Online supplemental manuscript:

Decreased capsaicin cough reflex sensitivity predicts hospitalization due to COPD

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Methods:

Subjects and the definition of COPD and comorbid asthma

We prospectively recruited patients with COPD who visited the Nagoya City University Hospital and Shizuoka General Hospital between June 2018 and January 2020. We enrolled patients in this study if their condition was stable at the time of their outpatient department visit. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 as follows: (1) a post-bronchodilator forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) ratio of less than 0.70 (fixed airflow limitation); (2) persistent respiratory symptoms such as dyspnea, cough, sputum production, or wheezing; and (3) significant exposure to noxious stimuli such as tobacco smoke or other environmental particles. We permitted to include patients with comorbid asthma, called “asthma-COPD overlap” in the present study. Patients were diagnosed with comorbid asthma if they had some clinical features of asthma, such as (1) variable or paroxysmal clinical symptoms, (2) a diagnosis of asthma before the age of 40 years, (3) elevated levels of fractional nitric oxide (FeNO) of 35 ppb or higher, and (4) a history of perennial allergic rhinitis, airway hyperresponsiveness, elevated peripheral blood eosinophils, and total or allergen-specific immunoglobulin E (IgE) levels, in addition to fixed airflow limitation¹. Conversely, patients were not recruited to the present
study when they denied participation in the study, had chronic respiratory diseases other than asthma, or had a post-bronchodilator FEV₁/FVC ratio of 0.7 or higher. Patients were not eligible for this study if they were in a nursing care home, were bedridden at home, had a history of aspiration pneumonia, or underwent tubal feeding. Patients who experienced respiratory infection within four weeks or hospitalization due to AE-COPD or CAP within 12 weeks prior to enrollment were also excluded because they may have affected the results of the capsaicin cough challenge test. This study was approved by the ethics committee of Nagoya City University (60-18-0012) and registered in the UMIN Clinical Trials Registry (Registry ID UMIN000032497). Written informed consent was obtained from all the participants.

**Measurements**

All patients underwent blood biomarker analyses (blood neutrophil and eosinophil counts, serum total IgE, albumin, lactate dehydrogenase, C-reactive protein, hemoglobin A1c, and plasma brain natriuretic peptide), echocardiography (ejection fraction), FeNO measurement, lung function test, computed tomography of the chest, and capsaicin cough challenge test at enrollment. Pre- and post-bronchodilator spirometry were performed according to the ATS/ERS recommendation using a Chestac-8900 (Chest Corp, Tokyo, Japan). The patients inhaled 400 µg of salbutamol using a spacer after
completing the first spirometry test. They underwent post-bronchodilator spirometry 15 min after inhalation of 400 µg salbutamol. Post-bronchodilator FEV₁ values were used. FeNO levels were determined at an expiratory flow rate of 50 ml/s using a Sievers NOA 280i chemiluminescence analyzer (GE Analytical Instruments, Boulder, CO, USA)³. They also completed the Leicester Cough Questionnaire, COPD Assessment Test, and modified Medical Research Council (mMRC) dyspnea scale at that time. The Leicester Cough Questionnaire contains 19 items with three subdomains: physical, social, and psychological, ranging from 3 to 21⁴. Higher scores indicate a better cough-specific QoL. This questionnaire was translated from English into Japanese using the international protocol of the International Quality of Life Assessment translation protocol⁵. The validity and reliability of the Japanese version of the LCQ⁴ has been confirmed in a previous study⁵. If patients expectorated sputum, they were cultured to assess the bacterial colonization of the airways. The COPD assessment test is a self-report questionnaire regarding the health status of patients with COPD, which includes eight items related to cough, phlegm, chest tightness, dyspnea, activities, confidence, sleep, and energy. It ranges from 0 to 40. Higher scores indicate worse health status⁶. The mMRC dyspnea scale is a five-point Likert scale that assesses dyspnea. It ranges from 0 (only breathlessness after heavy exercise) to 4 (too breathless to leave the house)⁷.
Capsaicin cough challenge test

The capsaicin cough challenge test was performed using an Astograph® (Chest, Tokyo, Japan) after blood collection, echocardiography, FeNO measurement, and spirometry. Detailed information on this method has been previously reported. Briefly, 10 doubling concentrations of capsaicin (0.61 to 312.5 µM) were inhaled for 15 s per concentration at 1-minute intervals in increasing order, following inhalation of physiological saline for 1 min. Saline was inhaled for 45 s until the initiation of the next inhalation of capsaicin to increase patient blindness. When patients coughed five or more times, the challenge ended at the end of the following saline inhalation for 45 s. The test ended when patients finished inhaling short-acting β₂ agonists (SABAs) for two min after the capsaicin cough challenge. The concentrations required to induce at least two (C2) and five (C5) coughs were recorded. Lower C2 and C5 values indicate heightened capsaicin cough reflex sensitivity. We planned to perform a capsaicin cough challenge test 1 year after enrollment in the study, but we could not evaluate capsaicin cough reflex sensitivity 1 year after enrollment because of the restriction of measurements due to the coronavirus disease 2019 pandemic.

Definition of AE-COPD and pneumonia

AE-COPD was defined as an acute worsening of respiratory symptoms that
resulted in additional treatments such as bronchodilators, antibiotics, and systemic corticosteroids. CAP was diagnosed when new infiltration was observed on chest radiography and/or computed tomography with two or more of the following findings: (1) fever (body temperature >37.5°C or <36.0°C), (2) leukocytosis or leukopenia (white blood cells >10,000/mm$^3$ or <4,000/mm$^3$), and (3) purulent tracheal aspirate and/or sputum.

**Statistical analysis**

Statistical analysis was performed using the JMP 14.3 software (SAS Institute Japan, Tokyo, Japan). Values are expressed as mean (standard deviation) for continuous variables and n (%) for categorical variables. We categorized patients into the hospitalization+ group (AE-COPD or CAP patients requiring hospitalization during 1 year after enrollment) and the hospitalization- group. Two-group comparisons were performed using unpaired t-test or Fisher's exact test, as appropriate. The values of C2 and C5 are expressed as the number of doubling concentrations. The number of doubling concentrations ranges from 1 to 10, with a high number indicating a decreased cough sensitivity of inhaled capsaicin. Corresponding inhalation concentration of capsaicin to the number of doubling concentration was shown as follows; e.g. 0.61 µM, 1.22 µM, 2.44 µM, 4.88 µM, 9.76 µM, 19.52 µM, 39.04 µM, 78.1 µM, 156.2 µM and, 312.5 µM.
correspond to 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 doubling concentrations, respectively. They reflect base2 logarithmic values of C5. Multivariate analysis was performed to determine the odds ratio for hospitalization. We adapted the number of doubling concentration of C5 when conducting the multivariate analysis because readers can easily understand the odds ratio for hospitalization if the concentration of C5 rises to the next concentration. A sensitivity analysis confined to patients who were hospitalized before enrollment was also performed to clarify the association between capsaicin cough reflex sensitivity and hospitalization in patients with COPD. Longitudinal data on capsaicin cough reflex sensitivity were compared using paired t-tests. A variable was considered significant if the p-value was ≤ 0.05. Some data were missing (n = 64 for total IgE, and n = 66 for CRP and ejection fraction). We handled missing data as a blank because these did not affect main results of this study.
References:


