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Impact of gastro-oesophageal reflux disease symptoms on chronic obstructive pulmonary disease exacerbation

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ABSTRACT

Background: The association between gastro-oesophageal reflux disease (GORD) and chronic obstructive pulmonary disease (COPD) exacerbation has so far remained unclear.

Objective: To prospectively establish the clinical significance of GORD symptoms on exacerbation.

Methods: In total, 82 COPD patients and 40 age-matched controls were enrolled in this study. Symptoms were evaluated by a questionnaire using the Frequency Scale for the Symptoms of GORD (FSSG). COPD patients were prospectively surveyed for 6 months, and episodes of exacerbation were identified using a diary based on modified Anthonisen's criteria. Exhaled breath condensate (EBC) pH was measured in both groups, and induced sputum was evaluated in COPD patients.

Results: Positive GORD symptoms were reported in 22 (26.8%) COPD patients and five (12.5%) controls ($p = 0.10$). The frequency of exacerbations was significantly associated with the FSSG score ($p = 0.03$, $r = 0.24$, 95% confidence interval (CI) = 0.02–0.43). Multiple regression analysis revealed that GORD symptoms were significantly associated with the occurrence of exacerbations ($p < 0.01$; relative risk (RR) = 6.55, 95% CI = 1.86–23.11). EBC pH was inversely correlated with FSSG score in both groups ($p = 0.01$, $r = –0.37$, 95% CI = –0.55 to –0.14 in COPD patients, and $p < 0.01$, $r = –0.45$, 95% CI = –0.67 to –0.16 in control subjects).

Conclusions: GORD symptoms were identified as an important factor associated with COPD exacerbation.

INTRODUCTION

Exacerbations of chronic obstructive pulmonary disease (COPD) are an important determinant of patient quality of life and can aggravate disease progression, which is associated with increased morbidity and use of health-care resources.[1] Several clinical backgrounds are reportedly associated with exacerbation frequency, including age, low forced expiratory volume in 1 s (FEV_1) and low body-mass index (BMI).[2,3]

Gastro-oesophageal reflux disease (GORD) is a relatively common condition, affecting 10–29% of the Western population and 5–14% of Japanese adults, and is associated with a variety of respiratory disorders.[4–7] GOR has been shown to worsen asthma control through oesophagobronchial reflex, and to heighten bronchial reactivity and microaspiration.[8–10] GOR has also been reported to be accompanied by neutrophilic airway inflammation.[11] We therefore hypothesized that GOR could act as a confounding factor of exacerbations through similar mechanisms in asthma and/or by increasing airway inflammation.

To our knowledge, only one previous study has investigated the relationship between GORD symptoms and exacerbations. This research relied on a retrospective analysis of the number of exacerbations based on patient recall during the previous year, and the mechanism by which GORD symptoms affect COPD exacerbation remains to be elucidated.[12]

In the current study, we prospectively examined the association between exacerbations and GORD symptoms using the Frequency Scale for the Symptoms of GORD (FSSG) for qualitative and quantitative analysis of symptoms. We also investigated the usefulness of EBC pH for reflecting airway inflammation and acid reflux, by analysing the association with GORD symptoms and inflammatory indices of induced sputum.[13,14]

METHODS

Subjects

Patients with moderate-to-severe COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease Guidelines (GOLD) 2003, were consecutively enrolled from the outpatient clinic of Kyoto University Hospital, Japan.[1] The exclusion criteria were as follows: smoking history < 20 packs per year; co-morbidities of respiratory disorders other than COPD; history of malignant disease within 5 years; active gastro-intestinal diseases other than GORD; initiation and withdrawal of medications related to COPD and GORD during the follow-up period; daily use of systemic corticosteroids; and current use of long-term oxygen therapy. In total, 40 controls from the Terada Clinic in Himeji, Japan, and the outpatient clinic at Kyoto University Hospital (n = 38 and 2, respectively) were enrolled (age = > 60 years). The exclusion criteria were as follows: comorbidities of respiratory disorders including COPD confirmed by medical history, physical examination, chest radiography and spirometry; history of malignant diseases within 5 years; active gastro-intestinal diseases other than GORD; and active infectious diseases. The research protocol was approved by the Ethics Committee of Kyoto University, and written informed consent was obtained from all subjects.

Definition of exacerbation and stable condition

Exacerbations were defined, according to the modified Anthonisen's criteria, as the occurrence of two or more of three major symptoms (that is, increase in dyspnoea, sputum purulence and increased sputum volume), or any one major symptom with any one minor symptom (that is, increase in nasal discharge, wheezing, sore throat, cough or fever) for at least two consecutive days.[15–17] Patients were issued with diaries in which each symptom was quantified on a graded scale from 1 to 5 (where 1 = much better than usual, 2 = better than usual, 3 = the same as usual, 4 = worse than usual and 5 = much worse than usual) based on previous recommendations.[17]

Exacerbations were identified using diaries at each visit to our clinic, and were confirmed by at least two respiratory physicians who were unaware of the EBC pH and the FSSG score. The end of an exacerbation was defined as the time point when the rolling 3-day mean symptom score returned to the pre-exacerbation level.[18] Stable conditions were defined as exacerbation-free intervals that lasted for more than 4 weeks, as confirmed in a diary, and episodes of worsening symptoms within 2 weeks that met the criteria of exacerbation were considered as one episode.[19]

Study protocol

The COPD and control subjects were compared using a cross-sectional survey. The association between GORD symptoms and exacerbations was investigated with a cohort survey. GORD symptom evaluation, EBC and sputum sampling, and lung function tests were performed on the same day under stable conditions. Subsequently, the numbers of exacerbations recorded in the diaries were calculated over a period of more than 6 months between 1 June 2006 and 31 December 2006.

GORD evaluation

GORD symptoms were evaluated with a self-reported FSSG questionnaire consisting of 12 items. The frequency of each item was quantified on a scale ranging from 0 to 4 points as follows: 0 = none (not in the past year); 1 = rarely (a few times in the past year); 2 = sometimes (a few times in the past month); 3 = often (a few times in the past week); 4 = always (everyday). The cut-off score for GORD symptoms was set at eight points.[13] The questionnaire included items related to two symptom subtypes: those associated with GOR (for example, “Do you get heartburn?”) and those associated with gastric dysmotility (for example, “Does your stomach get bloated?” and “Does your stomach ever feel heavy?”; see online appendix).[14] We also validated the FSSG results by evaluating the GORD symptoms with the Questionnaire for the Diagnosis of Reflux Disease (QUEST) using a cut-off score for GORD symptoms of four points.[19]

Lung-function tests

The FEV_1, forced vital capacity (FVC), VC, diffusing capacity of the lung for carbon monoxide (DL_{co}), residual volume (RV), total lung capacity (TLC) and arterial blood gas were measured. The predicted values for FEV_1 were calculated according to the method of the Japan Society of Chest Diseases.[20]

EBC sample collection and analysis of pH

EBC samples were collected in a liquid state during tidal breathing for 8 min, using a disposable portable collector (Rtube, Respiratory Research Inc, Charlottesville, VA, USA) under supervised conditions.[21] Each sample was bubbled through Argon gas (350 ml/min) for 8 min. The EBC pH was measured with a 9669-10D glass micro-pH electrode attached to an F 52 pH meter (Horiba Corporation, Kyoto, Japan) immediately after the argon flushing. The F 52 pH meter was calibrated at pH 4, pH 7 and pH 9 against standards, prior to each series of assays.

Sputum induction and processing

Sputum induction and processing were performed on subjects with an $FEV_1 > 1$ L according to the European Respiratory Society (ERS) recommendations, with slight modifications, following the evaluation of GORD symptoms and the sampling of EBC.[22–24] Briefly, subjects were assessed using spirometric tests (Chest MI Corp, Tokyo, Japan) 10 min after premedication with 200 μg inhaled salbutamol, and then inhaled 3% saline for 20 min using an ultrasonic nebuliser (MU-32, Azwell Inc, Osaka, Japan). Each collected sample was immediately separated from the contaminating saliva by visual examination, then mixed with 0.1% dithiothreitol (Suptasol, Oxoid Ltd, Hampshire, UK) and diluted with Dulbecco's phosphate buffered saline (PBS) according to the recommended methodology.[24] After centrifugation, cell differentiation was determined by counting at least 400 non-squamous cells stained using the Diffquik method. The supernatants were stored at –80°C. The levels of interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) in

the supernatants were measured using quantitative sandwich immunoassay techniques (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Statistical analyses were performed using JMP 6.0 (SAS Campus Drive, Cary, NC, USA). Data are presented as the mean ± standard deviation (SD) or as the median with the interquartile range (IQR) shown in parentheses. Statistical analyses were performed using parametric (Student's *t*-test and analysis of variance (ANOVA)) or nonparametric (Mann–Whitney two-sample test and Spearman's rank-correlation test) methods as appropriate. The relationship between the two groups was evaluated with a Chi-squared test. Logistic regression was used to test whether individual factors were associated with exacerbations. A p value < 0.05 was considered statistically significant.

RESULTS

The clinical characteristics of the subjects are shown in Table 1. In total, 82 COPD patients and 40 controls met the entry criteria. There was no significant difference in age between both groups.

Table 1. Patient characteristics

Characteristic	COPD patients (*n* = 82)	Healthy controls (n = 40)	*p* value
Age (years)	73.0 ± 8.0	70.9 ± 9.3	0.32
Gender (male:female)	77:5	19:21	< 0.01
Smoking status (current:former:never)	10:72:0	1:17:22	< 0.01
Number of cigarette packs*year	65.3 ± 37.8	11.2 ± 15.9	< 0.01
BMI (kg/m^2)	21.5 ± 3.0	24.1 ± 3.5	< 0.01
FEV_1 (L)	1.5 ± 0.6	2.2 ± 0.7	< 0.01
$\%FEV_1$ (% pred)	56.9 ± 20.4	101.3 ± 15.9	< 0.01
RV/TLC (%)	43.6 ± 7.7	ND	
DL_{CO}/V_A (ml/min/mmHg/L)	2.7 ± 1.0	ND	
PaO_2 (kPa)	8.3 ± 0.9	ND	
$PaCO_2$ (kPa)	4.6 ± 0.5	ND	

ND, not done; PaO_2, partial pressure of oxygen in arterial blood; $PaCO_2$, partial pressure of arterial carbon dioxide. Gender and smoking status were evaluated by the Chi-squared test. The number of cigarette packs*year was evaluated by the Mann–Whitney *U*-test. All other indices were evaluated by the unpaired *t*-test.

Positive GORD symptoms were reported in 22 (26.8%) COPD patients and five (12.5%) controls (Table 2). The total FSSG score was similar in both groups (4.9 ± 5.3 in COPD patients versus 3.0 ± 3.2 in controls, $p = 0.10$).

Table 2. GORD symptoms in COPD and healthy subjects

Subjects	Evaluation by FSSG		Prevalence	*p* value
	Positive	Negative		
Healthy	5	35	12.5%	0.10
COPD	22	60	26.8%	

Data were evaluated by the Chi-squared test. The relative risk (RR) for GORD symptoms in COPD patients compared with controls was 2.15 (95% CI = 0.88–5.25, $p = 0.10$).

The association between clinical indices and GORD symptoms in the COPD patients is shown in Table 1S (see online appendix). There was no significant difference between subjects with and without GORD symptoms in terms of age, gender, smoking status, BMI, lung function and medications.

The incidence of exacerbation was significantly higher in patients with GORD symptoms than in patients without such symptoms (RR = 1.93, 95% CI = 1.32–2.84; $p < 0.01$; Table 3). The average number of exacerbations during 6 months was 0.98 ± 1.38 in all patients, and those with GORD symptoms experienced significantly more episodes than those without (1.73 ± 1.58 versus 0.70 ± 1.20, $p < 0.01$).

Table 3. Associations between exacerbations and GORD symptoms in COPD patients

GORD symptoms	COPD exacerbation		Exacerbation frequency over 6 months
	Did not occur	Did occur	
Negative	36	24	0.70 ± 1.20
Positive	5	17	1.73 ± 1.58
***p* value**	< 0.01		< 0.01

Data were evaluated by the Chi-squared test and the unpaired *t*-test. The RR for the occurrence of exacerbation in patients with GORD symptoms compared with patients without such symptoms was 1.93 (95% CI = 1.32–2.84, $p < 0.01$).

Figure 1 shows the relationship between the FSSG score and the exacerbation frequency. The FSSG score was significantly correlated with the number of exacerbations ($r = 0.24$, 95% CI = 0.02–0.43; $p = 0.03$).

The known confounding factors for exacerbations are shown in Table 2S (see online appendix). The demographic and other clinical characteristics were similar between both groups.

The GORD symptoms were also identified by multiple regression analysis as a significant factor associated with the occurrence of exacerbation (RR = 6.55, 95% CI = 1.86 to 23.11; $p < 0.01$; Table 3S (see online appendix)).

To exclude the effects both by daily treatment with proton-pump inhibitors (PPIs) or H2-receptor antagonists (H_2RAs) and by seasonal variability of exacerbations (for example, the frequency of exacerbations might increase in winter in association with the occurrence of influenza epidemics), 52 patients who were not receiving anti-acid therapy among the sample population were surveyed for an additional 6 months (12 months in total). In those patients, GORD symptoms was also significantly associated with annual frequency; the frequency was 2.6

± 2.0 in subjects with GORD symptoms and 1.5 ± 1.7 in subjects without such symptoms (p = 0.048). The GORD symptoms were significantly related to frequent (three or more episodes per year) exacerbations (RR = 2.18, 95% CI = 1.10–5.70; p = 0.046 for frequent exacerbations in patients with versus without GORD symptoms).

The EBC pH was similar between the COPD patients and the controls. However, the EBC pH was significantly lower in subjects with GORD symptoms than in subjects without GORD symptoms (6.47 ± 1.22 versus 7.17 ± 1.05, p = 0.02 in COPD patients, and 6.34 ± 1.22 versus 7.22 ± 0.53, p = 0.03 in controls; Table 4S (see online appendix)). The EBC pH was inversely correlated with the FSSG score in both groups (r = –0.37, 95% CI = –0.55 to 0.34; p = 0.01 in COPD patients, and r = –0.45, 95% CI = –0.67 to 0.16; p < 0.01 in controls). However, no significant correlation was found between the EBC pH and the exacerbation frequency (r = 0.13, 95% CI = –0.11 to 0.35; p = 0.29).

To examine whether the EBC pH reflected tracheobronchial inflammation, sputum induction was performed in 42 patients, 35 of whom met the inclusion criterion for evaluating sputum as a lower respiratory tract sample (squamous cell contamination = < 20%). There was no significant correlation between the EBC pH and the sputum inflammatory indices, such as the differential cell counts, and the IL-8 and TNF-α concentrations (Table 5S (see online appendix)).

According to QUEST, the prevalence of positive GORD symptoms was 24.4% in COPD patients and 10.0% in controls, the RR of the exacerbation in patients with GORD symptoms compared with patients without such symptoms was 1.61 (95% CI = 1.07–2.41, p = 0.07), the EBC pH values of subjects with and without GORD symptoms were 6.56 ± 1.19 and 7.12 ± 1.09, respectively (p = 0.07), and the GORD symptoms were marginally associated with exacerbation occurrence (RR = 3.21, 95% CI = 1.11–13.27; p = 0.05).

DISCUSSION

This prospective cohort study demonstrated, for the first time, that COPD exacerbations are associated with GORD symptoms, the frequency of which is inversely correlated with the EBC pH.

A recent retrospective study suggested that GORD symptoms were associated with exacerbations; however, the subjects were asked to report the number of exacerbations that had occurred during the previous year, which was an approach that did not meet the criteria used in previous studies and resulted in a recall bias.[12] To resolve this problem, we conducted a prospective questionnaire-based study that allowed us to identify exacerbations according to modified Anthonisen's criteria.[15–17] In order to determine the associations among GORD symptoms, airway inflammation and exacerbation frequency, we also evaluated the EBC pH and induced sputum under stable conditions.

We found the EBC pH to be correlated inversely with the FSSG score in both groups. Recently, Hunt *et al.* reported that transient EBC acidification occurred during acid reflux cough, and that the EBC pH profile collected immediately subsequent to a coughing episode was a strong predictor of GOR in the diagnosis of chronic cough.[5] In our current study, we were unable to determine the time lag between the actual acid reflux and the sampling of EBC pH. However, we speculated that asymptomatic acid reflux occurred more frequently in accordance with increased GORD symptoms. This might have resulted in the sampling point of EBC being contiguous to the acid reflux as the GORD symptoms worsened, lowering the EBC pH. In several previous studies, EBC pH has been reported to reflect the lining fluid of the inflamed lower respiratory tract, and considerable variability has been seen among COPD patients. Kostikas *et al.* demonstrated an inverse correlation between EBC pH and the percentage of neutrophils in induced sputum in 20 COPD patients.[25] However, we found that none of the sputum inflammatory indices correlated

with the EBC pH (Table 5S (see online appendix)).[22–24,26] Moreover, the EBC pH was not correlated with the frequency of exacerbations, and was similar between COPD patients and controls. These findings suggest that the EBC pH might have reflected acid reflux rather than tracheobronchial inflammation under stable conditions in our sample population. Further examination is necessary to determine whether EBC pH is a marker of acid reflux.

From the several different questionnaires developed for the symptom-based diagnosis of GORD, we chose the FSSG for the current study for the following reasons.[12,13,19,27,28] First, GORD symptoms can be quantified with the FSSG, but not with QUEST. Second, symptoms related not only to acid reflux but also to gastric dysmotility can be evaluated with the FSSG; this is of particular importance, and a descriptive questionnaire that covers symptoms associated with both acid reflux and gastric dysmotility is useful for recognizing these conditions.[29,30] We found that both the FSSG and QUEST identified GORD symptoms to a similar level. However, although QUEST showed a similar tendency for associations with exacerbations, it failed to reach statistical significance. This discrepancy between the FSSG and QUEST might be due to differences in the characteristics of the questionnaires: the latter focuses on ulcer-like and reflux-like symptoms, and lacks items concerning dysmotility-like symptoms, whereas the former covers all symptom subtypes.[13,19]

The occurrence of GORD symptoms in our sample population was similar to those reported in previous studies. Although the prevalence of GORD symptoms was reportedly higher in COPD patients than controls, this trend did not achieve statistical significance for the following reasons.[4,27,28] First, the sample size was small. Second, the confounding factors of the prevalence of GORD, such as gender, smoking status and BMI, were not matched between the COPD patients and the controls.[31,32]

The symptom-based diagnosis of GORD could be affected by symptoms related to

comorbidities such as COPD. In COPD, the overinflation of the lung could cause dysmotility-like dyspepsia giving a sensation of gastric fullness without GORD. However, we did not find any significant associations between GORD symptoms and physiological indices such as BMI, %FEV_1 (an index of COPD severity), RV, TLC, RV/TLC (a physiological parameter related to overinflation) and DL_{CO}/V_A (reflecting parenchymal destruction and V/Q mismatch). Therefore, we believe that the GORD symptoms were not dependent on the COPD severity.

It is difficult to determine whether GORD itself directly affects exacerbation or merely coexists in patients who experience frequent exacerbations. In asthmatic patients, the coexistence of GORD was shown to be associated with deteriorating symptoms through several mechanisms, including oesophageal–bronchial reflex, heightened bronchial reactivity and microaspiration.[8–10,33] Carpagnano *et al.* reported that GOR is characterized by neutrophilic airway inflammation, although it does not aggravate pre-existing airway inflammation in asthma.[11] However, it remains unclear whether GOR itself alters lung function or the clinical course in COPD. Previous reports have shown that COPD patients lack a bronchoconstrictive reflex to distal oesophageal acidification.[34] Ravelli *et al.* used gastro-oesophageal ^{99}Tc scintigraphy with lung scanning to show that microaspiration of gastric contents occurred even if pathologic GOR was not detected with 24-h intra-oesophageal pH monitoring.[35] Moreover, to determine whether the EBC pH reflected GOR, and whether GOR or gastric dysmotility was more strongly associated with the frequency of exacerbations, we investigated the associations among the frequency of exacerbations, EBC pH, and the symptom subtypes of the FSSG associated with GOR and gastric dysmotility. The number of exacerbations was significantly correlated with those associated with gastric dysmotility but not with GOR (Figure 1S (see online appendix)), whereas the EBC pH was inversely correlated with those associated with GOR but not with gastric dysmotility (Figure 2S (see online appendix)). We also demonstrated that the sputum inflammatory indices under stable

conditions were similar between patients with GORD symptoms and those without (Table 6S (see online appendix)). Considering these findings, we speculated that GOR might occur frequently even under stable conditions that cause the EBC pH to fall without aggravating airway inflammation, whereas gastric dysmotility might predispose an individual to episodic aspiration of low-acidic gastric contents and induce exacerbations. Moreover, impaired gastric motility might disturb the clearance of swallowed contents from the pharynx to the oesophagus, leading to their aspiration into the tracheobronchial tree and thereby causing exacerbations. Further objective examinations are necessary to confirm the associations between GOR, impaired gastric motility and exacerbations.

There was a limitation to our study. We did not confirm GOR objectively using 24-h intra-oesophageal pH monitoring. The sensitivity and specificity of questionnaire-based diagnosis is not satisfactory compared with 24-h intra-oesophageal pH monitoring for the diagnosis of GORD; however, 24-h intra-oesophageal pH probes are of limited relevance to acid-reflux respiratory diseases, are uncomfortable for subjects and are generally reserved for research purposes.[5] We found an inverse correlation between the FSSG score and the EBC pH. Further investigations are thus necessary to determine whether the EBC pH reflects GOR or some aspects of airway pathophysiology that relate to exacerbation.

In conclusion, we found that COPD patients with GORD symptoms were more likely to experience exacerbations than those lacking these symptoms. GORD might increase the tracheobronchial aspiration of gastric juice directly and/or disturb the clearance of swallowed contents from the pharynx to the oesophagus indirectly, leading to frequent exacerbations. Further examinations are needed to clarify whether treatment for GORD symptoms ameliorates COPD exacerbations.

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COMPETING INTERESTS

This study is not associated with any competing interest.

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STATEMENT

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FIGURE LEGENDS

Figure 1. Correlation between exacerbation frequency and FSSG score. The FSSG score ($p = 0.03$, $r = 0.24$, 95% CI = 0.02–0.43) was correlated with the frequency of exacerbations.

Figure 1S. Correlation between exacerbation frequency and symptom subtypes of the FSSG. (a) The frequency of exacerbations was not correlated with the symptom score associated with GOR ($r = 0.16$, 95% CI = –0.06 to 0.36; $p = 0.16$). (b) The frequency of exacerbations was correlated with the symptom score associated with gastric dysmotility ($r = 0.27$, 95% CI = 0.06–0.46, $p = 0.01$).

Figure 2S. Correlation between EBC pH and symptom subtypes on FSSG. (a) The EBC pH was correlated inversely with the symptom score associated with GOR ($r = –0.42$, 95% CI = –0.60 to –0.21; $p < 0.001$). (b) The EBC pH was not correlated with the symptom score associated with gastric dysmotility ($r = –0.21$, 95% CI = –0.42 to 0.03; $p = 0.09$).

FIGURE 1. Correlation between frequency of exacerbations and FSSG score

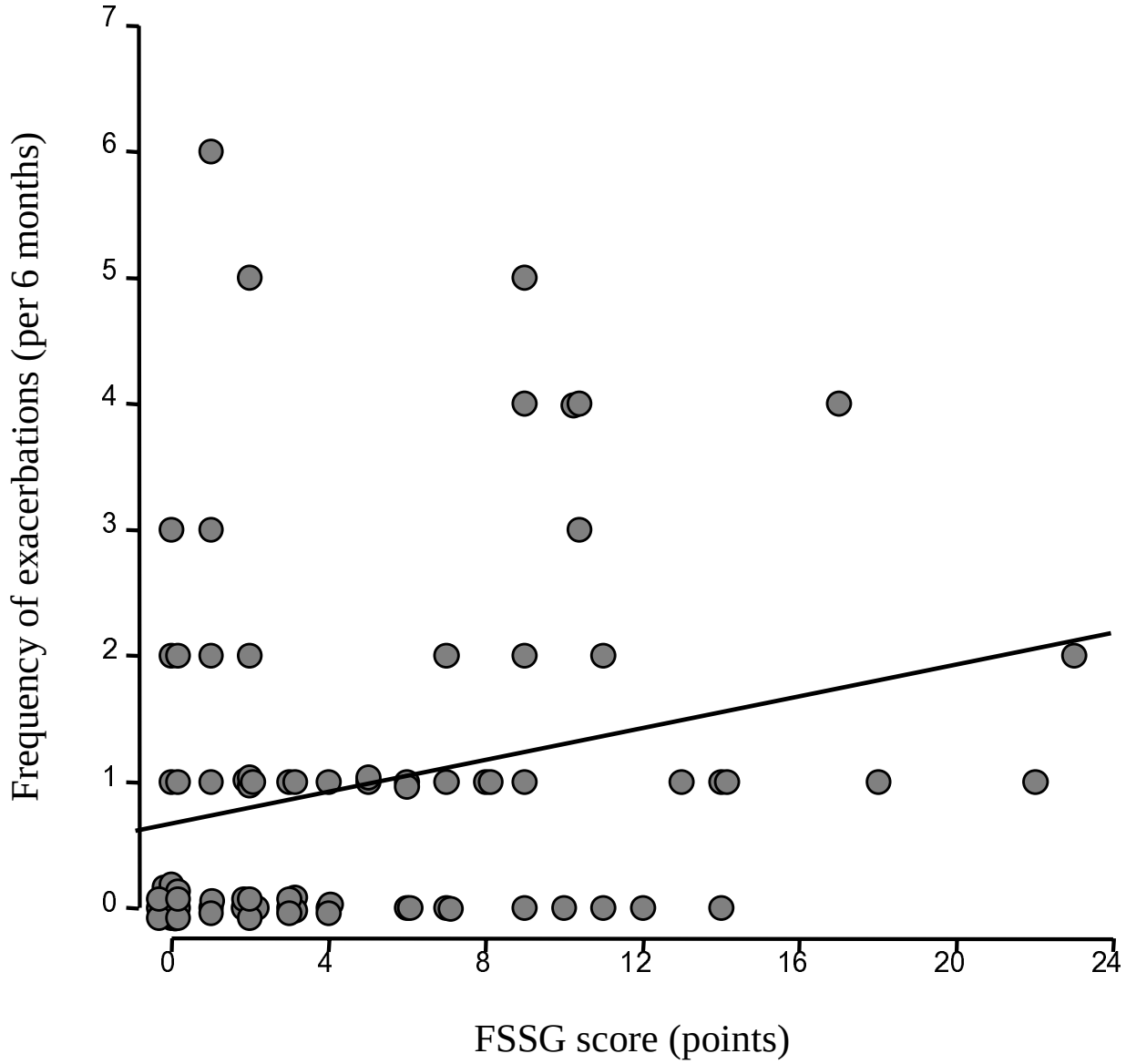

Table 1S. Associations between clinical indices and GORD symptoms in COPD patients

Characteristic	GORD symptoms		*p*-value
	Positive (*n* = 22)	Negative (*n* = 60)	
Age (years)	72.0 ± 5.3	71.7 ± 8.5	0.76
Sex (male:female)	20:2	57:3	0.61
Smoking status (current:former)	1:21	9:51	0.27
BMI (kg/m^2)	21.3 ± 3.1	21.5 ± 2.9	0.77
$\%FEV_1$ (% pred)	60.2 ± 17.5	55.8 ± 21.4	0.39
RV/TLC (%)	43.2 ± 8.9	43.7 ± 7.2	0.77
Inhaled corticosteroid use (D:ND)	8:14	23:37	> 0.99
Theophyline use (D:ND)	2:20	2:58	0.29
β-agonist use (D:ND)	13:9	41:19	0.44
Anti-cholinergics use (D:ND)	17:5	40:20	0.43
PPI or H_2RA use (PPI:H_2RA:ND)	1:5:16	8:6:46	0.21

PPI, proton pump inhibitor; H_2RA, type 2 histamine receptor antagonist, D, done; ND, not done

Age evaluated by Mann-Whitney's U test; BMI, $\%FEV_1$, and RV/TLC by unpaired t-test; the other indices by Chi-squared test.

Table 2S. Confounding factors of COPD exacerbation

Factor	Exacerbated (*n* = 41)	Non-exacerbated (*n* = 41)	*p*-value
Age (years)	73.7 ± 6.8	72.3 ± 9.1	0.41
Sex (male:female)	40:1	37:4	0.36
Smoking status (current:former)	4:37	6:35	0.74
BMI (kg/m^2)	21.5 ± 3.2	21.4 ± 2.8	0.90
$\%FEV_1$ (% pred)	56.9 ± 20.4	57.0 ± 20.7	0.98
RV/TLC (%)	42.7 ± 7.8	44.4 ± 7.5	0.30
DL_{CO}/V_A (ml/min/mmHg/L)	2.6 ± 0.9	2.9 ± 1.1	0.10
PaO_2 (kPa)	8.3 ± 0.9	8.3 ± 1.0	0.88
$PaCO_2$ (kPa)	4.6 ± 0.5	4.6 ± 0.5	0.61
Inhaled corticosteroid use (D:ND)	18:23	13:28	0.36
PPI or H_2RA use (PPI:H_2RA:ND)	6:7:28	3:4:34	0.30

PPI, proton pump inhibitor; H_2RA, type 2 histamine receptor antagonist; D, done; ND, not done

Gender, smoking status, inhaled corticosteroid use, and PPI or H_2RA use evaluated by Chi-squared test; the other indices by unpaired t-test.

Table 3S. Multivariable analysis of COPD exacerbation factors

Variable	OR	95% CI	*p*-value
GORD symptoms (positive:negative)	6.55	1.86–23.11	< 0.01
Age (years)	1.03	0.96–1.10	0.39
Sex (male:female)	5.18	0.36–74.47	0.23
Smoking status (current:former)	1.27	0.29–5.54	0.75
BMI (kg/m^2)	1.03	0.85–1.24	0.78
Inhaled corticosteroid use (D:ND)	1.53	0.52–4.52	0.45
$\%FEV_1$ (% pred)	0.99	0.96–1.02	0.43
PaO_2 (kPa)	1.02	0.96–1.09	0.54
$PaCO_2$ (kPa)	0.97	0.87–1.08	0.57

D, done; ND, not done, The coefficient determinant using all the variables was 0.12.

Table 4S. Association between EBC pH and GORD symptoms in COPD and control subjects

	GORD symptoms		*p*-value
	Negative	**Positive**	
Control	7.22 ± 0.52 (35)	6.34 ± 1.16 (5)	0.03
COPD	7.17 ± 1.05 (60)	6.47 ± 1.22 (22)	0.02

The EBC pH was similar between COPD and control (6.97 ± 1.14 versus 7.11 ± 0.68, $p = 0.55$)
EBC pH evaluated by Mann-Whitney's U test in healthy subjects, and unpaired t-test in COPD.
Number in parenthesis, Number of subjects

Table 5S. Correlation between sputum indices and EBC pH in COPD patients

Sputum indices	EBC pH		
	***r*-value**	**95%CI**	***p*-value**
Cell counts and differentiation			
Total cell counts (10^5/ml)	0.31	-0.03 – 0.59	0.07
Macrophage (%)	-0.01	-0.35 – 0.33	0.95
Neutrophil (%)	-0.06	-0.39 – 0.29	0.76
Eosinophil (%)	0.05	-0.30 – 0.38	0.79
Lymphocyte (%)	0.26	-0.09 – 0.55	0.14
Columnar epithelial cell (%)	-0.19	-0.50 – 0.16	0.28
Supernatant			
IL-8 (ng/ml)	0.17	-0.18 – 0.48	0.35
TNF-alpha (pg/ml)	-0.01	-0.35 – 0.33	0.95

Spearman's rank-correlation test.

Table 6S. Association between sputum indices and GORD symptoms in COPD patients

Indices	GORD symptoms		*p*-value
	Negative (n =25)	Positive (n = 10)	
Sputum cell counts and differentiation			
Total cell counts (10^5/ml)	32.2 ± 33.8	18.9 ± 8.9	0.78
Macrophage (%)	26.7 ± 13.7	25.0 ± 16.2	0.70
Neutrophil (%)	64.9 ± 13.3	63.9 ± 16.2	0.86
Eosinophil (%)	3.3 ± 2.7	2.3 ± 1.5	0.40
Lymphocyte (%)	2.5 ± 2.0	2.5 ± 2.1	0.97
Columnar epithelial cell (%)	5.0 ± 4.8	5.7 ± 4.7	0.70
Sputum supernatant			
IL-8 (ng/ml)	35.2 ± 41.1	27.5 ± 26.1	0.59
TNF-alpha (pg/ml)	21.9 ± 76.0	33.1 ± 31.5	0.86

Total cell counts and TNF-alpha level evaluated by Mann-Whitney's U test; the other indices by unpaired t-test.

FIGURE 1S. Correlation between frequency of exacerbations and symptom subtypes on FSSG

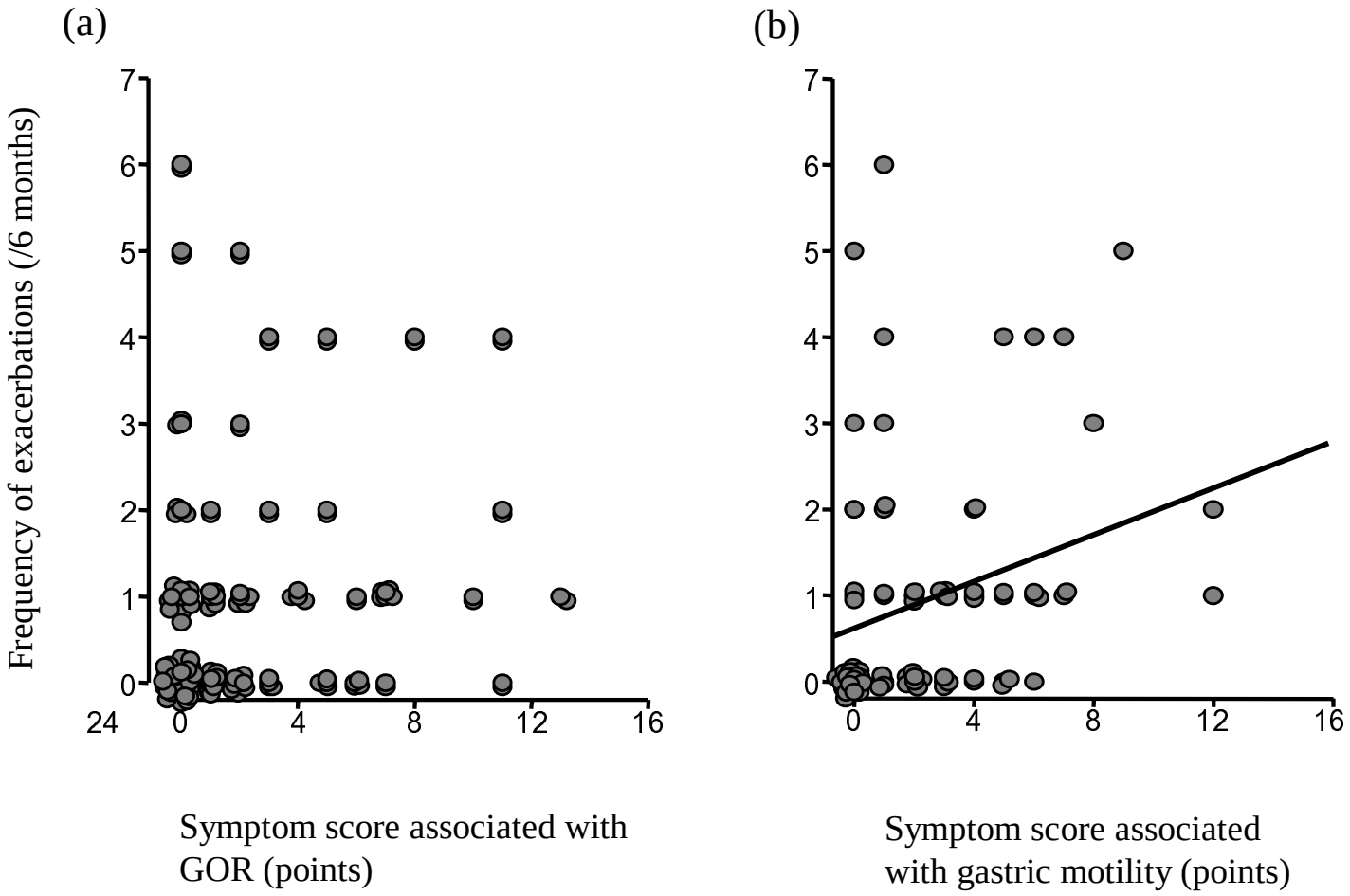

FIGURE 2S. Correlation between EBC pH and symptom subtypes on FSSG