Detection of type 2 biomarkers for response in COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is a heterogeneous inflammatory lung disease. It is important to identify patients who would respond to anti-inflammatory treatment. This prospective study aims to determine how inflammatory biomarkers could be used to predict the potential effect of inhaled corticosteroids (ICS) in terms of symptoms and lung function. We evaluated the levels of blood eosinophils, exhaled nitric oxide fraction at a flow rate of 50 ml s⁻¹ (FeNO), alveolar nitric oxide concentration (Calv), immunoglobulin E and atopy in 43 patients with symptomatic COPD and correlated these expression levels with the changes in the COPD Assessment Test (CAT) and lung function by 12 weeks of add-on therapy with ciclesonide 400 μg d⁻¹ on bronchodilators. The mean changes in the CAT score and FEV₁ were −1.4 points and +90 ml, respectively, with significant variation in the levels of change. The area under the receiver’s operating characteristic curve (AUC) for FeNO in predicting improvements in both the CAT score and FEV₁ was 0.92. The AUC for Calv and blood eosinophils was 0.82 and 0.65. Two cutoffs were chosen, one corresponding to a high value of FeNO associated with certainty for response inclusion (FeNO = 35 ppb; sensitivity = 0.67, specificity = 0.94; positive predictive value = 0.80) and the other with certainty for response exclusion (FeNO = 20 ppb; sensitivity = 1.00, specificity = 0.58, negative predictive value = 1.00). Baseline FeNO values were significantly correlated with changes in FEV₁ and CAT (all p < 0.0001). FeNO could be a valuable biomarker for identifying individuals who respond to steroid therapy among patients with symptomatic COPD in terms of symptoms and airflow limitation. The study was prospectively registered with the University Hospital Medical Information Network (UMIN) in Japan (protocol ID 000010711).

Introduction

Chronic obstructive pulmonary disease (COPD) develops from various causes and is a heterogeneous chronic inflammatory disease characterized by persistent respiratory symptoms and airflow limitation. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2019 guidelines documented that ‘COPD-associated inflammation has limited responsiveness to corticosteroids’ [1], and that the role of inhaled corticosteroids (ICS) in COPD remains controversial [2, 3]. In COPD, neutrophilic inflammation, which responds less to ICS compared with eosinophilic inflammation, is predominant [4]. A subgroup of patients with COPD was considered to benefit from ICS, while ICS for COPD patients has been reported to be associated with an increased risk of pneumonia [5, 6] and bone fracture [7]. Therefore, it is important to identify clinical parameters that predict favorable effects from ICS in patients with COPD.

Some COPD patients show gene expression of type 2 inflammation in the airway and this subset was associated with improvement of hyperinflation by ICS treatment [8], which raises the hypothesis that type 2 biomarkers might predict the responsiveness to ICS in COPD. In fact, type 2 inflammation biomarkers such
as sputum and blood eosinophils, exhaled nitric oxide fraction at a flow rate of 50 ml s\(^{-1}\) (FeNO), alveolar nitric oxide concentration (Calv), Immunoglobulin E (IgE) and atopy were reported to have potential in predicting the responsiveness to ICS in COPD\(^{[9–15]}\).

Nonetheless, no well-established biomarker for prescribing ICS in COPD has been identified. This prospective study comprehensively evaluated type 2 biomarkers such as blood eosinophils, FeNO, Calv, IgE and atopy, and how these biomarkers could predict favorable effects of ICS on symptoms and airflow limitation in patients with symptomatic COPD.

**Methods**

**Study design and patients**

Our analysis was based on data collected in the Detection of Biomarkers of Steroid Response in COPD (De-stress) single-arm prospective observational study. This study consisted of a 4 week observation period and 12 week treatment period (figure 1). COPD Assessment Test (CAT) and pulmonary function test were performed at the screening visit. After the observation period, inhaled ciclesonide 400 μg d\(^{-1}\) was administered for 12 weeks in addition to COPD maintenance treatment, and then various parameters were assessed at baseline and 12 weeks after treatment with inhaled ciclesonide. All COPD subjects were recruited from 1 June 2013 to 31 March 2015 at Wakayama Medical University Hospital. Eligibility criteria were as follows: (i) a clinical diagnosis of COPD; patients were 40 years of age or older, had a smoking history of more than 10 pack-years, had a post-bronchodilator forced expiratory volume in 1 s (FEV\(_1\))/forced vital capacity (FVC) ratio <70%, and FEV\(_1\) <80% of predicted; (ii) COPD Assessment Test (CAT) Score ≥10; (iii) they received long-acting muscarinic antagonist (LAMA), long-acting beta-agonist (LABA) or LAMA/LABA for more than 4 weeks by the time of the treatment period and (iv) ability to provide informed consent. Exclusion criteria were as follows: (i) a diagnosis of asthma or a diagnosis of any respiratory disease or condition other than COPD, which in the opinion of the investigator could influence the results; (ii) medication containing ICS (ICS or ICS/LABA) or oral corticosteroids within 4 weeks before screening and during the observation period; (iii) moderate to severe COPD exacerbations (administration of antibiotics and/or systemic steroids or requiring hospitalizations due to COPD) within 4 weeks before screening and during the observation period; (iv) receiving home oxygen therapy; (v) current smokers and (vi) individuals judged unsuitable. Since FeNO and blood eosinophils are affected by ICS, and chronically reduced levels of FeNO have been demonstrated in current smokers\(^{[16, 17]}\), we designed the protocol to exclude confounding factors as much as possible. The study was conducted in accordance with the Ethical Guidelines for Clinical Research of the Japanese Ministry of Health, Labor and Welfare and the Declaration of Helsinki. This study was approved by the ethics committee of Wakayama Medical University (IRB #526) and all patients gave written, informed consent. The study was prospectively registered with the University Hospital Medical Information Network (UMIN) in Japan (protocol ID #000010711).


Study assessments

FeNO and Calv were measured using a chemiluminescence-based exhaled NO analyzer (NA-623 N, Chest Co. and Kimoto Electric Co., Tokyo, Japan) in accordance with the recommendations of current guidelines [17]. Four separate, constant expiratory flow rates (50, 100, 175 and 370 ml s⁻¹) of exhaled NO were measured [18]. FeNO was presented as the exhaled nitric oxide (eNO) at a flow rate of 50 ml s⁻¹. Calv was calculated by a linear regression line method using a minimum of three expiratory flow rate data points [18]. Pulmonary function was measured using a dry-rolling seal spirometer (CHESTAC-8800; Chest Co., Tokyo, Japan). The improvement of airflow limitation was defined as an improvement of FEV₁ ≥ 200 ml. Subjects performed the CAT, which is a validated, simple questionnaire for assessing COPD symptoms [19]. CAT is composed of 8 questions associated with COPD symptoms such as dyspnea, cough, sputum production, fatigue and sleep disorder. A decrease of 2 points in the CAT score has been previously defined as the minimum clinically important difference (MCID) [20]. Blood eosinophil count, total IgE levels and specific IgE for common allergens including Dermatophagoides pteronyssinus, Dermatophagoides farina and house dust mite were examined. A specific IgE positive for at least one allergen was assumed to confirm the presence of atopy.

Statistical analysis

Wilcoxon signed rank test was used to compare paired data for each patient. All P-values were two sides and P-values of 0.05 or less were considered statistically significant. Receiver operating characteristics (ROC) analysis was performed to evaluate candidates for predicting improvement in both FEV₁ ≥ 200 ml and CAT score ≤ −2 points by ciclesonide. An optimal cutoff value was obtained from the highest sum of sensitivity and specificity. Spearman’s rank correlation was used to analyze the correlations between the variables. All analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan; http://jichi.ac.jp/saitama-sc/SaitamaHP.files/statmed EN.html; Kanda, 2012) [21], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0).

Results

A total of 46 patients were enrolled in the present study. Three patients failed to complete the treatment period (2 withdrew consent, 1 moved to another city), and 43 patients completed the study (figure 1). The baseline characteristics of the study subjects are shown in table 1. Most of the subjects were male and belonged to GOLD group B. 72% of the subjects were treated with combination therapy of long-acting bronchodilators (LAMA/LABA), and the others received monotherapy with LABA or LAMA. Only two patients were asthma–COPD overlap (ACO) according to the criteria of Global Initiative for Asthma (GINA)/Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations for ACO diagnosis [22].

Table 1. Baseline characteristics of the study subjects.

| Characteristic                  | n   | Mean ± SD |
|--------------------------------|-----|-----------|
| Gender (female/male), n         | 43  | 27/16     |
| Age (years)                     | 71.3| ± 6.1     |
| Body mass index (kg m⁻²)        | 22.2| ± 3.1     |
| Pack years (years)              | 49.4| ± 11.9    |
| GOLD stage (1/2/3/4), n         | 0/34| 9/0       |
| GOLD group (A/B/C/D), n         | 0/39| 0/4       |
| CAT (points)                    | 5.72| ± 3.9     |
| mMRC scale (0/1/2/3/4), n       | 0/5/26| 10/2     |
| Exacerbation history in a previous year, n (%) | 13(30.2) |
| Asthma-COPD overlap, n (%)      | 2(4.7) |

Table 2 shows changes in the CAT score, lung function and biomarkers in the subjects after add-on therapy of inhaled ciclesonide. The mean changes in the CAT score and FEV₁ were −1.4 points and +90 ml, respectively, with significant variation in the levels of change. FeNO and Calv significantly decreased after 12 weeks of inhaled ciclesonide, while the blood eosinophil count and percentage did not.

The area under the curve (AUC) for each candidate biomarker to predict improvements of FEV₁ ≥ 200 ml and CAT score ≤ −2 is shown in table 3. The AUC for FeNO was the highest (0.917) among the candidate biomarkers. The AUC for Calv, blood eosinophil count and IgE was 0.816, 0.648 and 0.726, respectively. Figure 2 shows the ROC curves for FeNO, blood eosinophil count and IgE.
**Table 2.** Outcomes before and after treatment with inhaled corticosteroid in 43 patients with COPD.

|                  | Before | After | Difference (range) | p-value |
|------------------|--------|-------|--------------------|---------|
| FVC (l)          | 3.32 ± 0.61 | 3.38 ± 0.61 | 0.06 (−0.29 to 0.51) | 0.046   |
| FEV₁ (l)         | 1.80 ± 0.40 | 1.89 ± 0.46 | 0.09 (−0.16 to 0.46) | <0.001  |
| CAT score (points) | 15.7 ± 3.9 | 14.3 ± 4.8 | −1.4 (−8 to 5) | 0.002   |
| Biomarkers       |        |       |                    |         |
| FeNO (ppb)       | 26.3 ± 14.5 | 17.9 ± 7.0 | −8.5 (−38 to 6) | <0.001  |
| Calv (ppb)       | 6.8 ± 3.0  | 5.0 ± 1.9 | −1.8 (−9.7 to 2.7) | <0.001  |
| Blood eosinophil count (cells μl⁻¹) | 270 ± 168 | 264 ± 168 | −6 (−309 to 541) | 0.740   |
| Blood eosinophil percentage (%) | 4.3 ± 2.4 | 4.3 ± 2.3 | −0.1 (−3.4 to 5.2) | 0.921   |
| IgE (IU ml⁻¹)    | 233 ± 200 | NA    | NA                |         |

**Notes.** Data are presented as mean ± SD. All spirometric data were post-bronchodilator measurements. Abbreviations: FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; CAT, Chronic Obstructive Lung Disease Assessment Test; FeNO, the exhaled nitric oxide fraction; Calv, alveolar nitric oxide concentration; IgE, immunoglobulin E. NA, not available.

Different values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood (LR+) and negative likelihood (LR−) for each cutoff value of FeNO for predicting favorable effects of ICS are listed in table 4. Based on a cutoff value of 35 ppb, which was the maximization of the sum of sensitivity and specificity, the sensitivity was 66.7% and the specificity was 93.5%. With a cutoff value of 20 ppb, which was the second largest of the sum of sensitivity and specificity, the sensitivity and specificity were 100.0% and 58.1%, respectively. The scatter diagram of FeNO in subjects with or without improvement in both CAT ≤ −2 and FEV₁ ≥ 200 ml is shown in figure 3. 80% of subjects with FeNO ≥ 35 ppb improved in both CAT ≤ −2 and FEV₁ ≥ 200 ml after treatment with inhaled ciclesonide, while no subjects with FeNO < 20 ppb did. The sensitivity, specificity, PPV, NPV, LR (+) and LR (−) for each cutoff value of the blood eosinophil count for predicting favorable effects of ICS is shown in table 5. A cutoff value of blood eosinophil count ≥ 300 μl⁻¹ showed sensitivity of 0.500 and specificity of 0.613. In addition, the combinations of biomarkers for predicting effects of ICS were assessed. However, no combinations of biomarkers were superior to FeNO alone in predicting the responsiveness of ICS (data not shown).
Baseline FeNO values were significantly correlated with changes in FEV₁ (\(\rho = 0.835, p < 0.0001\)) and CAT (\(\rho = -0.672, p < 0.0001\)) after treatment with inhaled ciclesonide (figures 4(A) and (B)). Changes in FeNO were significantly correlated with changes in FEV₁ (\(\rho = -0.780, p < 0.0001\)) and CAT (\(\rho = 0.556, p < 0.001\)) after treatment with inhaled ciclesonide (figures 4(C) and (D)).
Discussion

We have shown that FeNO could be a useful biomarker for predicting short-term improvements in symptoms and airflow limitation with ICS treatment in patients with symptomatic COPD. We propose two cutoff values for FeNO: 35 ppb is associated with certainty for response inclusion, and 20 ppb is associated with certainty for response exclusion.

In the present study, the changes in the CAT score and FEV$_1$ after ICS treatment were highly scattered, indicating the heterogeneity of COPD in terms of ICS responsiveness. Among the several candidate type2 inflammatory biomarkers, FeNO was identified as the most accurate predictor for benefits from ICS. Furthermore, the baseline FeNO values were significantly correlated with improvements in FEV$_1$ and CAT after treatment with ICS, supporting the usefulness of FeNO as a predictor of ICS responsiveness. FeNO previously predicted the improvement of FEV$_1$ with systemic corticosteroids in COPD [12]. Bronchial NO predicted improvement in symptoms and airflow limitation with ICS treatment [13]. Our results were consistent with those of previous studies. Although Calv was a good predictor as FeNO for the effectiveness of ICS, Calv is rarely available in general healthcare facilities. In contrast, FeNO is a simple, non-invasive method for assessing airway type 2 inflammation. Blood eosinophils, IgE and atopy were less likely to predict the efficacy of ICS compared to FeNO. Nonetheless, our results do not deny the potential of these blood biomarkers for predicting the effects of ICS in COPD. In fact, more than 60% of subjects with blood eosinophil counts \(\geq 300\) \(\mu\)l$^{-1}$ showed favorable effects from ICS in the present study. Previously, these blood biomarkers predicted the favorable effects of corticosteroids in COPD [14, 15]. Although Colak et al showed that the combination of blood eosinophil count and FeNO was useful in differentiating asthma from COPD [23], no combinations of the case that biomarkers were superior to FeNO alone in predicting the efficacy of ICS in our study. This might be due to the exclusion of current smokers. The FeNO levels in current smokers are reported to be lower than in

Figure 4. Correlations between baseline FeNO and changes in FEV$_1$ (A) and CAT (B) after treatment, and correlations between changes in FeNO and FEV$_1$ (C) and CAT (D) after treatment. Abbreviations: FeNO, the exhaled nitric oxide fraction; FEV$_1$, forced expiratory volume in 1 s; CAT, Chronic obstructive lung disease Assessment Test.
nonsmoking patients, probably through the reduced production and increased consumption of NO [16, 17]. Therefore, the usefulness of the combination of type 2 biomarkers in predicting the efficacy of ICS remains controversial.

In using FeNO to predict the ICS responsiveness, we proposed two cutoff values of FeNO; 35 ppb was an optimal cutoff value to include the response with near-certainty, and 20 ppb was an optimal cutoff value to exclude the response with near-certainty. The cutoff value of 35 ppb, which is currently recommended as a feature of asthma in the definition of ACO by the Japanese Respiratory Society (JRS) guidelines [24], showed the maximization of the sum of sensitivity (66.7%) and specificity (93.5%) in our study. This value was an optimal cutoff value to include the response with near-certainty. However, one-third of the COPD patients with FeNO < 35 ppb lose the opportunity to benefit from ICS. A cutoff value of 20 ppb, which was the second largest of the sum of sensitivity (100.0%) and specificity (58.1%) was an optimal cutoff value to exclude the response with near-certainty. In a previous report, the cutoff value of FeNO that predicted improvement of FEV1 by ICS was 26.8 ppb [25], and another study showed that the median FeNO in COPD patients with type 2 inflammation was 28 ppb [26], which were within the range from 20 to 35 ppb. The cutoff value of 50 ppb, which is currently recommended to be used to indicate that eosinophilic inflammation and responsiveness to corticosteroids are likely in the ATS guidelines [17], showed inadequate sensitivity (33.3%) to detect patients who respond to ICS in the present subjects. Moreover, our previous study demonstrated that the mean FeNO level of healthy adults was 16.9 ppb [27]. Considering these data, it is controversial whether ICS should be prescribed in patients within the range from 20 to 35 ppb whether. Blood biomarkers such as blood eosinophil count, IgE and atopy might still be strong candidates for predicting the efficacy of ICS, especially when the FeNO levels are within the range from 20 to 35 ppb. Recently, the breath volatile area has been attracting attention for the diagnosis of COPD and asthma [28, 29]. In the future, combining these breath and serum biomarkers may be useful to develop a clinical decision-making strategy for the appropriate use of ICS in COPD.

Importantly, most of subjects in our study belonged to GOLD B and ACO patients were very few. Our previous report showed that most COPD patients in Japan belonged to GOLD A or B, who are less likely to experience exacerbations but continue to have daily symptoms [30]. Generally, ICS is not recommended for GOLD B without the complication of asthma [1]. Nonetheless, in the present study, most of the GOLD B patients with elevated FeNO were associated with favorable effects from ICS, indicating that ICS might be beneficial in some of COPD patients irrespective of the exacerbation history. Furthermore, the definition of ACO is still ambiguous and ACO cannot be clinically separated from COPD [26]. Importantly, changes in FeNO were significantly correlated with changes in FEV1 and CAT after treatment with ICS, indicating that ICS might improve the symptoms and pulmonary function through suppression of type 2 airway inflammation in COPD. Prescribing ICS based on the presence of type 2 airway inflammation might be effective for improving both the symptoms and airflow obstructions in COPD regardless of the exacerbation history or clinically diagnosed asthma. This speculation is in agreement with the KRONOS trial that demonstrated the efficacy of triple therapy with budesonide/glycopyrrolate/formoterol fumarate therapy in patients with symptomatic COPD, who were included irrespective of their exacerbation history and lacked a current diagnosis of asthma [31].

There were several limitations to this study. Firstly, the number of study subjects was small, the participants were limited to ex-smokers alone, and our study was a single-arm study without a control group, and lacked a validation cohort. The usefulness of FeNO for many patients including current smokers and the determination of the appropriate cutoff value remain to be evaluated. Secondly, the evaluation of the effectiveness of ICS was limited in terms of symptoms and airflow limitation. Although in our study, none of the patients had an exacerbation during the treatment period, it is unclear whether ICS treatment guided by FeNO and Calv predicts the prevention of exacerbations. Interestingly, the exacerbation frequency in patients treated with ICS was lower in those who responded to short-term treatment with prednisolone than in non-responders [32]. Thus, it is possible that the presence of a high level of FeNO and Calv may also predict long-term benefits, including a reduction of exacerbations.

**Conclusion**

In summary, we performed a prospective study to detect biomarkers for predicting the short-term improvements in both QOL and airflow limitation by ICS in symptomatic COPD patients. FeNO could be a useful biomarker for prescribing ICS, and we propose two cutoff values of FeNO: 35 ppb is associated with certainty for response inclusion and 20 ppb is associated with certainty for response exclusion. However, further studies are needed to confirm the usefulness of FeNO for many patients including current smokers, and determine the appropriate cut-off value. Moreover, future prospective studies should be focused on detecting biomarkers for predicting the long-term prevention of exacerbations.

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