

1 **Online supplemental manuscript:**

2 **Decreased capsaicin cough reflex sensitivity predicts hospitalization due to COPD**

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17 **Methods:**

18 **Subjects and the definition of COPD and comorbid asthma**

19 We prospectively recruited patients with COPD who visited the Nagoya City
20 University Hospital and Shizuoka General Hospital between June 2018 and January 2020.
21 We enrolled patients in this study if their condition was stable at the time of their
22 outpatient department visit. COPD was diagnosed according to the Global Initiative for
23 Chronic Obstructive Lung Disease (GOLD) 2018 as follows: (1) a post-bronchodilator
24 forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) ratio of less than 0.70
25 (fixed airflow limitation); (2) persistent respiratory symptoms such as dyspnea, cough,
26 sputum production, or wheezing; and (3) significant exposure to noxious stimuli such as
27 tobacco smoke or other environmental particles. We permitted to include patients with
28 comorbid asthma, called “asthma-COPD overlap” in the present study. Patients were
29 diagnosed with comorbid asthma if they had some clinical features of asthma, such as (1)
30 variable or paroxysmal clinical symptoms, (2) a diagnosis of asthma before the age of 40
31 years, (3) elevated levels of fractional nitric oxide (FeNO) of 35 ppb or higher, and (4) a
32 history of perennial allergic rhinitis, airway hyperresponsiveness, elevated peripheral
33 blood eosinophils, and total or allergen-specific immunoglobulin E (IgE) levels, in
34 addition to fixed airflow limitation¹. Conversely, patients were not recruited to the present

35 study when they denied participation in the study, had chronic respiratory diseases other
36 than asthma, or had a post-bronchodilator FEV₁/FVC ratio of 0.7 or higher. Patients were
37 not eligible for this study if they were in a nursing care home, were bedridden at home,
38 had a history of aspiration pneumonia, or underwent tubal feeding. Patients who
39 experienced respiratory infection within four weeks or hospitalization due to AE-COPD
40 or CAP within 12 weeks prior to enrollment were also excluded because they may have
41 affected the results of the capsaicin cough challenge test. This study was approved by the
42 ethics committee of Nagoya City University (60-18-0012) and registered in the UMIN
43 Clinical Trials Registry (Registry ID UMIN000032497). Written informed consent was
44 obtained from all the participants.

45 **Measurements**

46 All patients underwent blood biomarker analyses (blood neutrophil and
47 eosinophil counts, serum total IgE, albumin, lactate dehydrogenase, C-reactive protein,
48 hemoglobin A1c, and plasma brain natriuretic peptide), echocardiography (ejection
49 fraction), FeNO measurement, lung function test, computed tomography of the chest, and
50 capsaicin cough challenge test at enrollment. Pre- and post-bronchodilator spirometry
51 were performed according to the ATS/ERS recommendation using a Chestac-8900 (Chest
52 Corp, Tokyo, Japan) ². The patients inhaled 400 µg of salbutamol using a spacer after

53 completing the first spirometry test. They underwent post-bronchodilator spirometry 15
54 min after inhalation of 400 µg salbutamol. Post-bronchodilator FEV₁ values were used.
55 FeNO levels were determined at an expiratory flow rate of 50 ml/s using a Sievers NOA
56 280i chemiluminescence analyzer (GE Analytical Instruments, Boulder, CO, USA) ³.

57 They also completed the Leicester Cough Questionnaire, COPD Assessment Test,
58 and modified Medical Research Council (mMRC) dyspnea scale at that time. The
59 Leicester Cough Questionnaire contains 19 items with three subdomains: physical, social,
60 and psychological, ranging from 3 to 21⁴. Higher scores indicate a better cough-specific
61 QoL. This questionnaire was translated from English into Japanese using the international
62 protocol of the International Quality of Life Assessment translation protocol⁵. The
63 validity and reliability of the Japanese version of the LCQ⁴ has been confirmed in a
64 previous study⁵. If patients expectorated sputum, they were cultured to assess the bacterial
65 colonization of the airways. The COPD assessment test is a self-report questionnaire
66 regarding the health status of patients with COPD, which includes eight items related to
67 cough, phlegm, chest tightness, dyspnea, activities, confidence, sleep, and energy. It
68 ranges from 0 to 40. Higher scores indicate worse health status⁶. The mMRC dyspnea
69 scale is a five-point Likert scale that assesses dyspnea. It ranges from 0 (only
70 breathlessness after heavy exercise) to 4 (too breathless to leave the house)⁷.

71 **Capsaicin cough challenge test**

72 The capsaicin cough challenge test was performed using an Astograph® (Chest,
73 Tokyo, Japan) after blood collection, echocardiography, FeNO measurement, and
74 spirometry. Detailed information on this method has been previously reported⁸. Briefly,
75 10 doubling concentrations of capsaicin (0.61 to 312.5 µM) were inhaled for 15 s per
76 concentration at 1-minute intervals in increasing order, following inhalation of
77 physiological saline for 1 min. Saline was inhaled for 45 s until the initiation of the next
78 inhalation of capsaicin to increase patient blindness. When patients coughed five or more
79 times, the challenge ended at the end of the following saline inhalation for 45 s. The test
80 ended when patients finished inhaling short-acting β₂ agonists (SABAs) for two min after
81 the capsaicin cough challenge. The concentrations required to induce at least two (C2)
82 and five (C5) coughs were recorded. Lower C2 and C5 values indicate heightened
83 capsaicin cough reflex sensitivity^{8 9}. We planned to perform a capsaicin cough challenge
84 test 1 year after enrollment in the study, but we could not evaluate capsaicin cough reflex
85 sensitivity 1 year after enrollment because of the restriction of measurements due to the
86 coronavirus disease 2019 pandemic.

87 **Definition of AE-COPD and pneumonia**

88 AE-COPD was defined as an acute worsening of respiratory symptoms that

89 resulted in additional treatments such as bronchodilators, antibiotics, and systemic
90 corticosteroids. CAP was diagnosed when new infiltration was observed on chest
91 radiography and/or computed tomography with two or more of the following findings:
92 (1) fever (body temperature $>37.5^{\circ}\text{C}$ or $<36.0^{\circ}\text{C}$), (2) leukocytosis or leukopenia (white
93 blood cells $>10,000/\text{mm}^3$ or $<4,000/\text{mm}^3$), and (3) purulent tracheal aspirate and/or
94 sputum¹⁰.

95 **Statistical analysis**

96 Statistical analysis was performed using the JMP 14.3 software (SAS Institute
97 Japan, Tokyo, Japan). Values are expressed as mean (standard deviation) for continuous
98 variables and n (%) for categorical variables. We categorized patients into the
99 hospitalization+ group (AE-COPD or CAP patients requiring hospitalization during 1
100 years after enrollment) and the hospitalization- group. Two-group comparisons were
101 performed using unpaired t-test or Fisher's exact test, as appropriate. The values of C2
102 and C5 are expressed as the number of doubling concentrations. The number of doubling
103 concentrations ranges from 1 to 10, with a high number indicating a decreased cough
104 sensitivity of inhaled capsaicin. Corresponding inhalation concentration of capsaicin to
105 the number of doubling concentration was shown as follows; e.g. 0.61 μM , 1.22 μM , 2.44
106 μM , 4.88 μM , 9.76 μM , 19.52 μM , 39.04 μM , 78.1 μM , 156.2 μM and, 312.5 μM

107 correspond to 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 doubling concentrations, respectively. They
108 reflect base2 logarithmic values of C5. Multivariate analysis was performed to determine
109 the odds ratio for hospitalization. We adapted the number of doubling concentration of
110 C5 when conducting the multivariate analysis because readers can easily understand the
111 odds ratio for hospitalization if the concentration of C5 rises to the next concentration. A
112 sensitivity analysis confined to patients who were hospitalized before enrollment was also
113 performed to clarify the association between capsaicin cough reflex sensitivity and
114 hospitalization in patients with COPD. Longitudinal data on capsaicin cough reflex
115 sensitivity were compared using paired t-tests. A variable was considered significant if
116 the p-value was ≤ 0.05 . Some data were missing (n = 64 for total IgE, and n = 66 for CRP
117 and ejection fraction). We handled missing data as a blank because these did not affect
118 main results of this study.

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