Research Article

Short-term responses to high-dose inhaled corticosteroid treatment in patients with chronic obstructive pulmonary disease with a fractional nitric oxide concentration over 35 parts per billion: A single-centre pre–post study

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Abstract

Introduction: There is currently no strategy for identifying chronic obstructive pulmonary disease (COPD) patients whose pulmonary function could benefit from inhaled corticosteroids. We investigated whether a 28-day regime of inhaled corticosteroids improved pulmonary function test results among COPD patients with a fractional exhaled nitric oxide concentration > 35 parts per billion.

Methods: This single-centre one-arm pre–post trial included COPD patients with a fractional exhaled nitric oxide concentration > 35 parts per billion treated at our institution from September 2018 to August 2019. Patients were administered budesonide (200 μg, 8 puffs daily) for 28 days. The primary outcome measure was the difference between the forced expiratory volume in 1 s (FEV1) at baseline and after 28 days of inhaled corticosteroid treatment. Secondary outcomes included differences in COPD Assessment Test scores, %FVC, and that between the percent forced vital capacity (%FVC) at baseline and after 28 days of treatment.

Results: Twenty patients completed the 28-day inhaled corticosteroid regime. The mean difference in FEV1 between day 1 and day 28 was 340 mL (95% confidence interval: −100 to 770 mL; p = 0.122). The mean differences in secondary outcomes were: %FVC, −0.16% (95% confidence interval [CI]: −2.84 to 2.53%; p = 0.905); %FEV1, 1.63% (95%CI: −4.56 to 7.81%; p = 0.589); COPD Assessment Test score, −2.50 (95%CI: −5.72 to 0.72; p = 0.121).

Conclusion: The 28-day course of inhaled corticosteroids yielded no significant difference in FEV1 for COPD patients with a fractional exhaled nitric oxide concentration > 35 parts per billion.

Trial registration: University Hospital Medical Information Network Center, UMIN000034005. Registered 3 September 2018, https://upload.umin.ac.jp/cgi-open-bin/ctrctr_view.cgi?recptno=R000038557

Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory disease caused by airflow limitation associate with a chronic inflammation [1]. According to the Global Initiative for Asthma statement, among patients with COPD, a sub-group of patients exhibit symptoms of a chronic airways disease that has features of both asthma and COPD, defined as asthma and COPD overlap (ACO) [2]. A previous meta-analysis reported a 27% – 28% ACO prevalence rate in
patients with COPD [3]. Compared to COPD patients, those with ACO were characterized by higher exacerbation rates and a poorer quality of life [4]. Although inhaled corticosteroids (ICSs) are assumed to improve the pulmonary function of ACO patients, the ACO diagnostic criteria are not yet clearly established [5,6].

Measuring fractional exhaled nitric oxide (FeNO) concentration is a non-invasive test related to sputum eosinophilia in patients with COPD [7]. According to the American Thoracic Society guidelines, an FeNO concentration > 50 parts per billion (ppb) is the recommended cut-off for diagnosing eosinophilic inflammation and steroid responsiveness for respiratory diseases [8]. However, in the Japanese Respiratory Society Guidelines for the Management of ACO, the recommended FeNO cut-offs > 35 ppb [9]. Neither of these FeNO cut-off values has been validated.

This study aimed to assess the efficacy of a 28-day course of ICS monotherapy to improve the pulmonary function test results, including forced expiratory volume in 1 s (FEV1), among COPD patients with FeNO concentrations > 35 ppb, the recommended cut-off according to the Japanese Respiratory Society Guidelines [8]. This study evaluated whether the cut-off of 35 ppb could be reasonable in the clinical practice.

Materials and methods
Study design and settings

This was a single-centre, pre–post, single-arm study conducted at the Department of Pulmonology in Kameda Medical Center, a tertiary care general hospital in a rural area of Japan. The department has 11 pulmonologists and cares for 500 patients with COPD annually. In 2017, the department began evaluating FeNO concentrations in patients with COPD with NIOX VERO® (Circassia Pharmaceuticals Inc., Morrisville, NC, USA) to detect eosinophilic inflammation. This study was conducted according to the Declaration of Helsinki and was approved by the Institutional Review Board of Kameda Medical Center (registration number: 18-053-181121). Before enrolling the first patient, this trial was registered with the University Hospital Medical Information Network Center (UMIN000034005). Version 1 of the study protocol was amended to correct a minor mistake; in this amendment, the secondary outcome was changed from “change of %VC” to “change of % forced vital capacity (FVC)”. The modified protocol was submitted to the institutional review board and the information was updated on the University Hospital Medical Information Network. All patients provided written informed consent before enrolment.

Participants

Stable patients with COPD, aged ≥ 40 years, who were not receiving ICS and had FeNO concentrations > 35 ppb at the time of visiting the Respiratory Department Clinic of the study hospital, were recruited for the study from 1 September 2018 to 31 August 2019. COPD diagnoses were made according to the following criteria: current or ex-smokers (≥ 10 pack-years) and post-bronchodilator FEV1 / FVC ratio < 0.7. Exclusion criteria included patients who were receiving oral corticosteroids or ICS within 1 month of inclusion in the trial, patients who were allergic to ICS, unstable COPD patients who experienced exacerbation within 4 weeks of trial enrolment, or those who exhibited severe intercurrent diseases or any other causes of airflow limitation, including asthma, or an untreated infection of the respiratory tract, clinically diagnosed by a physician. Patients who enrolled in other studies were also excluded.

Baseline variables

On the day of enrolment, the following baseline characteristics of study participants were recorded as part of routine assessment: age, sex, smoking status, medical history, medication for COPD, and the FeNO value. FeNO concentration measurements were performed using the NIVOX VERO® (Circassia Pharmaceuticals Inc., Morrisville, NC, USA) according to the recommendations of the American Thoracic Society and European Respiratory Society [10]. In addition, the following test results were recorded 2 weeks or less before enrolment: peripheral blood eosinophil count, total immunoglobulin E (IgE), sputum eosinophil count (low, < 1%; 1% ~ 5%, moderate; > 5%, high), and the post-bronchodilator reversibility of FEV1. Because of the small sample size in this trial, we did not examine specific IgE.

Treatment

Budesonide (Pulmicort Turbuhaler® , AstraZeneca, Cambridge, UK), a dry powder for oral inhalation (200 μg per dose, 200 metered doses), was used for ICS treatment. It was stored at room temperature (15–30°C) in the pharmacy at the Kameda Medical Center. After enrolment, the patients were instructed to revisit the outpatient clinic twice. During the first visit, the COPD Assessment Test (CAT) questionnaire was administered by a medical staff member, followed by an examination by a physician. A pulmonary function test was performed after bronchodilatation to obtain the “visit 1 value”. After completing the pulmonary function test, patients received budesonide (200 μg, 8 puffs daily for 28 days). The duration of ICS was based on the previous trials about ICS on patients with high sputum eosinophil counts [11-14]. Instructions for using budesonide were provided by both a doctor and a pharmacist. An asthma diary (produced by the Japan Allergy & Asthma Network Station) was provided to participants to ensure compliance with the ICS treatment regimen. Compliance and symptoms, including adverse events, were assessed using these daily diaries during the trial. ICS was discontinued at the participant’s request or due to a physician’s clinical judgment. During the trial, concomitant use of oral steroids or other types of ICS was prohibited.

At the second visit, 28 days after enrolment, and before
the consultation with a physician, the CAT questionnaire was again administered by a medical staff member. Subsequently, physicians evaluated the patients and ICS use was discontinued. Finally, after bronchodilation, a pulmonary function test was performed for each patient to assess improvement of FEV1 from the baseline; this also marked the completion of the trial.

Outcomes

Pulmonary function tests were performed with CHESTAC8800® (Chest M.I., Inc., Tokyo, Japan) according to recommendations of the American Thoracic Society [15]. FEV1, after bronchodilation was assessed after inhalation of a short-acting beta-adrenergic receptor antagonist, salbutamol (2 puffs, 100 μg/puff).

The primary outcome was defined as the difference between the FEV1 values after bronchodilation between visits 1 and 2. The secondary outcomes were changes in the CAT score, %FEV1, and %FVC between visits 1 and 2. Pulmonologists checked on the participant at least twice during the trial to assess the development of any adverse events. In addition, in case of any adverse effects among the trial participants, the on-duty pulmonologists were telephoned by the participant and the problem was addressed. During the trial, all adverse events were recorded on an electronic chart. Attending doctors managed the adverse events, and protocol treatment discontinuation was decided based on the clinical judgment of each doctor.

Data management and statistical analysis

Data were collected by a pulmonologist at Kameda Medical Center using the Research Electronic Data Capture [16] web-based application on visits 1 and 2. A list of personal identification numbers was kept in a depository at the clinic of the Kameda Medical Center.

This study had more than 80% statistical power at the 5% level to detect a 200-mL difference in FEV1 before and after the trial, assuming a standard deviation of 300 mL [11]. Considering a 10% dropout rate, a sample size of 23 patients was calculated to be sufficient to obtain statistically significant results. An examination of FeNO concentration was recommended for all COPD patients to achieve adequate and significant results. An examination of FeNO concentration was recommended for all COPD patients to achieve adequate and significant results. An examination of FeNO concentration was recommended for all COPD patients to achieve adequate and significant results.

All data were analysed using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria) [17]. The statistical analysis was performed by two physicians independently and double checked (Akihiro S and Atsushi S). A paired t-test was used to assess improvements in the primary and secondary outcome variables. Data from censored patients were not included in statistical analyses. Post-hoc subgroup analyses were performed in COPD patients with FeNO concentrations > 50 ppb, as described in the American Thoracic Society guidelines, in those with blood eosinophils > 300 × 10⁴/μL, and in those with non-specific IgE > 300 IU/mL [9]. All comparisons were two-tailed, and a p-value of < 0.05 was considered statistically significant.

Results

Twenty patients with COPD who had FeNO concentrations > 35 ppb were recruited and ICS therapy was initiated between September 2018 and May 2019. The recruitment ended after an adequate sample size of 20 was attained, without any dropouts in August 2019. No participant withdrew from the trial. The patient characteristics are summarized in Table 1. Nineteen of the 20 included patients were male (19; 95%). The mean age of the patients was 72 years (interquartile range, 68–75 years). The number of patients categorized by COPD stage, based on %FEV1 values, were as follows: Stage 1, 14 patients; Stage 2, 2 patients; Stage 3, 2 patients; and Stage 4, 2 patients. None of the patients had a medical history of asthma, atopic dermatitis, or allergic rhinitis. Although sputum induction was conducted, good quality sputum could not be collected in 50% of the patients. Only 1 patient demonstrated bronchodilator reversibility. Although all patients were provided with an asthma diary to promote compliance, half of the patients had missing data in the diary, either due to losing the diary or because they did not make daily entries. During the protocol, 5 patients experienced adverse events: 1 patient had mild nausea and 4 patients had mild hoarseness. The ICS dose was tapered from 8 times/day to 4 times/day in these patients.

The mean FEV1 difference between the day of ICS initiation and after 28 days of ICS treatment was 340 mL (95% confidence interval [CI]: −100 to 770 mL; p = 0.122; Figure 1). The mean differences in the secondary outcomes were as follows: %FVC, −16.0% (95% CI: −2.84 to 2.53%; p = 0.905); %FEV1, 1.63% (95% CI: −4.56 to 7.81%; p = 0.589); and CAT score, −2.50 (95% CI: −5.72 to 0.72; p = 0.121) (Figure 2).

Post-hoc subgroup analyses revealed that, in COPD patients with FeNO concentrations > 50 ppb, the mean FEV1 difference was 160 mL (95% CI: −100 to 1320; p = 0.750). In COPD patients with blood eosinophil concentrations > 300 × 10⁴/μL, the mean FEV1 difference was 250 mL (95% CI: −550 to 1050; p = 0.469). In COPD patients with IgE concentrations > 300 IU/mL, the mean FEV1 difference was 590 mL (95% CI: −320 to 1490; p = 0.169).

![Figure 1: CONSORT flowchart. We included 20 patients without any drop-out patients.](https://www.heighpubs.org/haard)
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Table 1: Patient characteristics.

| Case | Age (years) | Sex | Medical history | Smoking history (pack-years) | FeNO (ppb) | Total IgE (IU/mL) | Blood eosinophil count (10^4/μL) | Sputum eosinophil count | Bronchodilator reversibility | Baseline treatment for COPD |
|------|-------------|-----|-----------------|-----------------------------|------------|------------------|-------------------------------|--------------------------|--------------------------|--------------------------|
| 1    | 75          | Male| Oncocytic carcinoma with unknown primary origin | 52.5          | 74          | 418              | 221                          | NA                       | -                        | None                     |
| 2    | 74          | Male| Lung cancer     | 125           | 55          | 2010             | 138                          | Low                      | -                        | LABA/LAMA                 |
| 3    | 73          | Male| Asbestosis      | 50            | 38          | 281              | 544                          | NA                       | -                        | LABA/LAMA                 |
| 4    | 72          | Male| Obstructive sleep apnoea syndrome | 45          | 86          | 820              | 301                          | Moderate                 | -                        | None                     |
| 5    | 84          | Male| Diabetes mellitus, hypertension, aortic dissection, colon cancer | 96          | 46          | NA               | NA                           | NA                       | -                        | LABA/LAMA                 |
| 6    | 63          | Male| None            | 40            | 152         | 481              | 190                          | NA                       | -                        | None                     |
| 7    | 75          | Male| None            | 36            | 43          | 17               | 302                          | NA                       | -                        | None                     |
| 8    | 76          | Male| Prostate cancer | 53            | 41          | 616              | 120                          | NA                       | -                        | None                     |
| 9    | 78          | Female| Osteoporosis, hypertension, depression | 100         | 36          | NA               | 229                          | NA                       | -                        | None                     |
| 10   | 66          | Male| Colon cancer    | 120           | 37          | 54               | 238                          | NA                       | -                        | None                     |
| 11   | 78          | Male| Lung cancer     | 60            | 59          | 83               | 173                          | Low                      | -                        | LABA/LAMA                 |
| 12   | 71          | Male| None            | 80            | 83          | 53               | 121                          | High                     | -                        | LAMA                     |
| 13   | 74          | Male| None            | 45            | 39          | 228              | 495                          | Low                      | -                        | LABA                     |
| 14   | 68          | Male| Obstructive sleep apnoea syndrome, gastroesophageal reflex | 90          | 62          | 108              | 192                          | Moderate                 | -                        | LAMA                     |
| 15   | 74          | Male| Epilepsy, hyperlipidaemia, benign prostatic hyperplasia, hyperuricemia | 23          | 43          | 32               | 120                          | High                     | -                        | None                     |
| 16   | 72          | Male| Benign prostatic hyperplasia | 53            | 36          | 2022             | 388                          | High                     | -                        | LAMA                     |
| 17   | 64          | Male| None            | 44            | 41          | 48               | 341                          | NA                       | -                        | LAMA                     |
| 18   | 68          | Male| Cataract        | 88            | 46          | 140              | 114                          | High                     | -                        | LAMA                     |
| 19   | 63          | Male| Hyperlipidaemia | 32            | 300         | 573              | 1083                         | High                     | +                        | None                     |
| 20   | 59          | Male| None            | 25            | 37          | <1               | 45.1                         | NA                       | -                        | None                     |

FeNO, fractional nitric oxide concentration; ICS, inhaled corticosteroid; FEV1, percent predicted forced expiratory volume in 1 s; IgE, immunoglobulin E; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; NA, not available; COPD, chronic obstructive pulmonary disorder.

Figure 2: Predicted FEV1 pre- and post-therapy (1 month) with an inhaled corticosteroid. FEV1, forced expiratory volume in 1 s.

Our study revealed heterogeneous effects of inhaled corticosteroid on patients with chronic obstructive pulmonary disease with a fractional nitric oxide concentration over 35 parts per billion.

Discussion

This pre–post trial aimed to assess whether a 28-day ICS course changed the pulmonary function test results and the quality of life in COPD patients with FeNO concentrations > 35 ppb. No significant differences were noted in FEV1 values after a 28-day ICS course in these patients. Similarly, the present study did not reveal any changes in secondary outcomes, including FVC, %FEV1, or CAT scores.

Notably, 16.3% of COPD patients had FeNO levels > 35 ppb [18]. High FeNO levels have been associated with high sputum eosinophil counts and improved responses to bronchodilators, such as short-acting beta-adrenergic agonists, in COPD patients [19]. In a previous prospective trial, fluticasone/salmeterol improved FEV1 by more than 200 mL in 4 of 7 (57.1%) COPD patients with FeNO concentrations > 35 ppb [20]. Since the type of treatment, i.e., ICS or long-acting beta-adrenergic agonists, associated with FEV1 improvements could not be determined, ICS alone were used. In the current trial, there were no significant differences in FEV1 after bronchodilation in both the entire study population (FeNO > 35 ppb) and FeNO > 50 ppb groups. According to these results, the standard deviation of 435 mL was greater than our hypothesized 200 mL, and a larger sample size or targeted patients based on demographic features such as FEV1 and severity is needed to assess improvements in pulmonary function test results after ICS administration in COPD patients with FeNO concentrations > 35 ppb. We could not make a conclusion of our hypothesis. Nonetheless, the present results could be utilized to calculate the sample size in future clinical trials.

Previous studies have investigated the usefulness of biomarkers, including blood eosinophil and sputum eosinophil...
counts, to detect ICS responsiveness in COPD patients. Regarding sputum eosinophil counts, previous studies used the post-bronchodilator FEV₁ as the primary outcome, and the use of oral steroids and ICS yielded improved post-bronchodilator FEV₁ values after approximately 1 month of treatment [11-14]. However, in routine clinical settings, it is often difficult to collect good quality lower respiratory tract sputum specimens to calculate eosinophil counts in most COPD patients [21]. In this pre–post trial, saline induction was used to attempt sputum collection, as described previously; however, sputum collection was only successful in 10 patients, yielding inconclusive findings [22]. Additionally, although the blood eosinophil count is correlated with sputum eosinophil counts [12], recent clinical studies indicated that the usefulness of blood eosinophils as a biomarker for ICS responsiveness remains controversial [23, 24]. The present subgroup analysis results among patients with blood eosinophils > 300 × 10⁴/μL did not reveal any significant FEV₁ improvements.

In previous studies, the total IgE level was high among ACO patients, and similar to FeNO, this measure was considered as a biomarker for detecting eosinophilic inflammation in COPD patients [18,25]. However, these studies included patients with a history of asthma, atopic dermatitis, and allergic rhinitis that could have increased the total IgE level, regardless of COPD [26]. In this trial, an IgE cut-off value of 300 IU/mL was used in accordance with the reference range of our medical laboratory, which was higher than that used previously [18]. Patients with known confounders, such as asthma, atopic dermatitis, or allergic rhinitis, were not included. Our post-hoc subgroup analysis did not reveal any significant differences in FEV₁ after 28 days of ICS use in patients with total IgE levels > 300 IU/mL. Only 1 of the 7 patients demonstrated improvements in FEV₁ with ICS use. Therefore, currently, ICS administration does not conclusively improve FEV₁ in COPD patients with a high total IgE level.

This trial had several limitations. First, it targeted only a small proportion of the Japanese population, without any control participants, in a single centre. Clinical studies with different ethnicities and control groups are warranted to analyse precise pharmacological responses. Second, only the short-term effects of ICS were investigated. Changes in FEV₁ have typically been used as a surrogate endpoint in COPD patients; however, this strategy may only assess a single aspect of COPD [27]. Therefore, further investigations should determine whether an FEV₁ improvement is a valid surrogate marker for long-term effects, including hospitalization and mortality, in ACO patients. Third, patient compliance with ICS use could not be accurately assessed. Asthma diaries were distributed to all patients, but some patients did not use them. The patients were called during the trial and they confirmed their compliance, but half of the included patients did not follow the full protocol. Therefore, compliance-assurance measures will be needed in future studies.

Conclusion

This study investigated whether a 28-day ICS regime improved pulmonary function test results among COPD patients with FeNO concentrations >35 ppb. No significant improvements in FEV₁ were demonstrated after 1 month of high-dose ICS use in these patients. Further trials with a larger population are needed to assess the efficacy of ICS in COPD patients with FeNO >35 ppb.

Declarations

Funding: We obtained funding from the department of pulmonology in Kameda Medical Center.

Conflicts of interest: K.N. and A.M. received speaker's fee from Astra Zeneca. Other authors have no conflicts of interest.

Ethics approval: This study was approved by the institutional review board of Kameda Medical Center (registration number: 18-053-181121).

Consent for publication: Consent for publication was obtained from all subjects.

Availability of data: Data of our study is uploaded at the Dryad online repository, (https://doi.org/10.5061/dryad.v15dv41sc).

Author contributions: All authors contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

References

1. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, et al. International variation in the prevalence of COPD (the GOLD Study): a population-based prevalence study. Lancet. 2007; 370: 741. PubMed: https://pubmed.ncbi.nlm.nih.gov/17765523/

2. Boulet LP, Reddel HK, Bateman E, Pedersen S, FitzGerald JM, et al. The Global Initiative for Asthma (GINA): 25 years later. Eur Respir J. 2019; 29:1900598. PubMed: https://pubmed.ncbi.nlm.nih.gov/31273040/

3. Aleshabanat A, Zafari Z, Albaynany Q, Daifi M, FitzGerald JM. Asthma and COPD Overlap Syndrome (ACOS): A systematic review and meta-analysis. PLoS One. 2015; 10: e0136065. PubMed: https://pubmed.ncbi.nlm.nih.gov/26336076/

4. Hardin M, Cho M, McDonald ML, et al. The clinical and genetic features of COPD-asthma overlap syndrome. Eur Respir J. 2014; 44: 341-350. PubMed: https://pubmed.ncbi.nlm.nih.gov/24876173/

5. Singh D, Agusti A, Anzueto A, Beaty T, Ramsdell J, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. Eur Respir J. 2019; 53: 1900164. PubMed: https://pubmed.ncbi.nlm.nih.gov/24876173/

6. Soler-Cataluña JJ, Novella L, Soler C, Nieto ML, Esteban V, et al. Clinical characteristics and risk of exacerbations associated with different diagnostic criteria of asthma-COPD overlap. Arch Bronconeumol. 2020; 56: 282-290. PubMed: https://pubmed.ncbi.nlm.nih.gov/31784349/

7. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, et al. What
is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J. 2016; 48: 664-673. PubMed: https://pubmed.ncbi.nlm.nih.gov/27338195/  
8. The Japanese Respiratory Society Guidelines for the Management of Asthma and COPD Overlap. 2018.  
9. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011; 184: 602-615. PubMed: https://pubmed.ncbi.nlm.nih.gov/21885636/  
10. American Thoracic Society. European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005; 171: 912-930. PubMed: https://pubmed.ncbi.nlm.nih.gov/15817806/  
11. Leigh R, Pizzichini MM, Morris MM, Maltais F, Hargreave FE, et al. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. Eur Respir J. 2006; 27:964-971. PubMed: https://pubmed.ncbi.nlm.nih.gov/16446316/  
12. Brightling CE, McKenna S, Hargadon B, Birring S, Green R, et al. Sputum eosinophilia and the short-term response to inhaled mometasone in chronic obstructive pulmonary disease. Thorax. 2005; 60: 193-198. PubMed: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1747331/  
13. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, et al. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. Lancet. 2000; 356:1480-1485. PubMed: https://pubmed.ncbi.nlm.nih.gov/11081531/  
14. Pizzichini E, Pizzichini MM, Gibson P, Parameswaran K, Gleich GJ, et al. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. Am J Respir Crit Care Med. 1998; 158: 1511-1517. PubMed: https://pubmed.ncbi.nlm.nih.gov/9817701/  
15. Culver BH, Graham BL, Coates AL, Wanger J, Berry CE, et al. Recommendations for a standardized pulmonary function report. An official American Thoracic Society Technical statement. Am J Respir Crit Care Med. 2017; 196: 1463-1472. PubMed: https://pubmed.ncbi.nlm.nih.gov/29192635/  
16. projectredcap.org [Internet]. Tennessee: Vanderbilt University; c2004. https://projectredcap.org/.  
17. r-project.org. The R Foundation. 2018. https://www.r-project.org/  
18. Tamada T, Sugiiro H, Takahashi T, Matsunaga K, Kimura K, et al. Biomarker-based detection of asthma-COPD overlap syndrome in COPD populations. Int J Chron Obstruct Pulmon Dis. 2015; 10: 2169-2176. PubMed: https://pubmed.ncbi.nlm.nih.gov/26491283/  
19. Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, et al. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2000; 162: 1773-1777. PubMed: https://pubmed.ncbi.nlm.nih.gov/11069811/  
20. Akamatsu K, Matsunaga K, Sugiiro H, Koarai A, Hirano T, et al. Improvement of airflow limitation by fluticasone propionate/salmeterol in chronic obstructive pulmonary disease: What is the specific marker? Front Pharmacol. 2011; 2: 36. PubMed: https://pubmed.ncbi.nlm.nih.gov/21811461/  
21. Szefl  er SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, et al. Asthma outcomes: biomarkers. J Allergy Clin Immunol. 2012; 129: S9-S23. PubMed: https://pubmed.ncbi.nlm.nih.gov/22386512/  
22. Pizzichini MM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, et al. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. Am J Respir Crit Care Med. 1997; 155: 1501-1508. PubMed: https://pubmed.ncbi.nlm.nih.gov/9154849/  
23. Roche N, Chapman KR, Vogelmeier CF, Herth FJF, Thach C, et al. Blood eosinophils and response to maintenance chronic obstructive pulmonary disease treatment. Data from the FLAME trial. Am J Respir Crit Care Med. 2017; 195: 1189-1197. PubMed: https://pubmed.ncbi.nlm.nih.gov/28278391/  
24. Watz H, Tetzlaff K, Wouters EF, Kirsten A, Magnusson H, et al. Blood eosinophil count and exacerbations in severe chronic obstructive pulmonary disease after withdrawal of inhaled corticosteroids: a post-hoc analysis of the WISDOM trial. Lancet Respir Med. 2016; 45: 390-398. PubMed: https://pubmed.ncbi.nlm.nih.gov/27066739/  
25. Kalinina EP, Denisenko YK, Vitkina TI, Lobanova EG, Novgorodtseva TP, et al. The mechanisms of the regulation of immune response in patients with comorbidity of chronic obstructive pulmonary disease and asthma. Can Respir J. 2016; 2016: 4503267. PubMed: https://pubmed.ncbi.nlm.nih.gov/27660519/  
26. Ansotegui IJ, Melioli G, Canonica GW, Caraballo L, Villa E, et al. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. World Allergy Organ J. 2020; 13: 100080. PubMed: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7044795/  
27. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, et al. Evaluation of COPD longitudinally to identify predictive surrogate end-points (ECLIPSE). Eur Respir J. 2008; 31: 869-873. PubMed: https://pubmed.ncbi.nlm.nih.gov/18216052/