174 (81 never-smoked, 41 ex-smokers, and 52 current-smoking patients) completed the 6-month ICS treatment. Another 40 (21 never-smoked, 11 ex-smokers, and 8 current-smoking patients) failed to follow up. The patients

From the ¹Clinical Research Center, Fukuoka National Hospital, Fukuoka, Japan; ²Second Department of Internal Medicine, School of Medicine, Nagasaki University, Nagasaki, Japan

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Address correspondence to Terufumi Shimoda, M.D., Clinical Research Center, Fukuoka National Hospital, 4–39-1 Yakatabaru, Minami-ku, Fukuoka 811-1394, Japan
E-mail address: t-shimoda@mfukuoka2.hosp.go.jp

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who were ex-smokers were excluded from the analysis due to variations in the amount and duration of smoking and the timing of cessation. Accordingly, the patients with asthma were analyzed according to two subgroups: never smoked ($n = 81$) and current smoker ($n = 52$).

Thirty-four healthy control volunteers without asthma were also evaluated according to two subgroups: never smoked ($n = 20$) and current smoker ($n = 14$). Bronchial asthma was diagnosed according to the Global Initiative for Asthma Guidelines.¹ Specifically, patients were required to meet the following three eligibility criteria to enter the study: (1) their disease should be reversible with at least 12% and 200-mL improvements in forced expiratory volume in 1 second (FEV₁) after bronchodilator therapy, (2) provocative concentrations of acetylcholine that produced a 20% fall in FEV₁ (PC₂₀) should be $\leq 8000 \mu\text{g/mL}$ in a test of bronchial hyperresponsiveness to inhaled acetylcholine, and (3) patients had to have a history of wheezing and/or dyspnea. All antiasthma medications were withheld for at least 24 hours before the measurements were obtained. The study protocol received approval from the ethics committee of the Fukuoka National Hospital; all the participants received verbal and written study information before giving their informed consent (approval number 20–14). T. Shimoda designed the study, participated in the analysis of the data, and wrote the manuscript. Y. Obase performed the statistical analysis of the data. R. Kishikawa assisted in the design of the study and performed the clinical examinations. T. Iwanaga coordinated the patient recruitment process.

Study Design

Respiratory function tests, bronchial hyperresponsiveness, measurement of fractional exhaled nitric oxide (FeNO) concentration, and sputum induction with the use of inhaled 3% hypertonic saline solution were performed on all patients with asthma in the same order at baseline and after 6 months of treatment with $800 \mu\text{g/day}$ of budesonide. With the exception of bronchial hyperresponsiveness, the same set of examinations was performed in the same manner for all controls.

Measurement of Flow Volume Curves

Flow volume curves were measured with a spirometer (Chest Graph HI-701; Chest M.I., Tokyo, Japan). The results are expressed as the percentage of predicted values based on relevant reference standards.¹¹

Measurement of Bronchial Hyperresponsiveness to Acetylcholine

The challenge test was performed by using a standardized methodology. The subjects inhaled acetylcho-

line aerosol from a hand-held nebulizer (PARI BOY 038; PARI GmbH, Starnberg, Germany) with tidal breathing for 2 minutes. Isotonic saline solution was inhaled first as a control. This was followed by progressive doubling of concentrations of acetylcholine, from 0.039 to 20 mg/mL. The FEV₁ was measured after each inhalation by using a spirometer (Chest Graph HI-701). The test was continued until the FEV₁ had fallen by $>20\%$ or until the maximal concentration of acetylcholine had been administered. Bronchial hyperresponsiveness was expressed as the PC₂₀. The subjects with a PC₂₀ of $<8000 \mu\text{g/mL}$ were considered to have bronchial hyperresponsiveness according to the criteria of the American Thoracic Society.¹²

Measurement of Exhaled Nitric Oxide Concentration

FeNO was measured according to the guidelines of the American Thoracic Society by using the single-breath method (online measurement) with a fast-response (0.02 second) chemiluminescence analyzer (NOA 280; Sievers Instruments Inc., Boulder, CO).¹³ All measurements were made by using a mouth pressure of 16 cm H₂O, which corresponds to an expiratory flow of 50 mL/s. FeNO was recorded as the average of three values. Values of ≥ 20 parts per billion (ppb) were judged to be abnormal.

Sputum Induction and Processing

Sputum Induction. Sputum was induced by inhalation of 5 mL of 3% NaCl solution by using a small ultrasonic nebulizer (Nescosonic nebulizer, UN-511; Nesco Medlab, Jakarta, Indonesia). The subjects were asked to cough during and after inhalation and to expectorate into empty containers. Sputum was induced over 20 minutes.

Sputum Processing. Sputum was processed within 30 minutes according to the method described by Metso *et al.*,¹⁴ and both cells for cytospin and the cell-free supernatant were collected. The cell suspension was cytocentrifuged (Cytospin 3; Shandon, Astmoor, U.K.) onto microscope slides (Super frost S2441; Matsunami Glass Ind., Ltd., Kishikawa, Japan) at 450 rpm for 6 minutes. Cytospins from the sputum were allowed to air-dry for 30 minutes and were then stained by using the Giemsa stain method. At least 400 nonsquamous cells, including eosinophils, neutrophils, lymphocytes, macrophages, and ciliated epithelial cells, were counted differentially. The results are expressed as percentages of total nonsquamous counts.

Statistical Analysis

StatMate IV (ATMS Co., Ltd., Tokyo, Japan) was used to perform a *t*-test to compare data before and

after the treatment as well as the changes between the never-smoked and current-smoker subgroups; $p < 0.05$ was considered statistically significant. StatMate IV was also used for comparisons between the never-smoked and current-smoker subgroups of the patients with asthma and the controls, and between the subjects with asthma and the control subjects in the never-smoked and current-smoker subgroups. The χ^2 test was used for sex, disease type, and severity differences, and the t -test was used for age, smoking history, body mass index, duration of asthma, immunoglobulin E (IgE), respiratory function, PC₂₀, FeNO, and frequencies of macrophages, neutrophils, and eosinophils; $p < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Subgroup analysis of the patients with asthma revealed that there were more men in the current-smoker group ($p < 0.001$), with a longer duration of illness ($p < 0.05$) compared with the never-smoked group. The never-smoked group included a higher proportion of patients with mild asthma ($p < 0.05$). The never-smoked group also had higher FEV₁ to forced vital capacity (FVC), flow at 50% forced vital capacity (V50% predicted) and flow at 25% forced vital capacity (V25% predicted), PC₂₀, FeNO, and sputum eosinophil values ($p < 0.05$ to $p < 0.01$, each, Table 1). None of the 34 control subjects (20 nonsmokers and 14 smokers) had atopic predisposition. Among the controls, there were more women in the never-smoked group than in the current-smoker group ($p < 0.01$). The current-smoker group had higher levels of IgE and sputum neutrophils ($p < 0.05$ and $p < 0.001$). The never-smoked group had higher levels of sputum macrophages ($p < 0.001$). Among the never-smoked subjects, the controls showed higher pulmonary function and sputum macrophage values than the patients with asthma ($p < 0.001$). The patients with asthma had higher FeNO, IgE, sputum neutrophil, and sputum eosinophil levels ($p < 0.05$ and $p < 0.001$, respectively, Table 1). Among the current-smoker subjects, the patients with asthma were older ($p < 0.01$). The controls exhibited higher pulmonary function and sputum macrophage levels ($p < 0.001$). The patients with asthma exhibited higher FeNO, IgE, and sputum eosinophil levels ($p < 0.001$) (Table 1).

Respiratory Function

Among the patients with asthma, the never-smoked group showed a significant increase in FEV₁:FVC after treatment ($79 \pm 8\%$) relative to baseline ($73 \pm 12\%$) ($p < 0.001$). The current-smoker group also exhibited a significant increase in FEV₁:FVC after treatment ($73 \pm 11\%$) relative to baseline ($69 \pm 12\%$) ($p < 0.001$). The

change from baseline was not significantly different between the never-smoked ($10 \pm 18\%$) and current-smoker groups ($7 \pm 11\%$) ($p = 0.10$).

The never-smoked group exhibited a significant increase in FEV₁% predicted after treatment ($96 \pm 12\%$) relative to baseline ($84 \pm 17\%$) ($p < 0.001$). There also was a significant increase in the current-smoker group FEV₁% predicted after treatment ($89 \pm 17\%$) relative to baseline ($81 \pm 20\%$) ($p < .001$). The change from baseline was significantly greater in the never-smoked group ($19 \pm 31\%$) than in the current-smoker group ($12 \pm 17\%$) ($p < 0.05$) (Fig. 1).

The never-smoked group exhibited a significant increase in V50% predicted after treatment ($71 \pm 22\%$) relative to baseline ($57 \pm 24\%$) ($p < 0.001$). Similarly, there was a significant increase in the current-smoker group V50% predicted after treatment ($59 \pm 24\%$) relative to baseline ($50 \pm 24\%$) ($p < 0.001$). The change from baseline was significantly greater in the never-smoked group ($79 \pm 45\%$) than in the current-smoker group ($52 \pm 26\%$) ($p < 0.05$).

The never-smoked group exhibited a significant increase in V25% predicted after treatment ($55 \pm 22\%$) relative to baseline ($42 \pm 21\%$) ($p < 0.001$). Likewise, there was a significant increase in the current-smoker group V25% predicted after treatment ($43 \pm 19\%$) relative to baseline ($35 \pm 18\%$) ($p < 0.001$). The change from baseline was significantly greater in the never-smoked group ($79 \pm 56\%$) than in the current-smoker group ($52 \pm 33\%$) ($p < 0.05$) (Fig. 2).

Bronchial Hyperresponsiveness and FeNO

In the patients with asthma, there was a significant increase in the never-smoked group PC₂₀ after treatment ($4887 \pm 6205 \mu\text{g/mL}$) relative to baseline ($841 \pm 1126 \mu\text{g/mL}$) ($p < 0.001$). The current-smoker group also exhibited a significant increase in PC₂₀ after treatment ($2874 \pm 4655 \mu\text{g/mL}$) relative to baseline ($528 \pm 709 \mu\text{g/mL}$) ($p < 0.001$). The change from baseline was significantly greater in the never-smoked group ($1145 \pm 2295\%$) than in the current-smoker group ($535 \pm 795\%$) ($p < 0.05$).

There also was significant improvement in the FeNO value in the never-smoked group after treatment ($38 \pm 33 \text{ ppb}$) relative to baseline ($108 \pm 88 \text{ ppb}$) ($p < 0.001$). The current-smoker group also exhibited a significant improvement in FeNO after treatment ($33 \pm 28 \text{ ppb}$) relative to baseline ($77 \pm 55 \text{ ppb}$) ($p < 0.001$). The change from baseline was not significantly different between the never-smoked ($49 \pm 39\%$) and current-smoker groups ($42 \pm 42\%$) ($p = 0.16$) (Fig. 3).

Cellular Composition of Induced Sputum

In the patients with asthma, the never-smoked group exhibited a significant increase in sputum macro-

Table 1 Patient characteristics

	Asthma		Healthy Volunteer		Asthma; Never vs Current, <i>p</i> Value	Healthy Volunteer; Never vs Current, <i>p</i> Value	Never Smoked; Asthma vs Healthy Volunteer, <i>p</i> Value	Current Smoker; Asthma vs Healthy Volunteer, <i>p</i> Value
	Never Smoked (<i>n</i> = 81)	Current Smoker (<i>n</i> = 52)	Never Smoked (<i>n</i> = 20)	Current Smoker (<i>n</i> = 14)				
M/F	20/61	33/19	2/18	7/7	<0.001	<0.01	0.15	0.36
Age, y	41 ± 12	42 ± 13	41 ± 15	35 ± 6	0.33	0.07	0.5	<0.01
BMI, kg/m ²	23 ± 3	23 ± 4	22 ± 3	23 ± 4	0.5	0.19	0.09	0.5
Pack-years, no.	0	28 ± 14	0	25 ± 12	—	—	—	0.23
Disease duration, y	6 ± 10	10 ± 12	—	—	<0.05	—	—	—
Severity, mild/ moderate, no.	70/11	38/14	—	—	<0.05	—	—	—
Atopic/nonatopic, no.	50/31	39/13	0/20	0/14	0.11	—	<0.001	<0.001
IgE, IU/mL	338 ± 450	601 ± 810	39 ± 40	78 ± 67	<0.05	<0.05	<0.001	<0.001
FEV ₁ :FVC, %	73 ± 12	69 ± 12	83 ± 6	84 ± 7	<0.05	0.4	<0.001	<0.001
FEV ₁ %predicted	84 ± 17	81 ± 20	98 ± 15	97 ± 17	0.24	0.44	<0.001	<0.01
V50 %predicted	57 ± 24	50 ± 24	88 ± 22	93 ± 27	<0.05	0.25	<0.001	<0.001
V25 %predicted	42 ± 21	35 ± 18	70 ± 27	73 ± 31	<0.05	0.38	<0.001	<0.001
%DLco	100 ± 18	105 ± 20	96 ± 18	100 ± 18				
%DLco/VA	101 ± 18	103 ± 19	94 ± 13	96 ± 16				
PC ₂₀ , µg/mL	841 ± 1126	526 ± 709	N.D.	N.D.	<0.05	—	—	—
FeNO, ppb	108 ± 88	77 ± 55	13 ± 3	14 ± 4	<0.01	0.18	<0.001	<0.001
Macrophage, %	32 ± 22	30 ± 17	70 ± 16	40 ± 16	0.34	<0.001	<0.001	<0.05
Neutrophil, %	32 ± 24	44 ± 22	22 ± 9	53 ± 20	<0.05	<0.001	<0.001	0.07
Eosinophil, %	32 ± 24	21 ± 18	0.2 ± 0.4	0.4 ± 0.5	<0.05	0.11	<0.001	<0.001

BBMI = body mass index; IgE = immunoglobulin E; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; V50% predicted = flow at 50% forced vital capacity; V25% predicted = flow at 25% forced vital capacity; %DLco = % diffusing capacity for carbon monoxide; %DLco/VA = % diffusing capacity for carbon monoxide/volume of alveolar; PC₂₀ = provocative concentrations of acetylcholine that produced a 20% fall in FEV₁; N.D. = not done; FeNO = fractional exhaled nitric oxide.

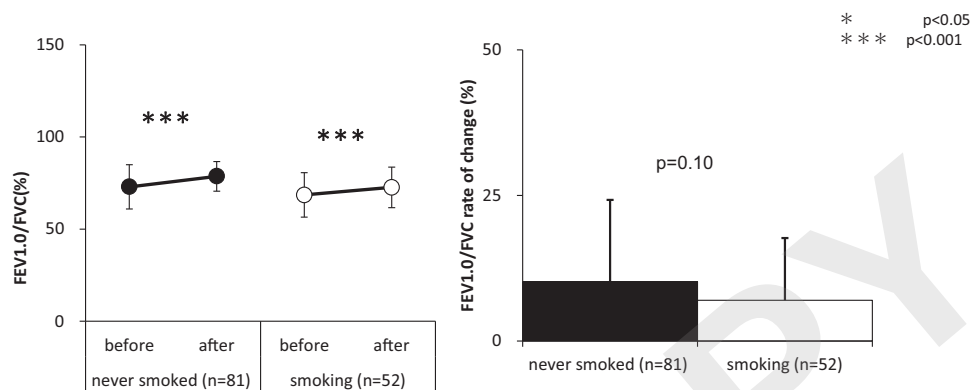


Figure 1. Analysis of data regarding changes in respiratory function (forced expiratory volume in 1 second [FEV₁] to forced vital capacity [FVC], FEV₁% predicted) in patients who were never-smokers and current-smokers before and after treatment. Respiratory function was examined before and after treatment, and the changes between the patients who were never-smokers and current-smokers were compared.

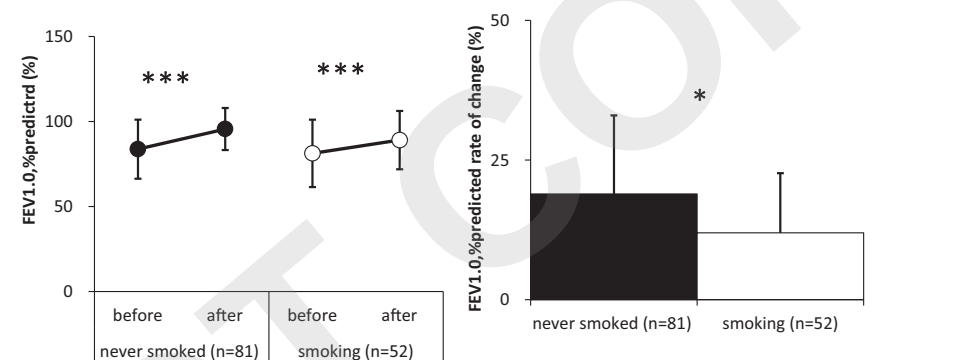
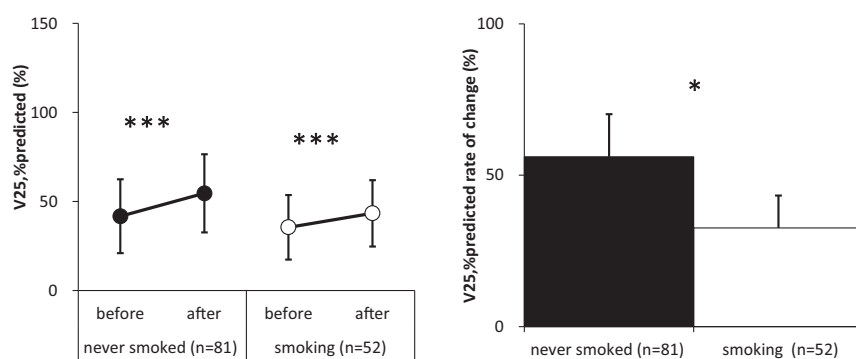
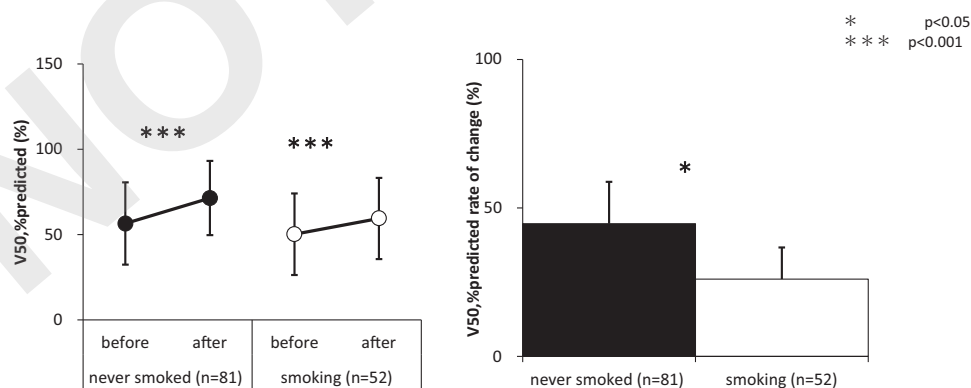


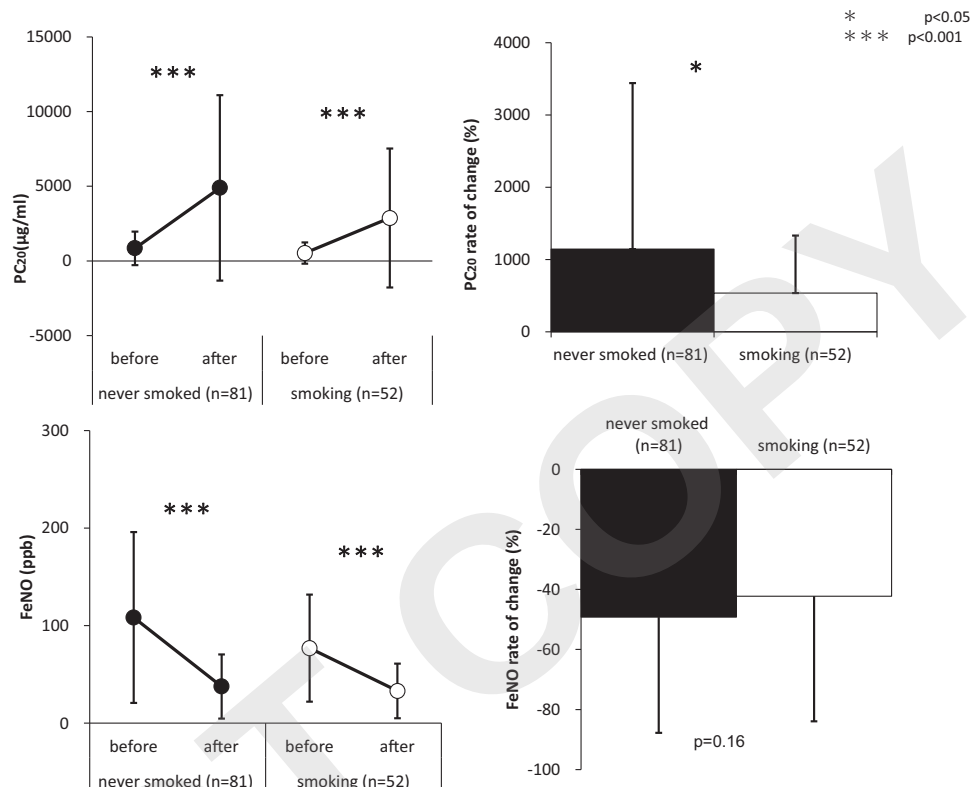
Figure 2. Analysis of data regarding changes in respiratory function (Flow at 50% forced vital capacity [V50] % predicted, flow at 25% forced vital capacity [V25] % predicted) in patients who were never-smokers and patients who were current-smokers before and after treatment. Respiratory function was examined before and after treatment, and the changes between the patients who were never-smokers and patients who were current-smokers were compared.



phages after treatment ($53 \pm 22\%$) relative to baseline ($32 \pm 22\%$) ($p < 0.001$). There was no significant difference in the current-smoker group sputum macrophage level after treatment ($33 \pm 19\%$) compared with

baseline ($30 \pm 17\%$) ($p = 0.14$). The change from baseline was significantly greater in the never-smoked group ($171 \pm 255\%$) than in the current-smoker group ($34 \pm 90\%$) ($p < 0.001$). There also was a significant

Figure 3. Analysis of data regarding changes in provocative concentrations of acetylcholine that produced a 20% fall in FEV₁ (PC₂₀) and fractional exhaled nitric oxide (FeNO) in patients who were never-smokers and patients who were current-smokers before and after treatment. PC₂₀ and FeNO were examined before and after treatment, and the changes between the patients who were never-smokers and patients who were current-smokers were compared.



increase in the percentage of sputum neutrophils in the never-smoked group after treatment ($38 \pm 23\%$) relative to baseline ($32 \pm 24\%$) ($p < 0.05$).

The current-smoker group also exhibited a significant increase in the percentage of sputum neutrophils after treatment ($54 \pm 19\%$) relative to baseline ($44 \pm 22\%$) ($p < 0.05$). The change from baseline was not significantly different between the never-smoked ($82 \pm 174\%$) and current-smoker groups ($60 \pm 122\%$) ($p = 0.27$). The never-smoked group demonstrated a significant decrease in the percentage of sputum eosinophils after treatment ($5 \pm 7\%$) relative to baseline ($32 \pm 24\%$) ($p < 0.05$). There also was a significant decrease in the percentage of sputum eosinophils in the current-smoker group after treatment ($7 \pm 13\%$) relative to baseline ($21 \pm 18\%$) ($p < 0.05$). The change from baseline was significantly greater in the never-smoked group ($80 \pm 33\%$) than in the current-smoker group ($57 \pm 50\%$) ($p < 0.05$) (Fig. 4).

DISCUSSION

Smoking is known to affect the control of asthma¹⁵ and worsen asthma severity by worsening the respiratory function and symptoms of patients with asthma.¹⁶ Patients with asthma and who smoke visit emergency departments frequently¹⁷ and have a higher risk of death from an asthma attack.¹⁸ The present study demonstrated that patients with asthma who were non-smoking tended to have mild disease, whereas those

who smoked had a significantly higher rate of moderate disease, which provided additional evidence that smoking exacerbated asthma conditions. Smoking has also been reported to cause neutrophilic inflammation in addition to eosinophilic inflammation in patients with asthma.¹⁹ Our present study also demonstrated neutrophilic inflammation and eosinophilic inflammation in smokers with asthma.

Patients with asthma also exhibit an elevated proportion of sputum neutrophils when data are compared according to smoking status.²⁰ Indeed, our present study demonstrated that there was a higher percentage of sputum neutrophils in patients with asthma and who smoked. In their 10-year study of adult patients with asthma, Lange *et al.*³ demonstrated that the reduction in FEV₁ was smaller by ~ 15 – 20 mL/y in patients with asthma who had been using ICS than in those who had not used ICS and that smokers have a greater reduction in respiratory function than do nonsmokers. O'Byrne *et al.*²¹ found that lung function was more significantly reduced in smokers than in nonsmokers. Furthermore, ICS treatment is known to improve the percentage of sputum neutrophils in patients with asthma and who were nonsmokers but not in patients with asthma and who smoke.²²

Smoking is also considered to reduce the efficacy of ICS *via* the induction of neutrophilic inflammation.²³ Chalmers *et al.*²² demonstrated that ICS therapy improved bronchial hyperresponsiveness in nonsmokers

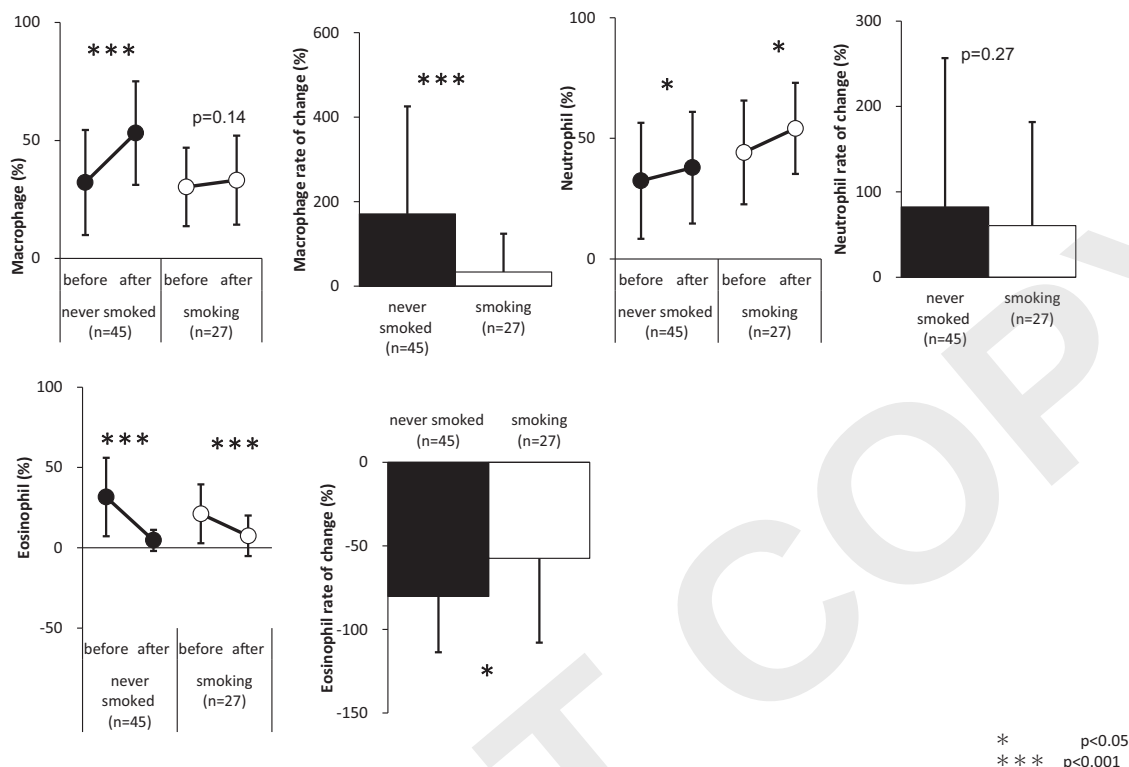


Figure 4. Analysis of data regarding changes in sputum macrophages, neutrophils and eosinophils in patients who were never smokers and patients who were current smokers before and after treatment. Sputum macrophages, neutrophils and eosinophils were examined before and after treatment, and the changes between the patients who were never smokers and patients who were current smokers were compared.

but not in smokers. Our present study indicated that smoking reduced the efficacy of ICS by causing neutrophilic inflammation in addition to eosinophilic inflammation in smokers with asthma. Although the mechanism of steroid resistance in smokers with asthma has yet to be elucidated, the potential causes include the following: (1) increased airway mucosal permeability²⁴ and increased bronchial secretions²⁵; (2) downregulation of β 2-adrenergic receptor function²⁶; (3) increased airway neutrophil or CD8⁺ lymphocyte numbers^{27,28} and reduced airway eosinophil numbers^{23,29}; increased production of interleukin (IL) 4, IL-8, and tumor necrosis factor α ,^{30–32} decreased production of IL-10,³³ and nitrosative stress³⁴; (4) overexpression of glucocorticoid receptor β ^{35,36} and reduced expression of glucocorticoid receptor α ³⁷; (5) overexpression of signal transduction-activated factor³⁸; and (6) reduced histone deacetylase activity³⁹ and increased p38 mitogen-activated protein kinase activity⁴⁰ for corticosteroid cell-signaling systems.⁴¹

ICS therapy improves asthma conditions in non-smokers by reducing bronchial hyperresponsiveness and sputum eosinophils but not in patients with asthma who smoke.^{23,42} However, our results demonstrated improvements in bronchial hyperresponsiveness and the sputum eosinophil percentage even in smokers with asthma, although to a lesser extent than

that observed in nonsmokers. These effects were likely observed because the study subjects had mild-to-moderate asthma in which the airway had not been significantly remodeled and the disease process remained reversible even in smokers. The present study has some limitations. The study manner was case-control design only in patients with mild-to-moderate asthma, and only budesonide inhalation was used for the treatment, which means that the ICS particle difference effect and patient severities were not considered.⁴³

CONCLUSION

Our study indicated that smokers with asthma experienced not only eosinophilic but also neutrophilic inflammation. ICS therapy seemed to be less effective for improving the respiratory function in smokers than in nonsmokers because it suppressed eosinophilic inflammation but not neutrophilic inflammation. Thus, our study demonstrated that smoking reduced the efficacy of ICS and that it is important to stop smoking to prevent the progression of asthma symptoms and airway remodeling.

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