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Running title: diurnal variation of fractional exhaled nitric oxide

Usefulness of diurnal variation of fractional exhaled nitric oxide for predicting early therapeutic response to asthma treatment

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ABSTRACT

OBJECTIVE: Fractional exhaled nitric oxide (FeNO) is considered to be an adjunct for asthma management, although its usefulness remains controversial. Therefore, it may be necessary for new approaches to use FeNO for asthma management. We evaluated whether diurnal variations of FeNO can predict response to asthma treatment.

METHODS: This pilot study consisted of 22 uncontrolled asthmatics and 16 healthy subjects. FeNO and peak expiratory flow (PEF) were measured by themselves twice daily at home for three weeks (asthmatics) or two weeks (healthy subjects), and daily mean and diurnal variations of FeNO and PEF levels were calculated. In uncontrolled asthmatics, treatment was intensified a week after study entry, and then control status was re-evaluated after three to four weeks. Asthmatics were then divided into two groups; good or poor responders.

RESULTS: Diurnal variations of FeNO levels, as well as daily mean FeNO and PEF levels, in uncontrolled asthmatics before intensive treatment were significantly higher than those in healthy subjects, regardless of treatment response ($p < 0.01$). Furthermore, in the good responders, diurnal variations of FeNO levels were significantly decreased in the 1st week ($p < 0.05$) of intensive treatment, whereas the daily mean FeNO levels significantly dropped in the 2nd week ($p < 0.05$). In the poor responders, no such changes were observed in FeNO levels. In terms of PEF, only the daily mean levels were significantly elevated after the initiation of intensive treatment, regardless of treatment response.

CONCLUSIONS: Diurnal variations of FeNO may contribute to predicting early therapeutic response to asthma treatment.

Keywords : asthma, fractional exhaled nitric oxide, home monitoring, diurnal variation, therapeutic response

Abbreviations

PEF: peak expiratory flow

FeNO: fractional exhaled nitric oxide

ICS: inhaled corticosteroids

FEV₁: forced expiratory volume in one second

GINA: Global Initiative for Asthma

ATS: American Thoracic Society

LABA: long-acting β 2-agonist

SABA: short-acting β 2-agonist

ERS: European Respiratory Society

A-NO: ambient nitric oxide

Introduction

It has been reported that between 20% and 50% of asthmatic subjects are uncontrolled ⁽¹⁾. Therefore, painstaking assessments, such as domiciliary symptoms and/or peak expiratory flow (PEF) monitoring, have been recommended to achieve comprehensive asthma management ⁽²⁾. However, this assessment does not directly reflect airway inflammation, which is the fundamental condition of asthma pathophysiology. From this point of view, tools for directly assessing airway inflammation, such as fractional exhaled nitric oxide (FeNO), are necessary ⁽³⁻⁷⁾.

At present, it is controversial whether FeNO can be a useful biomarker of asthma management ⁽⁷⁾. In fact, some previous reports have suggested that FeNO measurements contribute to the reduction of both the maintenance doses of inhaled corticosteroids (ICS) and frequency of exacerbations ^(8,9). Others have reported that FeNO measurements, either alone or in addition to conventional measures, have not resulted in significant improvement in asthma management ^(10,11). Given the circumstances, new approaches are needed.

To date, the usefulness of domiciliary FeNO monitoring for asthma management has been previously reported ^(12,13). Anderson et al. found that FeNO levels in the morning were significantly higher than those in the evening, and that there was a significant dose response of diurnal FeNO to ICS ⁽¹²⁾. Furthermore, we have also previously reported that there were significantly larger diurnal FeNO variations in uncontrolled asthmatic subjects compared to subjects with stable controlled asthma. In addition, the diurnal FeNO variations provided more accurate information for predicting uncontrolled asthma and future risks of asthma exacerbation ⁽¹³⁾. These findings indicate the potential of domiciliary FeNO monitoring for asthma management, including therapeutic response to asthma treatment. However, the type of patient who has a good therapeutic response to asthma treatment with domiciliary FeNO monitoring has yet to be elucidated. Therefore, the aim of the current study was to evaluate whether diurnal variations of FeNO levels, as well as absolute FeNO levels, can be used to predict better treatment response in uncontrolled asthma.

Methods

Subjects

Asthmatic subjects with uncontrolled status were recruited from an outpatient clinic at Fukushima Medical University Hospital, Fukushima, Japan. Healthy subjects with normal spirometry and no respiratory disorders were also recruited from the staff of Fukushima Medical University. The diagnosis of asthma was made by respiratory physicians according to a clinical history of characteristic symptoms (i.e. dry cough, wheezing, chest tightness and breathlessness), as well as a history of more than one of the following; forced expiratory volume in one second (FEV₁) reversibility $\geq 12\%$, positive bronchial hyperresponsiveness, or proportion of eosinophils in sputum $\geq 3\%$. If the subject has sputum eosinophilia, but does not have airflow reversibility or bronchial hyperresponsiveness, $\geq 20\%$ PEF variabilities were confirmed. Asthmatic subjects were excluded if they were current smokers, had concomitant respiratory diseases other than asthma, or had a respiratory tract infection within four weeks before study entry. The healthy subjects were defined as having a smoking history of < 5 pack-years, no respiratory diseases and normal spirometry. The present study was conducted in accordance with the principles of the Declaration of Helsinki, approved by the Ethics Committee of Fukushima Medical University (NO. 1721), and registered to UMIN (UMIN000010675). All participants provided written informed consent to this study.

Definition of asthma control status

The uncontrolled asthmatic subjects included in the present study were those with either partly- or poorly-controlled asthma based on the Global Initiative for Asthma (GINA) guidelines⁽²⁾. In brief, poorly-controlled asthmatic subjects were defined as having three or more features of the following, within four weeks prior to recruitment: (1) daily symptoms lasting more than two days in a row; (2) any limitation of activities; (3) any nocturnal symptoms or awakening; (4) need for reliever/rescue treatment more than twice a week; and/or (5) spirometry (PEF or FEV₁) $< 80\%$ predicted or personal best (if known). Asthmatic subjects who had two or fewer of these features were classified as having partly-controlled asthma.

Study design

For the healthy subjects (Figure 1a), spirometry was performed at study entry according to the American Thoracic Society (ATS) guidelines⁽¹⁴⁾. Next, a portable FeNO monitor (NObreath[®]; Bedfont Scientific Ltd, Rochester, UK), and PEF monitor (PIKO-1[®]; nSpire Health Ltd, London, UK) were provided to measure FeNO and PEF levels twice a day over two weeks at home. For the uncontrolled asthmatic subjects (Figure 1b), FeNO measurements and spirometry⁽¹⁴⁾ were performed at study entry, in that order, and then asthma control status was evaluated in line with the GINA guidelines⁽²⁾. Thereafter, like with the healthy subjects, NObreath[®] and PIKO-1[®] were provided so the subjects could measure their FeNO and PEF levels twice a day over three weeks. During the course of the three-week period, the asthmatic subjects were asked to continue their treatment in the same manner for one week after study entry. Then, at the second visit (a week after study entry), their asthma treatment was intensified with increased ICS doses either alone or in addition to long-acting β_2 -agonist (LABA) based on the GINA recommendations for a stepwise approach to ongoing treatment⁽²⁾. Briefly, if asthmatic subjects had symptoms twice a month or more, but less than 4–5 days a week, low dose ICS were added to the baseline treatment. If asthmatic subjects had symptoms most days, or nocturnal symptoms once a week or more, either low or medium dose of ICS with LABA were prescribed with increasing the doses of the subjects' baseline treatments. If the asthmatic subjects had daily symptoms, nocturnal symptoms once a week or more, and had low lung function, either medium or high dose of ICS with LABA were taken with increasing the doses of the subjects' baseline treatments. At the third visit (three to four weeks after study entry), the asthmatic subjects brought the monitors back and underwent spirometry. Their control status was then assessed based on their symptoms and rescue uses of short-acting β_2 agonist (SABA) in order to evaluate the response to intensive treatment.

FeNO and PEF measurements

FeNO and PEF measurements were conducted in line with the ATS / European Respiratory Society (ERS) recommendations^(14, 15) using portable handheld analyzers (NObreath[®] and PiKo-1[®]). At the first visit, all subjects were instructed on how to measure FeNO and PEF by expert physicians using instruction brochures with photos. The physicians supervised the subjects performing FeNO and PEF measurements by themselves until they could perform the measurements perfectly. The brief instructions of FeNO and PEF measurements were as follows: in terms of FeNO measurements, an ambient nitric oxide (A-NO) was first measured and written down on a recording sheet in order to minimize atmospheric effects⁽¹⁶⁾. If an A-NO level was ≥ 40 ppb, the subjects were asked to ventilate the room and repeat A-NO measurements until the level dropped down to < 40 ppb. When an A-NO level was < 40 ppb, daily FeNO measurements were subsequently started at a constant flow of 50 ml/s based on the ATS / ERS recommendations⁽¹⁵⁾. At least two successive measurements were made on each occasion, and the levels were written down on the recording sheet. In terms of PEF measurements, three successive measurements using PiKo-1[®] were performed after FeNO measurements⁽¹⁴⁾. The best of the three readings was automatically recorded on the monitor, and all measurements were transferred to a computer when the subjects returned the monitor. In order to avoid measurement bias, the subjects were asked to successively measure FeNO and PEF in the same place twice a day, in the morning between 07:00 h and 10:00 h, and in the evening between 18:00 h and 21:00 h. The subjects were also asked not to take their usual medications prior to performing FeNO and PEF measurements, and to always measure FeNO before PEF.

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 21, IBM, Chicago IL, USA), and Graph Pad Prism (version 5, Graph Pad Software, San Diego, USA). The sample size was determined using G*power software. Based on our previous study⁽¹³⁾, we determined that a total of 32 subjects (16 asthmatic subjects and 16 healthy subjects) were needed for 85% power at a significance alpha level of 0.05 in order to detect a 50% difference in diurnal FeNO levels between the healthy and asthmatic subjects. Data are shown as mean (95% confidential interval). The morning and evening FeNO levels were each calculated as the means of two or three accurately measured levels. The morning and evening PEF levels were each the best of three measurements using programmed computer software for the PiKo-1[®]. We used two ways to assess the daily fluctuations of FeNO and PEF in relation to their predictive effectiveness of asthma treatment (Table 1). In brief, each daily mean FeNO level and PEF level was defined as the mean of the morning and evening levels. Diurnal variation of each FeNO and PEF level was defined as the difference between the highest and lowest measurements in a day. Data of daily means as well as diurnal variations of FeNO and PEF levels in the uncontrolled asthmatic subjects were assessed for three periods: untreated week; 1st week; and 2nd week of intensive treatment (Figure 1). Moreover, for the evaluation of response to intensive treatment, the uncontrolled asthmatic subjects were classified into two groups, according to their symptoms and rescue uses of SABA. Subjects who did not have any daily or nocturnal symptoms, limited activity, or rescue uses of SABA during the week before Visit 3 were defined as 'good responders'. On the other hand, subjects who experienced either the above-mentioned symptoms, limited activity, or rescue uses of SABA during the week before Visit 3 were defined as 'poor responders'. Daily means and diurnal variations of FeNO and PEF before and after intensive treatment in the uncontrolled asthmatic subjects were compared using the Wilcoxon signed rank sum test. The Mann-Whitney U test and χ^2 test were performed to compare continuous and categorical parameters, respectively, between the uncontrolled asthmatic subjects and healthy subjects. A two-tailed p values of < 0.05 was considered statistically significant.

Results

Study profile

In total, 32 uncontrolled asthmatic subjects and 16 healthy subjects were screened in the study. All 16 healthy subjects completed the study protocol to the end. Among the asthmatic subjects, one discontinued the study because of consent withdrawal, 3 were unable to measure FeNO levels routinely at home for personal reasons, and we excluded 6 because of high ambient NO, which can influence actual FeNO readings. Finally, 22 uncontrolled asthmatic subjects were analysed (Figure 2), of whom 21 were referred from non-respiratory specialists to our clinic because of poor control of their asthma. Only 3 out of 21 subjects were prescribed ICS, and the others were treated with SABA, LABA, long acting muscarinic antagonist, leukotriene receptor antagonist LTRA), and/or antitussives. Only one subject, regularly visited our outpatient clinic, was treated with ICS/LABA, although his asthma control was poor.

Characteristics of subjects

In total, 22 uncontrolled asthmatic subjects (13 good responders and 9 poor responders) and 16 healthy subjects were enrolled in the present study. Their characteristics are summarized in Table 2. The uncontrolled asthmatic group consisted of 14 partly-controlled and 8 poorly-controlled subjects. The uncontrolled asthmatic subjects were significantly older, had lower FVC and FEV₁ levels, and had higher FeNO levels compared to the healthy subjects ($p < 0.05$). In the asthmatic subjects, there were no significant differences in ICS doses, FeNO or total IgE levels between the good and poor responders.

Example of diurnal variation of FeNO

An example of individual diurnal variation of FeNO was shown in Figure 3. In a healthy subject, FeNO levels remained almost constant throughout the two weeks, and diurnal variations of FeNO, represented by the gray areas, were also small (Figure 3a). On the other hand, in both a good and poor responder, daily FeNO (Figure 3(b,d)) levels were fluctuated, and diurnal variations of FeNO were greater before intensive treatment compared to those in a healthy subject. Once intensive treatment was initiated, diurnal variations of FeNO in a good responder (Figure 3c) were gradually reduced, while those in a poor responder (Figure 3e) did not change significantly.

Example of diurnal variation of PEF

An example of individual diurnal variations of PEF was shown in Figure 4. In a healthy subject, PEF levels remained almost constant throughout the two weeks like those of FeNO, and diurnal variations of PEF (Figure 4a), indicated by the gray areas, were also small. In an asthmatic subjects, daily PEF (Figure 4(b,d)) levels were fluctuated and diurnal variations of PEF were greater compared to those in a healthy subject. However, this trend did not change after intensive treatment in both a good and poor responder (Figure 4(c,e)).

Comparison of FeNO and PEF between healthy subjects and uncontrolled asthmatic subjects with good response and poor response

As expected, the daily means (good responder, mean 62.1 ppb, 95%CI 23.9–100.4 ppb; poor responder, mean 43.4 ppb, 95%CI 22.2–64.7 ppb; healthy, 14.5 ppb, 95%CI 12.0–17.1 ppb; $p < 0.001$) as well as the diurnal variations (good responder, mean 16.6 ppb, 95%CI 8.6–24.6 ppb; poor responder, 11.1 ppb, 95%CI 7.1–15.1 ppb; healthy 5.12 ppb, 95%CI 4.09–6.04 ppb; $p < 0.001$ and $p < 0.01$, respectively) of FeNO levels over a week in the uncontrolled asthmatic subjects before intensive treatment were significantly higher than those in the healthy subjects regardless of

treatment response (Figure 5(a,b)). Similarly, the daily mean PEF levels in the uncontrolled asthmatic subjects before intensive treatment were significantly lower than those in the healthy subjects, regardless of response to asthma treatment (good responder, mean 344 L/min, 95%CI 265–423 L/min; poor responder, mean 266 L/min, 95%CI 159–373 L/min; healthy, 512 L/min, 95%CI 460–563 L/min; $p < 0.01$ and $p < 0.001$, respectively) (Figure 5c) although the diurnal variations of PEF levels were not (good responder, mean 48.0 L/min, 95%CI 30.7–65.4 L/min; poor responder, mean 35.7 L/min, 95%CI 18.5–52.9 L/min; healthy, 30.4 L/min, 95%CI 21.4–39.2 L/min) (Figure 5d).

Comparison of daily mean and diurnal variations of FeNO levels before and after intensive treatment in uncontrolled asthmatic subjects

Regardless of treatment response, the daily mean as well as diurnal variation of FeNO levels at the 2nd week of intensive treatment were significantly lower than those at study entry ($p < 0.05$). Interestingly, the diurnal variations of FeNO levels in the 1st week of intensive treatment significantly dropped from those before intensive treatment in the good responders only (mean 9.4 ppb, 95%CI 5.0–13.8 ppb vs mean 16.6 ppb, 95%CI 8.6–24.6 ppb; $p < 0.05$) (Figure 6c), but not in poor responders (mean 11.1 ppb, 95%CI 6.2–16.0 ppb vs mean 11.1 ppb, 95%CI 7.1–15.1 ppb) (Figure 6d). On the other hand, the daily mean FeNO levels in the 1st week of intensive treatment did not change significantly from those before intensive treatment in both the good (mean 40.8 ppb, 95%CI 24.3–57.3 ppb vs mean 62.1 ppb, 95%CI 23.9–100.4 ppb) and poor responders (mean 37.2 ppb, 95%CI 19.2–55.2 ppb vs mean 43.4 ppb, 95%CI 22.2–64.7 ppb) (Figure 6(a,b)). Thereafter, in the 2nd week of intensive treatment, the daily mean FeNO levels were significantly lower than those in the 1st week of intensive treatment in the good responders only (mean 32.7 ppb, 95%CI 21.5–43.9 ppb vs mean 40.8 ppb, 95%CI 24.3–57.3 ppb; $p < 0.05$) (Figure 6a).

Comparison of daily mean and diurnal variations of PEF levels before and after intensive treatment in uncontrolled asthmatic subjects

In terms of PEF, the daily mean PEF levels in the 1st week of intensive treatment significantly rose compared to those at study entry not only in the good responders (mean 386 L/min, 95%CI 326–446 L/min vs 344 L/min, 95%CI 265–423 L/min; $p < 0.01$), but also in the poor responders (mean 315 L/min, 95%CI 219–411 L/min vs 277 L/min, 95%CI 198–356 L/min; $p < 0.01$) (Figure 7(a,b)). Thereafter, only the good responders kept the daily mean PEF levels steady in the 2nd week of intensive treatment. There were no significant changes in the diurnal variations of PEF levels before and after intensive treatment, regardless of response to intensive treatment (Figure 7(c,d)).

Discussion

The aim of this pilot study was to evaluate the clinical usefulness of diurnal variations of FeNO as a biomarker for predicting early response to asthma treatment. In the patients with uncontrolled asthma, the diurnal variations, as well as the daily mean FeNO levels were significantly higher than those in the healthy subjects, regardless of response to intensive asthma treatment. Regarding the time courses of FeNO after intensive treatment, the diurnal variations of FeNO levels significantly decreased during the 1st week of intensive treatment, whereas the daily mean FeNO levels significantly dropped during the 2nd week, but not during the 1st week, in the good responder group. These observations were not seen in the poor responder group. With regard to the time courses of PEF, the daily mean PEF levels were elevated significantly after intensive treatment, regardless of treatment response.

In the present study, the diurnal variations of FeNO levels in the uncontrolled asthmatic subjects were greater when compared to the healthy subjects. This finding is consistent with those in our previous study, which demonstrated that asthmatic subjects, especially uncontrolled asthmatic subjects, had greater diurnal FeNO variations compared to healthy subjects⁽¹³⁾. Several studies have revealed that airway inflammation in asthma has a diurnal variation^(17,18). In fact, the frequency of asthma exacerbation is known to be higher from midnight to early morning and lower during the day. The possible reasons for this condition are considered to be the decreased levels of cortisol, which lead to the activation of inflammatory cells such as eosinophils⁽¹⁹⁾ and mast cells⁽²⁰⁾, and also to be the activated parasympathetic nerves which cause bronchial constriction during the night time⁽²¹⁾. Furthermore, Kraft et al. reported that CD4+ lymphocytes were increased in the alveolar tissues at night, which led to the elevation of alveolar tissue eosinophils and the overnight decrement in lung function⁽¹⁸⁾. In support of these findings, sputum eosinophils were reported to be significantly higher at around 4 am than at around 4 pm⁽²²⁾. Taking the above-mentioned findings into consideration, it may be reasonable that FeNO levels, as an index of eosinophilic airway inflammation, show a diurnal variation, and that diurnal variations in asthmatic patients with asthma are higher than those in healthy people.

Interestingly, the time courses of FeNO levels expressed as either daily mean or diurnal variations differed depending on how asthmatic subjects responded to asthma treatment, whereas these trends were not observed in the time courses of PEF levels in this study. Some studies have reported good relationships between diurnal PEF variations, control status and exacerbations in asthma^(23, 24), whereas others have suggested only limited usefulness of diurnal PEF variations for asthma management^(25, 26). Therefore, it may be reasonable to conclude that the diurnal variations in FeNO, rather than PEF, are more closely related to early therapeutic response to anti-inflammatory treatment for asthma, since FeNO is known as a faster-responding biomarker directly reflecting airway inflammation, compared to other conventional markers, such as symptoms and spirometry⁽²⁷⁾. To date, there has only been one report that monitored the time course of FeNO levels after ICS treatment⁽¹²⁾. In that study, absolute FeNO levels showed a significant decrease of up to 75% from the baseline level at the 2nd week of ICS treatment, but not during the 1st week. This result is consistent with our finding that daily mean FeNO levels significantly dropped during the 2nd week of intensive treatment. On the other hand, to the best of our knowledge, there have been no reports that prospectively compared diurnal variations of FeNO levels before and after treatment. In the present study, we found that diurnal variations of FeNO levels significantly decreased during the 1st week of intensive treatment in the good responder group only, but not in the poor responder group, indicating that diurnal variations of FeNO levels can be more sensitive and rapid than daily mean FeNO levels when evaluating early therapeutic response to asthma treatment.

There are some limitations to the present study. Firstly, the number of enrolled subjects was small, which may have reduced the statistical power. Practically, it takes a lot of time to recruit a large number of subjects, because portable FeNO devices are still expensive, limiting their use as a home monitoring device. However, the present study is a pilot study, and a home-based FeNO monitoring system is now being developed⁽²⁸⁾. We believe that large prospective observational studies using the home-monitoring system will lead to the clinical application of diurnal FeNO variations to predict early response to asthma treatment in the near future. Secondly, we did not assess adherence to treatment. However, it is unlikely that there was poor adherence in the current study's population, since all participants provided FeNO and PEF measurements on a twice-daily basis, and had to take their inhaler treatments after these measurements. Thirdly, we were unable to confirm whether the subjects could accurately measure FeNO and PEF in accordance with how they had been instructed. However, all subjects were instructed at the study entry until their physicians were satisfied that they could perfectly perform measurements. In addition, we confirmed that FeNO and PEF levels at each consecutive measurement were acceptable with < 10% variations. Therefore, measurement bias is unlikely.

In conclusion, diurnal variations of FeNO may provide useful information regarding the prediction of early therapeutic response to asthma treatment. Further prospective large observational studies are needed to examine whether either domiciliary FeNO monitoring alone, or with a symptom diary and PEF, can result in better asthma management.

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Author contributions;

MU and JS contributed equally to this work. MU recruited patients, analyzed and interpreted the data, and drafted the manuscript. JS conceived and designed the study, recruited patients, analyzed and interpreted the data, and drafted and edited the manuscript. SS, AF, YSu, MR, TO, HT, NW, MS, JM, TKa, TU, RT, YS, TKo, KH, HM, TN, KK, and YT recruited patients, and interpreted the data. MM and YSh interpreted the data, and critically revised the manuscript. All authors approved the final version of the manuscript.

Declaration of Interest

MU received grants from JSPS KAKENHI. None of the rest of the authors received grants related with submitted work, and have no relevant conflicts of interests.

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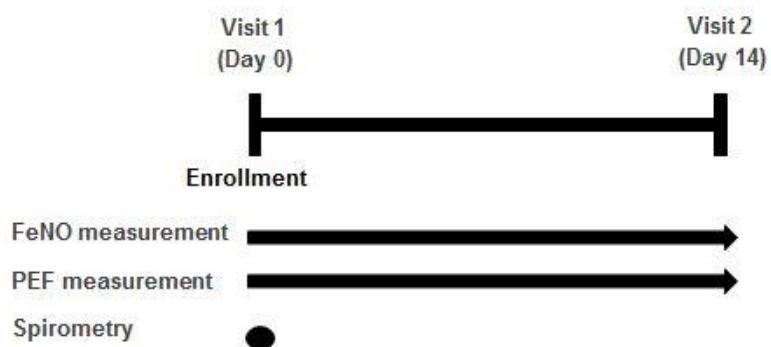
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a) Healthy subjects



b) Asthmatic subjects

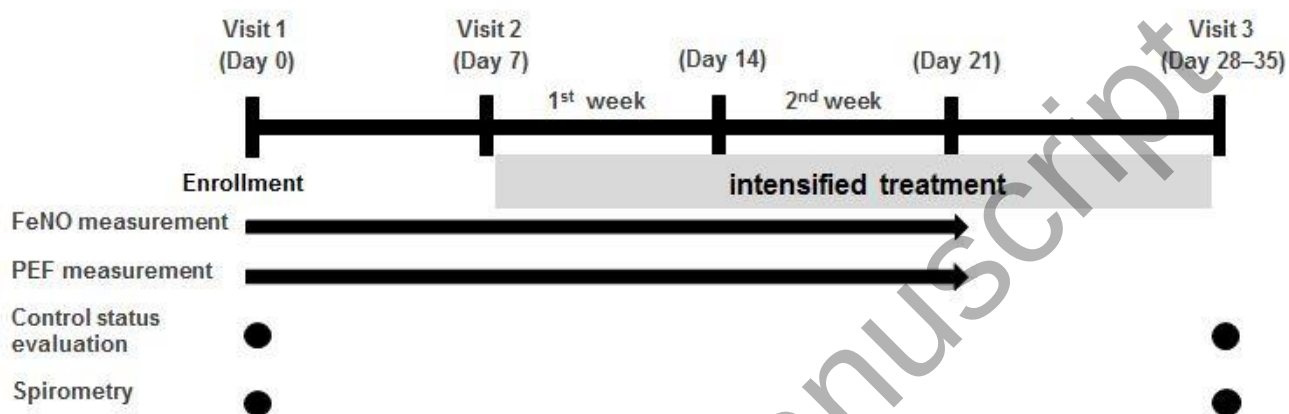


FIGURE 1. Study of a) healthy subjects, and b) asthmatic subjects. FeNO: fractional exhaled nitric oxide; PEF: peak expiratory flow.

a) Healthy subjects



b) Asthmatic subjects

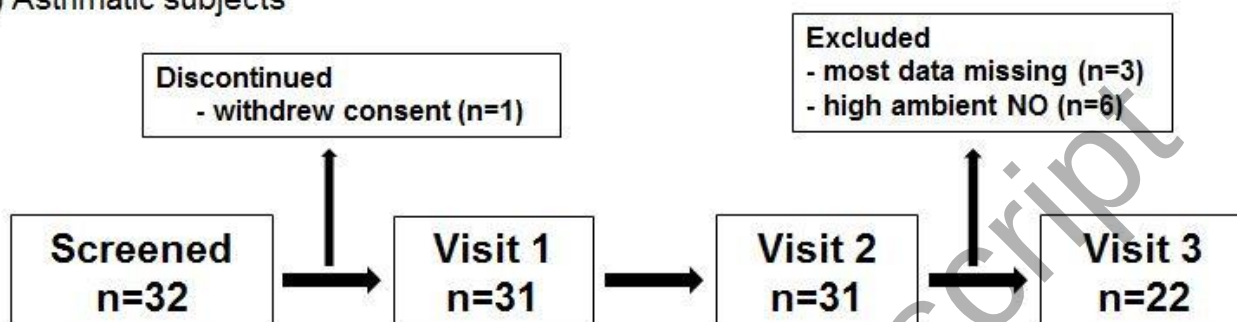


FIGURE 2. Study profile of a) healthy subjects, and b) asthmatic subjects.

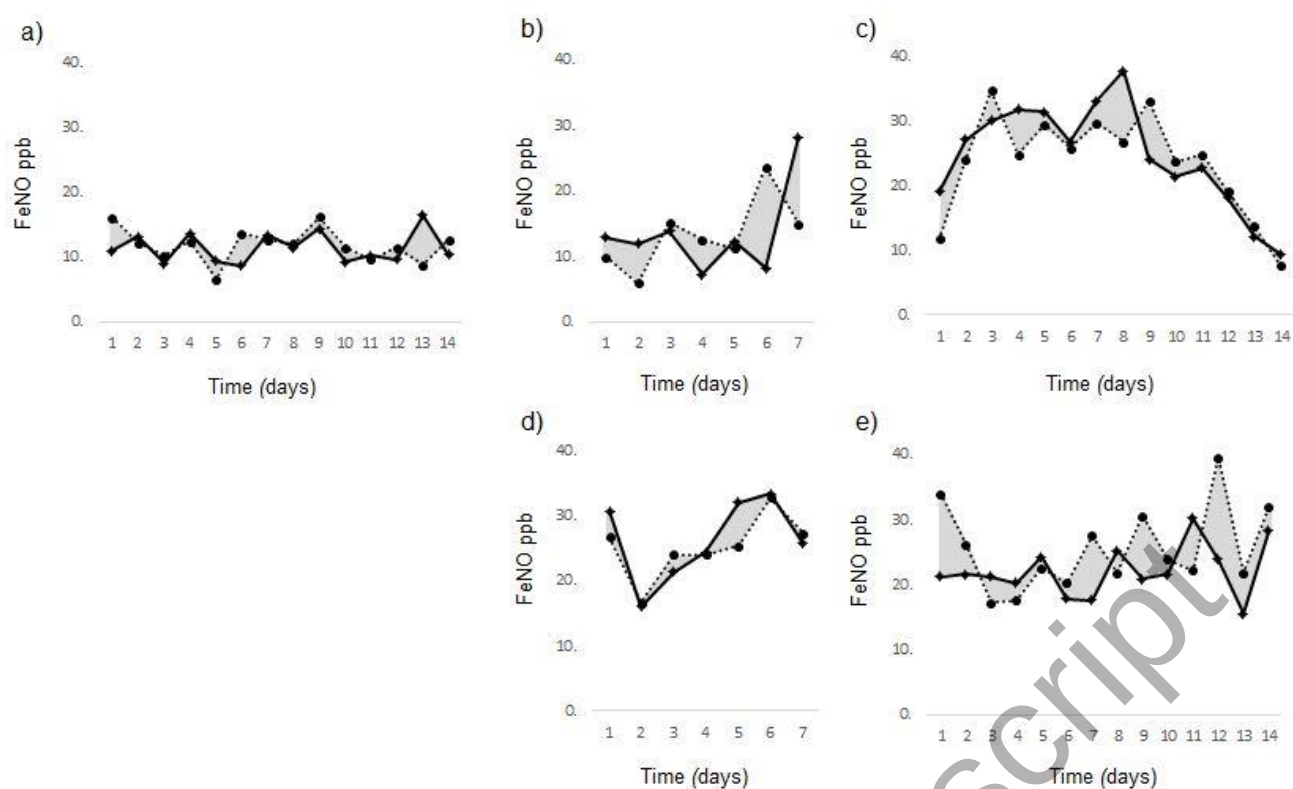


FIGURE 3. Examples of typical individual patterns of fractional exhaled nitric oxide (FeNO) levels in: a) a healthy subject over 2 weeks; b) a good responder over 1 week before intensive treatment; c) a good responder over 2 weeks after intensive treatment; d) a poor responder over 1 week before intensive treatment; and e) a poor responder over 2 weeks after intensive treatment. Morning FeNO levels are connected with dotted lines, and evening FeNO levels with solid lines.

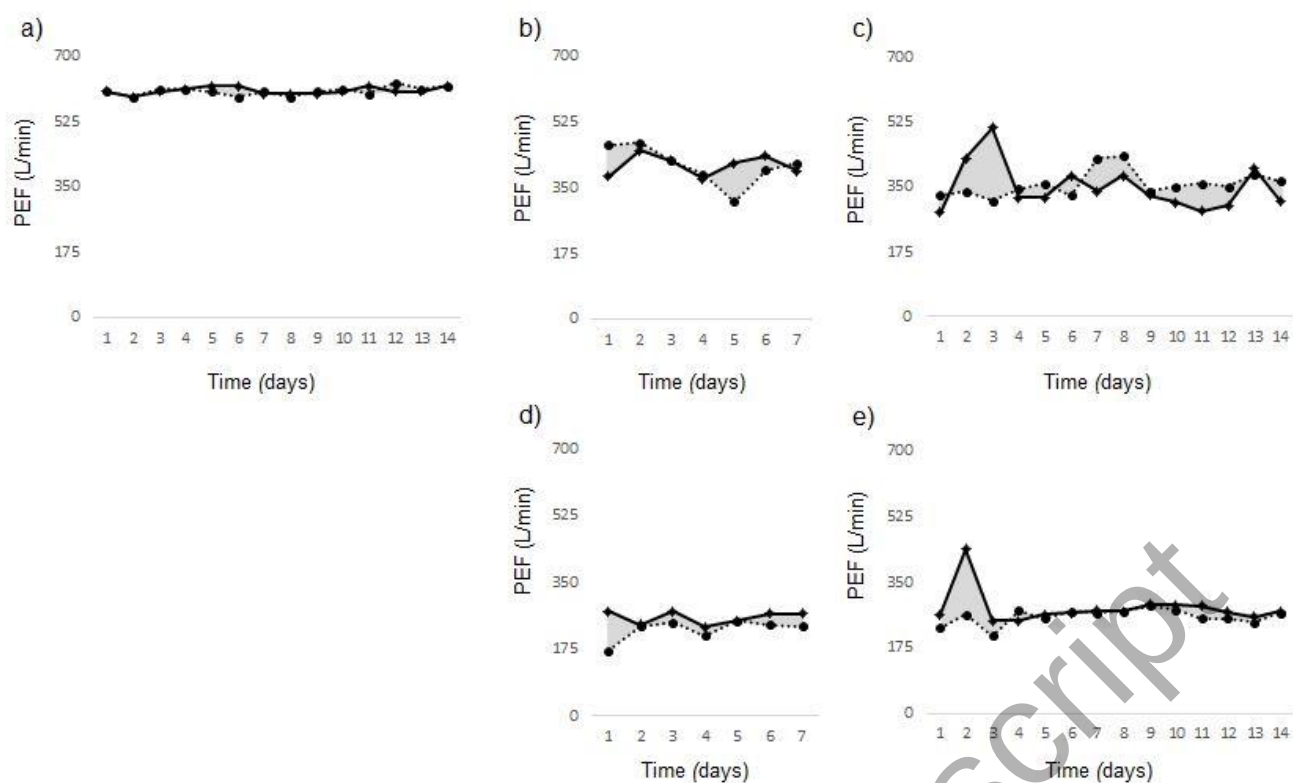


FIGURE 4. Examples of typical individual patterns of peak expiratory flow (PEF) levels in: a) a healthy subject over 2 weeks; b) a good responder over 1 week before intensive treatment; c) a good responder over 2 weeks after intensive treatment; d) a poor responder over 1 week before intensive treatment; and e) a poor responder over 2 weeks after intensive treatment. Morning PEF levels are connected with dotted lines, and evening PEF levels with solid lines.

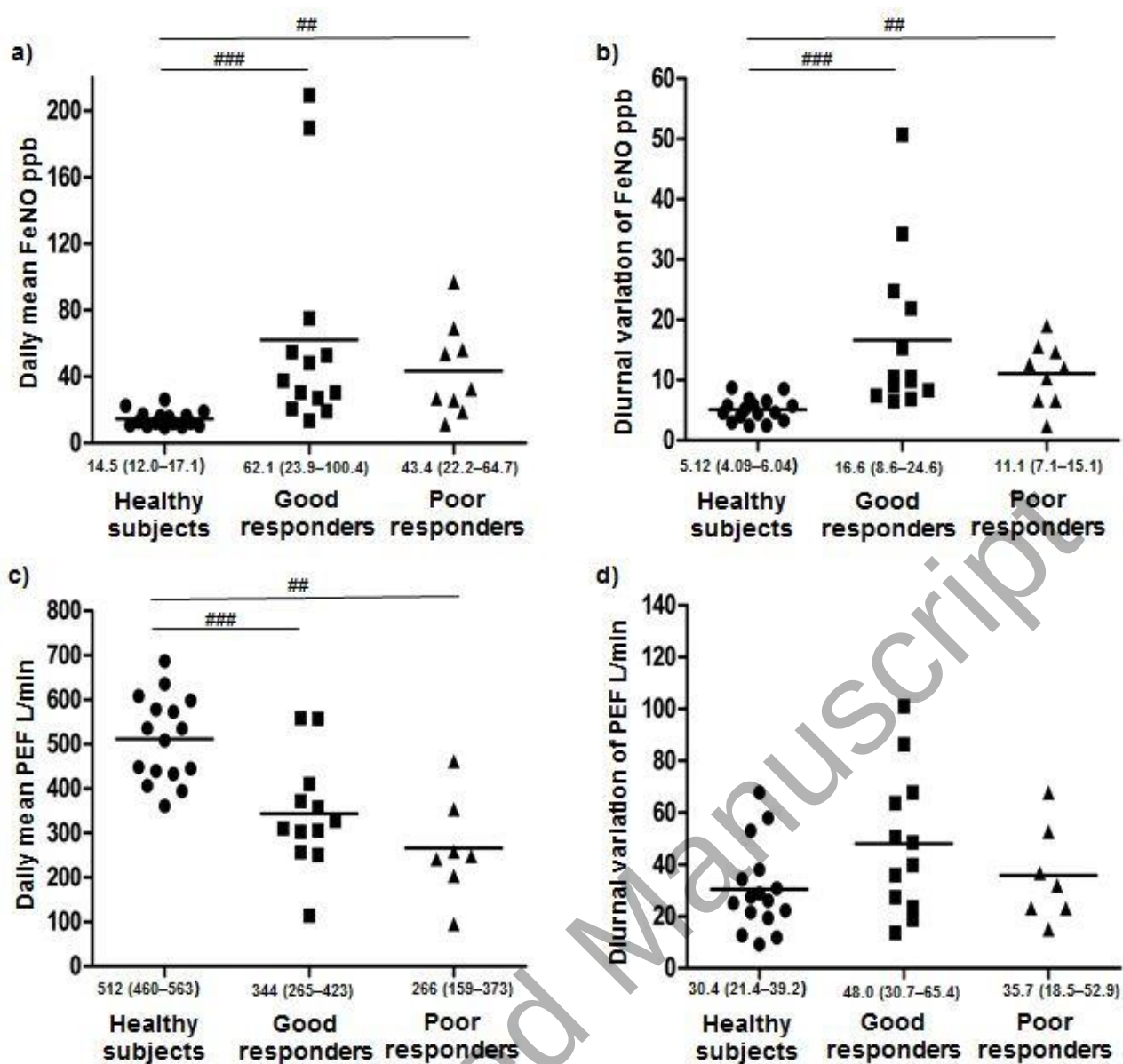


FIGURE 5. Daily mean a) fractional exhaled nitric oxide (FeNO) and c) peak expiratory flow (PEF) levels, and diurnal variations of b) FeNO and d) PEF levels in healthy subjects, good responders, and poor responders before intensive treatment. Horizontal bars represent mean. #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$.

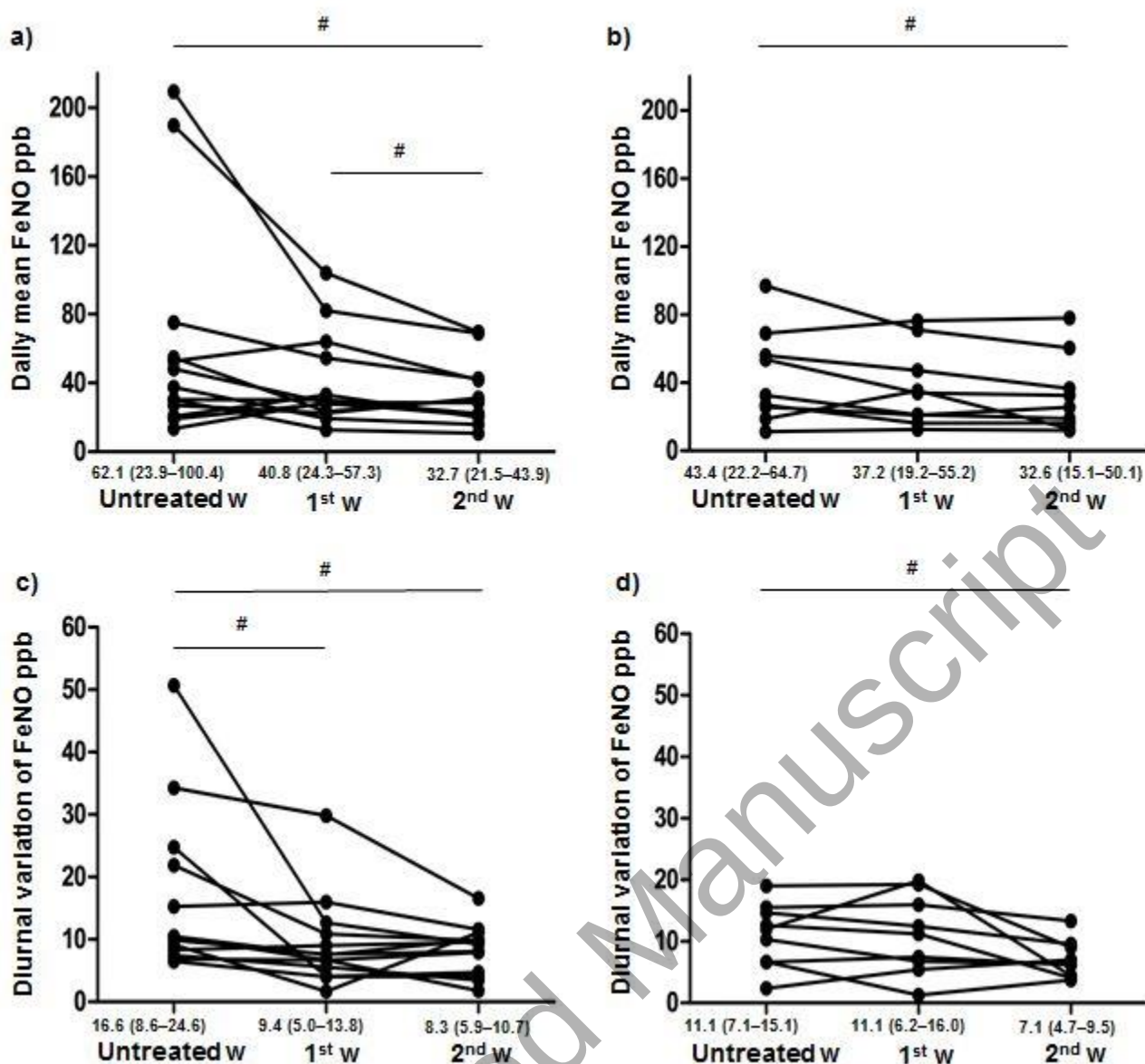


FIGURE 6. Daily mean fractional exhaled nitric oxide (FeNO) levels in a) good responders and b) poor responders, and diurnal variations of FeNO levels in c) good responders and d) poor responders before intensive treatment, in the 1st week and 2nd week of intensive treatment. Untreated w, 1 week before intensive treatment; 1st w, 1st week of intensive treatment; 2nd w, 2nd week of intensive treatment. #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$.

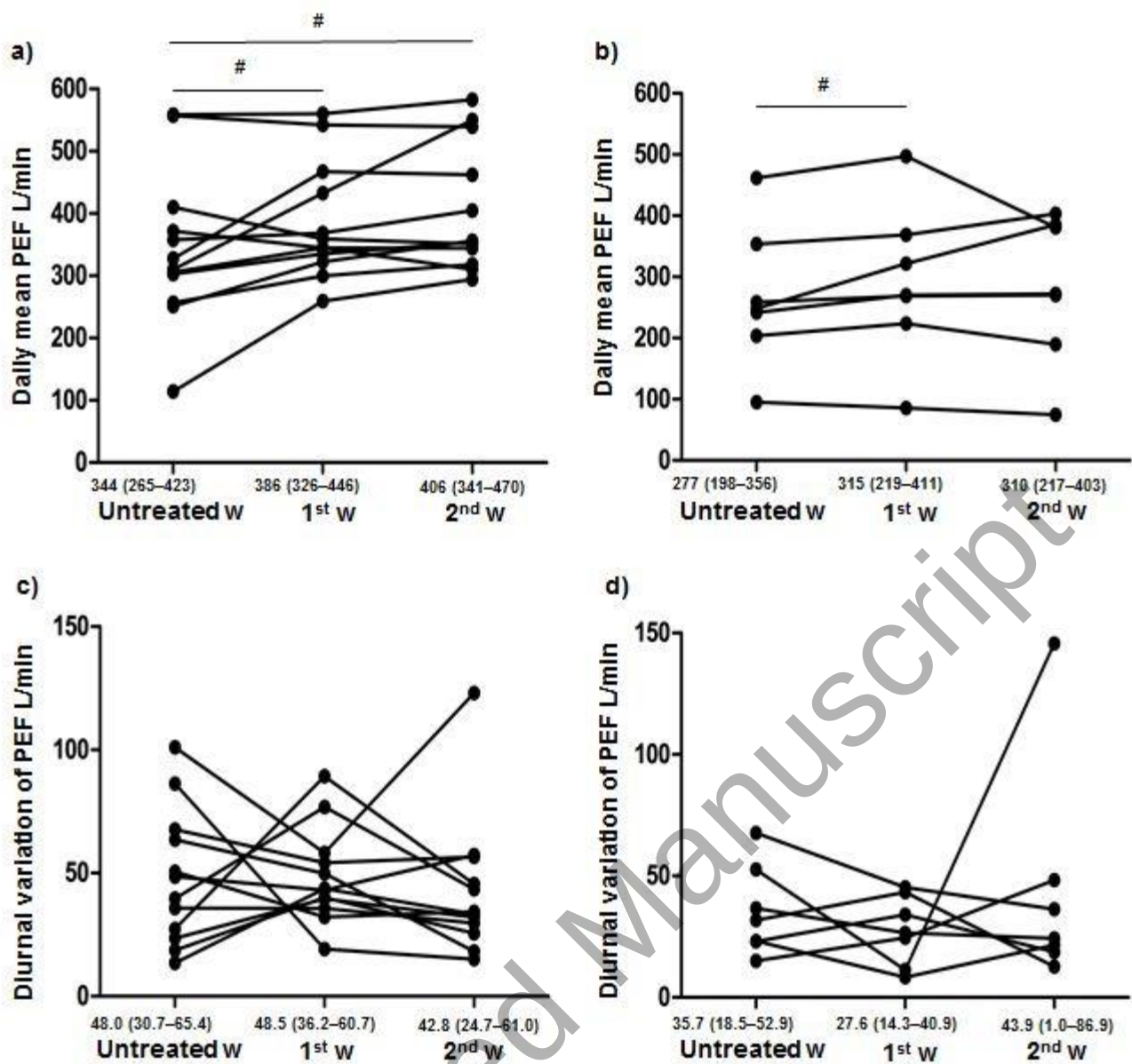


FIGURE 7. Daily mean peak expiratory flow (PEF) levels in a) good responders and b) poor responders, and diurnal variations of PEF levels in c) good responders and d) poor responders before intensive treatment, in the 1st week and 2nd week of intensive treatment. Untreated w, 1 week before intensive treatment; 1st w, 1st week of intensive treatment; 2nd w, 2nd week of intensive treatment. #: $p < 0.05$; ##: $p < 0.01$; ###: $p < 0.001$.

TABLE 1. Evaluation indexes of fractional exhaled nitric oxide (FeNO) and peak expiratory flow (PEF) for analysis.

Index name	Formula
FeNO	
Daily mean FeNO	$[\text{Day 1}(\text{Morning} + \text{Evening})/2 + \text{Day 2}(\text{Morning} + \text{Evening})/2 + \dots + \text{Day n}(\text{Morning} + \text{Evening})] / n$
Diurnal variation of FeNO	$[\text{Day 1} \text{Morning} - \text{Evening} + \text{Day 2} \text{Morning} - \text{Evening} + \dots + \text{Day n} \text{Morning} - \text{Evening}] / n$
PEF	
Daily mean PEF	$[\text{Day 1}(\text{Morning} + \text{Evening})/2 + \text{Day 2}(\text{Morning} + \text{Evening})/2 + \dots + \text{Day n}(\text{Morning} + \text{Evening})] / n$
Diurnal variation of PEF	$[\text{Day 1} \text{Morning} - \text{Evening} + \text{Day 2} \text{Morning} - \text{Evening} + \dots + \text{Day n} \text{Morning} - \text{Evening}] / n$

TABLE 2. Subject characteristics.

	Healthy subjects	Good responders	Poor responders
Subjects n	16	13	9
Age, years	36.3 [31.5–41.1]	61.6 [54.4–68.9] ***	72.2 [66.3–78.1] ***,#
Male / female n/n	9 / 7	7 / 5	7 / 3
BMI kg/m²	22.3 [20.8–23.8]	23.3 [21.7–24.9]	25.5 [23.0–28.0] *
Control (partly / poorly)	NA	10 / 3	4 / 5
Total IgE	NA	231.8 [28.7–435.0]	360.3 [-44.1–764.8]
Atopy %	NA	61.5	55.6
Allergic rhinitis %	NA	33.8	33.3
FVC L	3.95 [3.53–4.36]	3.25 [2.69–3.82] *	2.68 [2.09–3.28] **,#
FVC % predicted	109.5 [104.4–114.6]	103.7 [95.7–111.7]	86.0 [70.9–101.1] **,#
FEV₁ L	3.25 [2.93–3.57]	2.42 [2.00–2.85] **	1.70 [1.39–2.01] ***,#
FEV₁ % predicted	101.0 [95.6–106.3]	94.1 [86.0–102.3]	69.8 [57.3–82.3] ***,##
FEV₁/FVC %	82.8 [79.4–86.2]	75.4 [68.5–82.3] *	65.5 [55.8–75.1] **
FeNO ppb	12.2 [9.1–15.4]	99.1 [31.9–166.3] *	104.2 [48.1–160.2] **
Untreated / treated	NA	7 / 6	4 / 5
Medication taken			
Inhaled corticosteroid	NA	1 [7.7]	3 [33.3]
Long-acting β_2 -agonist	NA	4 [30.8]	4 [44.4]
Leukotriene antagonist	NA	3 [23.1]	2 [22.2]
Theophylline	NA	2 [15.4]	2 [22.2]
Long-acting muscarinic antagonist	NA	0 [0.0]	1 [11.1]
Started ICS μg (FP equivalent)	NA	684.6 [464.2–905.1]	711.1 [506.1–916.1]

Data are presented as mean [95% confidence interval] or n [%], unless otherwise stated. BMI: body mass index; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; FeNO: fractional exhaled nitric oxide; ICS: inhaled corticosteroid; FP: fluticasone propionate; NA: not applicable. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ versus healthy subjects. #: $p < 0.05$; #: $p < 0.01$; ###: $p < 0.001$ versus good responders.

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