

Bronchoalveolar pH and inflammatory biomarkers in patients with acute exacerbation of chronic obstructive pulmonary disease

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Emilija Lozo Vukovac^{1,2}, Kornelija Miše^{1,2},
Ivan Gudelj^{1,2}, Irena Perić^{1,2},
Darko Duplancić^{2,3}, Ivica Vuković^{2,3},
Zoran Vučinović⁴ and Mislav Lozo³

Abstract

Objectives: This study aimed to directly measure pH in the lungs, determine lactate dehydrogenase (LDH), C-reactive protein (CRP), and glucose levels in serum and bronchoalveolar aspirate, and identify bacterial pathogens from bronchoalveolar fluid during acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

Methods: We performed an observational, analytical case–control study from February 2015 to March 2017. We included 84 patients with AECOPD and 42 with stable chronic obstructive pulmonary disease (COPD). All participants underwent detailed medical anamnesis, a clinical examination, chest radiography, spirometry, an arterial blood gas test, bronchoscopy, bacterial culture, and serum/bronchiolar aspirate laboratory testing.

Results: The mean pH of bronchoalveolar fluid was significantly higher in patients with AECOPD than in patients with stable COPD. The mean lung pH value, bronchoalveolar and serum LDH levels, and serum CRP levels in patients with isolated bacteria were higher than those in patients without isolated bacteria in the AECOPD patient group. Lung pH values in patients with AECOPD were significantly correlated with bronchoalveolar LDH and glucose levels.

¹Department of Pulmonary Diseases, University Hospital Center Split, Split, Croatia

²University of Split School of Medicine, Split, Croatia

³Department of Cardiology, University Hospital Center Split, Split, Croatia

⁴Department of Endocrinology, University Hospital Center Split, Split, Croatia

Corresponding author:

Emilija Lozo Vukovac, Department of Pulmonary Diseases, University Hospital Center Split, Spinciceva I, 21 000 Split, Croatia.

Email: emilija.lozo@gmail.com



Conclusions: AECOPD is associated with local cell and tissue injury in the lungs, especially in the presence of bacterial pathogens, which is accompanied by a low systemic inflammatory response.

Keywords

Chronic obstructive pulmonary disease, disease exacerbation, C-reactive protein, lactate dehydrogenase, glucose, bronchoalveolar aspirate

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Introduction

Chronic obstructive pulmonary disease (COPD) is an important global health issue that is predicted to be the third cause of death in the world by 2020. COPD is described as a chronic and progressive inflammatory lung disease, which causes persistent respiratory symptoms and airflow limitation.¹⁻³ Development of COPD is related to smoking, air pollution, genetic factors, and infections. Increased levels of inflammatory mediators and cytokines are also observed in COPD as part of systemic inflammation.⁴

COPD is a progressive disease, and patients experience periods of increased COPD symptoms called exacerbations.⁵ Acute exacerbation of COPD (AECOPD) are periods of this disease with worsening of respiratory symptoms, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD).⁶ Exacerbations are often triggered by respiratory pathogens, such as bacteria or viruses. AECOPD is usually accompanied by normal or slightly elevated blood test results, such as C-reactive protein (CRP) levels, the sedimentation rate, and lactate dehydrogenase (LDH) levels.

Recently, our research group showed that bronchoalveolar pH can also reflect the local inflammatory burden in patients

with idiopathic pulmonary fibrosis (IPF) and those with gastroesophageal reflux.^{7,8} Lung pH has most commonly been investigated using non-invasive sampling methods of the lower respiratory tract in humans. However, direct measurement of lung pH from the peripheral branches of bronchi has not been performed in patients with AECOPD.

In this study, we aimed to examine systemic and local inflammatory processes in patients with AECOPD by determining inflammatory biomarkers, bronchoalveolar pH, and bacterial culture from bronchoalveolar fluid. We hypothesized that the local pH of patients with AECOPD is more alkaline compared with those with stable COPD, as well as more alkaline in bacteria-isolated versus non-isolated bacteria patients with AECOPD.

Methods

The study was performed at the University Hospital Center Split, Department of Pulmonary Diseases, from February 2015 to March 2017. The clinical diagnosis of AECOPD was determined by an experienced pulmonologist according to previously published GOLD criteria as described above.⁶ COPD was confirmed by spirometry, and it was defined by an forced expiratory volume in 1 second/forced vital

capacity (FEV_1/FVC) < 0.70 after inhalation of nebulized salbutamol (400 mcg), which proves permanent limitation of lung airflow. We included patients in whom exacerbation began no more than 3 days before admission to the Emergency Department. All patients were diagnosed with COPD in the moderate or severe stage (GOLD B, C, D) within 5 years before admission to hospital, by following the recommendations of GOLD.⁶ All patients who received antibiotics and/or systemic corticosteroids at least 2 months before admission were excluded from the study. Participants underwent detailed medical anamnesis, a clinical examination, chest radiography, spirometry, an arterial blood gas test, bronchoscopy, bacterial culture, and serum/bile acid laboratory testing. The study was granted approval by the University Hospital Center Split Ethics Committee. To be included in this study and for presentation of data in this paper, all of the participants signed an informed consent. All experimental procedures were performed following the Helsinki Declaration.

During admission, FVC and FEV_1 values were obtained by a plethysmograph (Masterlab circa 2000; Jaeger, Würzburg, Germany). A blood gas electrolyte analyzer (GEM[®] Premier[™] 3000; Instrumentation Laboratory, Lexington MA, USA) was used to perform arterial blood gas analysis. Blood samples were withdrawn anaerobically from the radial artery. The partial tension of arterial CO_2 ($PaCO_2$) and partial tension of arterial O_2 (PaO_2) were analyzed.

Before the beginning of antibiotic treatment, patients underwent a video-bronchoscopy procedure. For this procedure, we used a video-bronchoscope (Olympus BF 1T160; Olympus Corp., Tokyo, Japan). As preparation for the bronchoscopy procedure, the patients were sedated with 2% lidocaine local anesthesia. We used test strips (Multistix 10 SG;

Bayer-Diagnostics, Leverkusen, Germany) to measure lung pH within the range of 4.5 to 8.0 as reported in our previous studies.^{7,8} Briefly, a piece of test strip was inserted by forceps for 15 to 20 seconds into the right lower lobe peripheral branch (DB9, DB10). The forceps with the strip were retracted from the working channel of the bronchoscope, and a pH value reading was obtained at the same moment. The color of strips changed from orange to green to blue in 0.5 pH value increments. The pH value was always read and verified by the same three medical staff, including one doctor and two assistants. The pH strip was advanced through a biopsy/suction channel of the fiber bronchoscope to avoid any contamination of the pH strip from the bronchial tree and other structures during the procedure. A catheter (18 cm long, 2 mm wide) was used for deep catheter aspiration to obtain aspirate from DB9 or DB10 peripheral bronchial branches. After the lumen of the catheter was completely filled with the aspirated fluid, the assistant used 1.5 mL of distilled water to extract the aspirate from the catheter. A minimum amount of distilled water was used to avoid any effect on inflammation markers, which were analyzed later.

Bronchoalveolar aspirate lactate dehydrogenase (BA-LDH), C-reactive protein (BA-CRP), and glucose (BA-Glu) levels were measured using an Architect c8000 clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA) within 1 hour. Levels of these parameters were also measured in the serum of participants (S-LDH, S-CRP, and S-Glu, respectively). Finally, identification of microorganisms from the bronchoalveolar aspirate was performed according to standard methods.⁹ Bacteria were only regarded as clinically significant in case of reaching $> 10^3$ cfu/mL. One pulmonologist (K.M.) with more than 30 years of

bronchoscopic experience conducted all endoscopic procedures in all of the subjects. There were no periprocedural complications or serious adverse effects related to study procedures.

For statistical analysis, the Student's *t*-test (analysis of variance) was used to compare group differences in quantitative variables (normally distributed data). The Mann-Whitney test was used for data that were not normally distributed. We also used Pearson's correlation coefficient (*r*) to determine the correlation of bronchoalveolar pH with bronchoalveolar LDH levels, bronchoalveolar glucose levels, and FEV₁. All analyses were conducted using Statistica 8 software (StatSoft Inc., Tulsa, OK, USA). A *p* value < 0.05 was considered statistically significant.

Results

A total of 84 patients were included with AECOPD without concomitant pneumonia or other significant respiratory disease other than COPD (e.g., pulmonary fibrosis, asthma, or bronchiectasis) who were consecutively admitted to hospital. We also included 42 age- and sex-matched patients with stable COPD (Figure 1). Anthropometric data are presented in Table 1. FEV₁ predicted was significantly lower in patients with AECOPD compared with those with stable COPD (*p* = 0.014, Table 1). PaO₂ (*p* = 0.006) was significantly lower in patients with AECOPD, while PaCO₂ (*p* = 0.019) was higher than that in patients with stable COPD (Table 1). The mean pH of bronchoalveolar fluid in patients with AECOPD was 6.89 ± 0.53,

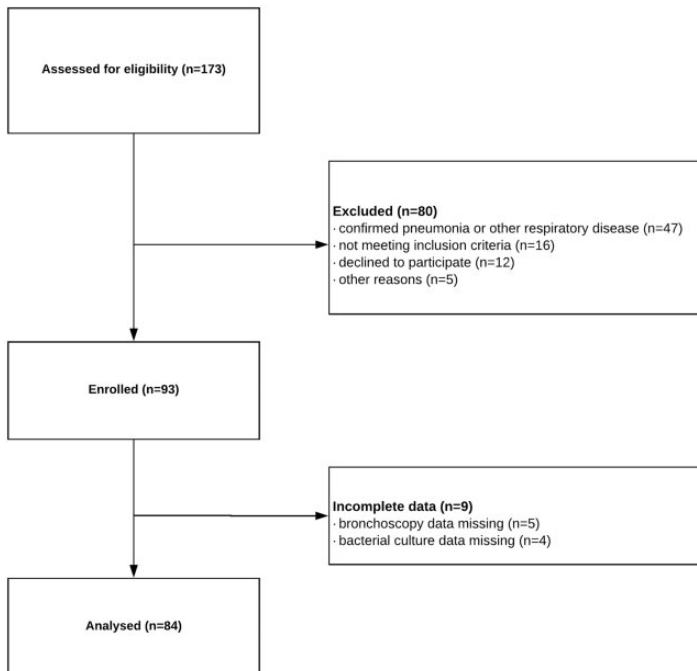


Figure 1. A flow diagram of all assessed patients (included and excluded). This figure shows the number of patients assessed for eligibility and number of patients completing the study. Only patients with acute exacerbation of chronic obstructive disease, who had completed the whole study protocol, were included in the present analysis.

Table 1. Anthropometrics, smoking status, pulmonary test function, and arterial blood gas data.

	AECOPD (n = 84)	Stable COPD (n = 42)	p
Age (years)	61.9 ± 8.1	62.4 ± 11.6	0.545
Sex, male	58 (69%)	16 (64%)	0.637
BMI (kg/m ²)	25.4 ± 5.2	24.7 ± 3.7	0.604
Current smoker	30 (35.7%)	14 (33.3%)	0.625
Pack years of smoking (current smokers)	38.6 ± 21.4	35.2 ± 18.9	0.593
Former smoker	45 (53.6%)	23 (54.8%)	0.609
Pack years of smoking (former smokers)	34.1 ± 23.1	32.8 ± 20.4	0.534
Non-smoker	9 (10.7%)	5 (11.9%)	0.612
FVC (L)	2.37 ± 0.73	3.14 ± 0.96	0.331
FVC (% predicted)	60.1 ± 15.9	85.7 ± 13.9	0.104
FEV ₁ (L)	1.27 ± 0.41	2.07 ± 0.68	0.156
FEV ₁ (% predicted)	41.02 ± 11.5	79.4 ± 11.2	0.014
FEV ₁ /FVC (%)	53.58 ± 14.4	66.52 ± 4.92	0.215
PaO ₂ (kPa)	8.6 ± 1.5	12.1 ± 0.61	0.006
PaCO ₂ (kPa)	5.7 ± 0.9	4.52 ± 0.39	0.019
Arterial blood pH	7.29 ± 0.3	7.47 ± 0.4	0.052

Data are presented as mean ± standard deviation or n (%). AECOPD, acute exacerbation of chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second; PaO₂, partial pressure of arterial oxygen; PaCO₂, partial pressure of arterial carbon dioxide. Statistical significance for group comparisons was tested using the Student's t-test or one sample t-test.

Table 2. Bronchoalveolar pH and other variables in bronchoalveolar fluid and serum in patients with AECOPD versus those with stable COPD.

	Bacteria isolated in AECOPD		Stable COPD controls (n = 42)
	No (n = 24)	Yes (n = 60)	
Lung pH	6.31 ± 0.55	7.13 ± 0.52*	6.21 ± 0.37‡
BA-LDH (U/L)	361 ± 250	1381 ± 1621*	124 ± 50 ^{†‡}
S-LDH (U/L)	173 ± 36	188 ± 24*	160 ± 25‡
BA-CRP (mg/L)	0.17 ± 0.34	0.14 ± 0.26	0.12 ± 0.11
S-CRP (mg/L)	16.8 ± 11.6	29.4 ± 11.2*	4.3 ± 2.6 ^{†‡}
BA-Glu (mmol/L)	1.6 ± 0.7	0.9 ± 0.7*	1.6 ± 0.6‡
S-Glu (mmol/L)	5.8 ± 0.7	6.1 ± 1.4	5.6 ± 1.2‡

Data are presented as mean ± standard deviation. AECOPD, acute exacerbation of chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; BA-LDH, bronchoalveolar lactate dehydrogenase; S-LDH, serum lactate dehydrogenase; BA-CRP, bronchoalveolar C-reactive protein; S-CRP, serum C-reactive protein; BA-Glu, bronchoalveolar glucose; S-Glu, serum glucose. **p* < 0.05, non-isolated bacteria versus isolated bacteria; [†]*p* < 0.05, non-isolated bacteria versus stable COPD controls; [‡]*p* < 0.05, isolated bacteria versus stable COPD controls.

which was significantly higher than that of patients with stable COPD (6.21 ± 0.37, *p* < 0.001). The mean pH of bronchoalveolar fluid in patients without isolated bacteria was significantly lower than that in

patients with isolated bacteria in the AECOPD patient group (*p* < 0.05, Table 2). No significant difference was found between lung pH in AECOPD patients according to smoking status (ex-smokers

versus current smokers: 6.67 ± 0.67 and 6.99 ± 0.63 , $p = 0.238$, respectively).

BA-LDH, S-LDH, and S-CRP levels were significantly lower in patients without isolated bacteria compared with those with isolated bacteria in the AECOPD patient group ($p < 0.05$; $p = 0.042$; $p = 0.028$, respectively) (Table 2). BA-Glu levels were significantly higher in patients without isolated bacteria compared with those with isolated bacteria in the AECOPD patient group ($p = 0.014$). No significant differences were found in BA-CRP and S-Glu levels between the AECOPD groups (Table 2). Patients with AECOPD had higher BA-LDH and S-CRP levels compared with patients with stable COPD, and this difference was more pronounced if a bacterial pathogen was isolated (all $p < 0.05$, Table 2). Significant differences were found in lung pH, and S-LDH, BA-Glu, and S-Glu levels ($p < 0.05$, $p < 0.05$, $p = 0.012$, and $p = 0.032$) only in patients

with isolated bacteria and AECOPD compared with patients with stable COPD (Table 2). Lung pH values were significantly correlated with BA-LDH levels ($r = 0.629$, $p < 0.001$) and BA-Glu levels ($r = 0.573$, $p < 0.001$) (Figures 2, 3). Lung pH was not correlated with FEV₁ in the AECOPD patient group ($r = -0.198$, $p = 0.070$). Furthermore, lung pH was not correlated with FEV₁ in the stable COPD patient group ($r = -0.093$, $p = 0.402$).

In 60 of 84 (71%) patients the following bacteria were isolated: *Haemophilus influenzae* (n = 15, 25%), *Streptococcus pneumoniae* (n = 11, 18%), *Moraxella catarrhalis* (n = 8, 13%), *Pseudomonas aeruginosa* (n = 6, 10%), *Escherichia coli* (n = 4, 7%), *Klebsiella pneumoniae*, Methicillin-sensitive *Staphylococcus aureus*, *Proteus mirabilis*, and anaerobic bacteria (n = 3, 5%), *Enterobacter cloacae* and *Serratia marcescens* (n = 2, 3%). Lung pH and levels of bronchoalveolar and serum parameters for

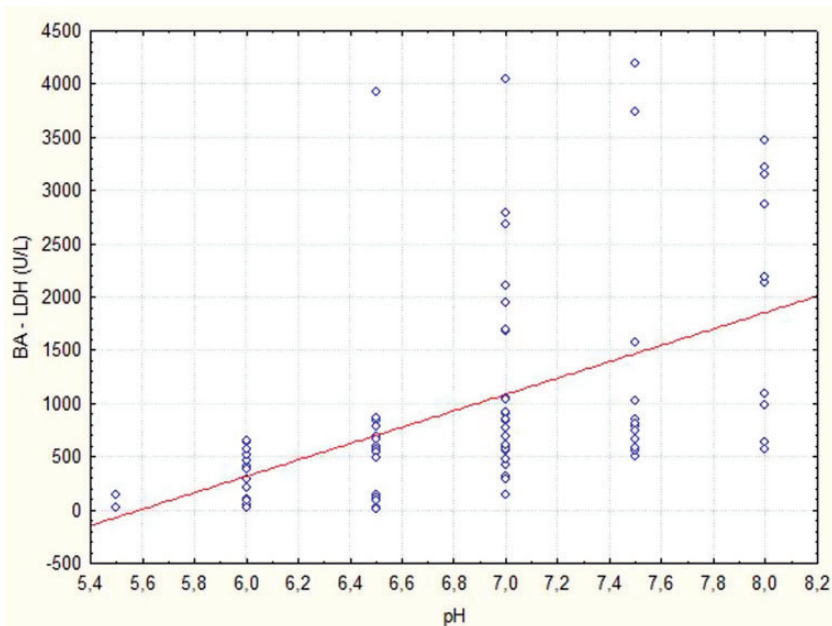


Figure 2. Relationship between bronchoalveolar lactate dehydrogenase (BA-LDH) and pH at acute exacerbation of chronic obstructive pulmonary disease.

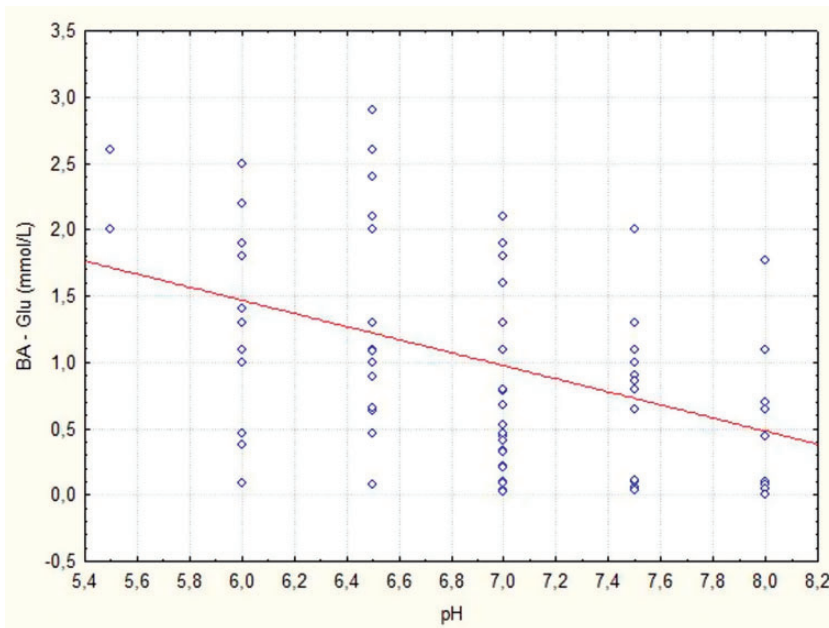


Figure 3. Relationship between bronchoalveolar glucose (Ba-Glu) levels and pH at acute exacerbation of chronic obstructive pulmonary disease.

the five most frequently isolated bacterial pathogens are shown in Table 3.

Discussion

This study showed that pH of bronchoalveolar fluid in patients with AECOPD was more alkaline compared with that in patients with stable COPD. Furthermore, the mean lung pH value in patients with isolated bacteria was higher than that in patients without isolated bacteria in the AECOPD patient group. These findings confirmed our hypothesis. In patients with stable COPD, the mean bronchoalveolar pH was 6.21 ± 0.37 and in patients with AECOPD it was 6.89 ± 0.53 , while pH was even more alkaline in the presence of bacteria. We speculate that the inflammatory process accompanied by bacterial metabolites contributed to the increase in pH. Previous studies have shown that sputum in patients with chronic bronchitis is

alkaline (7.6 and 7.8)¹⁰ and that the lower airway mucus pH is alkaline in patients with cystic fibrosis.¹¹ Lung pH in patients with pneumonia is approximately 7.3,¹² and patients with rhinitis have nasal mucosa pH values ranging from 7.2 to 8.3.^{13,14} These studies indicate that there is an association between alkaline mucosal pH and inflamed respiratory tract tissue. Our findings of arterial blood pH showed that there was a 3% lower arterial blood pH in patients with AECOPD compared with those with stable COPD, which is consistent with findings by Grumelli.¹⁵

Alkalinity in the lungs contributes to local tissue damage by promoting cellular injury, apoptosis, necrosis, and inflammation.¹⁰ In our study, amore alkaline bronchoalveolar pH values were significantly correlated with higher BA-LDH values, which indicated more intense tissue damage. Higher lung pH in exacerbation contributes to surfactant structural

Table 3. Lung pH and levels of parameters measured in bronchoalveolar fluid and serum for the five most frequently isolated bacterial pathogens.

	Lung pH	BA-LDH	S-LDH	BA-CRP	S-CRP	BA-Glu	S-Glu
<i>Haemophilus influenzae</i> (n = 15)	7.37 ± 0.64	2912 ± 2505	186 ± 19	0.03 ± 0.06	27.3 ± 13.5	0.9 ± 0.7	6.3 ± 1.4
<i>Streptococcus pneumoniae</i> (n = 11)	7.14 ± 0.56	959 ± 829	197 ± 28	0.21 ± 0.33	17.8 ± 8.7	1.1 ± 0.6	6 ± 1.4
<i>Moraxella catarrhalis</i> (n = 8)	7.25 ± 0.27	859 ± 133	186 ± 26	0.12 ± 0.20	15.1 ± 8.6	0.8 ± 0.7	5.8 ± 1.2
<i>Pseudomonas aeruginosa</i> (n = 6)	7.01 ± 0.45	1457 ± 1256	198 ± 21	0.09 ± 0.19	18.7 ± 9.8	0.8 ± 0.3	6.1 ± 1
<i>Escherichia coli</i> (n = 4)	6.75 ± 0.29	673 ± 121	186 ± 38	0.23 ± 0.37	27.1 ± 14.7	1.1 ± 1.3	6.2 ± 0.9

Data are presented as mean ± standard deviation. BA-LDH, bronchoalveolar lactate dehydrogenase; S-LDH, serum lactate dehydrogenase; BA-CRP, bronchoalveolar C-reactive protein; S-CRP, serum C-reactive protein; BA-Glu, bronchoalveolar glucose; S-Glu, serum glucose.

changes, which can result in ventilatory problems (microatelectasis). This could explain fluctuations in the patient's state.¹¹ To date, lung pH has most commonly been investigated in exhaled breath condensate (EBC), which represents a sampling method of the lower respiratory tract that is not invasive.¹² Several studies have shown that EBC pH is more acidic in patients with AECOPD/COPD compared with the healthy population.^{13,14} However, there are difficulties encountered in sampling and analysis of EBC. Detection and quantification of biomarkers in EBC, including cytokines, nitric oxide and pH, are compromised by many factors. These factors include the condenser type, cigarette smoking, ambient temperature, contamination of saliva, indoor humidity, liquid intake, volatile oral contaminants, and exhaled carbon dioxide as potential confounders, especially in patients with acute or chronic hypercapnic pulmonary insufficiency, which is often present in AECOPD and even in stable COPD.^{9,16–20} EBC is

only used in experimental conditions and is not validated for use in clinical practice. Based on these findings, we consider that direct bronchoalveolar fluid sampling provides a more accurate assessment of local lung pH and inflammatory biomarkers compared with EBC sampling. Bronchoalveolar fluid sampling thus provides better information for evaluating local lung tissue damage.

To define different phenotypes of COPD and to assess the therapy response, biomarkers of respiratory tract inflammation and oxidative stress could be useful. Our measurements of LDH and CRP levels from serum are not valid local pulmonary inflammation and tissue damage indicators. Obtaining these biomarkers from bronchoalveolar aspirate may represent a more specific alternative.

LDH is a cytoplasmic enzyme that is found in the majority of body cells, including the lungs. Detection of higher than normal range LDH values is used to identify cellular damage or death.²¹ The highest

BA-LDH values in the present study were observed in patients with isolated *Haemophilus influenzae* and *Pseudomonas aeruginosa*, which indicated the most active local inflammation, cell injury, and necrosis when the most pathogenic bacteria were present. If a bacterial pathogen was isolated, 7.4 times higher values of LDH were found in bronchoalveolar aspirate compared with serum, while if no bacterial pathogen was isolated, this ratio was only 2.1. In patients with stable COPD, mean S-LDH and BA-LDH levels were 160 ± 25 and 124 ± 50 UL, respectively. Compared with these values, S-LDH and BA-LDH in patients with non-isolated bacteria in the AECOPD patient group were 1.1 and 2.9 times higher, respectively; and if a bacterial pathogen was isolated, this ratio was 1.2 and 11.2, respectively. Higher BA-LDH levels in bronchoalveolar aspirate in patients with AECOPD, especially if a bacterial pathogen was isolated, compared with patients with stable COPD suggests the presence of local inflammation, cell injury, necrosis, and disease activity. Our finding indicates that BA-LDH is a relevant biomarker of tissue damage in the lungs and it may reliably be used to estimate bacterial infection in AECOPD. However, only 9% of patients had S-LDH values ≥ 220 U/L (above the reference range). This finding indicated that in patients with AECOPD, S-LDH levels were relatively normal, suggesting a low systemic inflammatory response.

CRP is a marker of inflammation, and CRP levels are increased during underlying inflammatory conditions.²² Some studies have shown an association of higher systemic CRP levels and other inflammatory biomarkers in patients with COPD compared with healthy patients.²³⁻²⁵ A study by Bircan et al.²⁶ showed that increased CRP values in serum are an indicator of COPD exacerbation caused by a respiratory infection. In the present study, we found

slight, but significantly higher S-CRP levels in patients with AECOPD compared with those with stable COPD, which were even higher if a bacterial pathogen was isolated. Recently, Peng et al.²⁷ showed that, to identify the bacterial origin of AECOPD, the cut-off value of CRP was 19.65 mg/L. S-CRP appears to be a better indicator of a systemic inflammatory response in patients with AECOPD than S-LDH. However, BA-CRP levels were low in our study. Our findings suggest that BA-CRP levels should not be measured because their levels were low or even hardly detectable, with no difference in measured levels among the groups.

While blood glucose levels are not related to mortality, hospital stay duration, or re-hospitalization in patients with AECOPD,²⁸ detection of glucose in bronchoalveolar aspirate is associated with the presence of pathogenic bacteria.^{29,30} In the present study, if bacteria were isolated in patients with AECOPD, BA-Glu levels were 1.8 times lower than those in patients without bacterial isolation, while no difference was found in S-Glu levels. Therefore, results for BA-Glu levels could also serve as a good predictor of bacterial presence.

We also investigated bacterial pathogens to evaluate the level of inflammation and pH values in relation to bacterial pathogens. This study was based on identification of bacterial etiology directly from bronchoalveolar aspirate, and not from sputum, as performed in previous studies.^{31,32} Bacterial etiology is predominant as an origin of inflammation in AECOPD. In this study, bacterial pathogens were found in 71% of patients in contrast to 24% in study by Boixeda et al.³³ We believe that the reason for this difference between studies was due to direct analysis of bronchoalveolar fluid instead of sputum samples because sputum samples are vulnerable to sampling errors. We isolated 42 (70%) Gram-negative bacteria and 18 (30%)

Gram-positive bacteria. Microbiological patterns were similar as shown by Siddiqi et al.³⁴

There are two possible limitations in the present study. First, our study was performed on a relatively low number of subjects and thus the results have to be viewed with caution. Second, a semi-quantitative technique (pH test strips) was used to directly measure pH in the lungs, and ideally, a more precise method to determine pH would be better. However, we believe that the technique that we used is an acceptable substitute. Recently, this technique has been shown many times to be efficient in studies performed by our research group.^{12,13}

In conclusion, this study shows a strong pathological process in the lungs of patients with COPD during AECOPD. This pathological process is present, even in cases with scarce clinical symptoms of deterioration or scant or no pathological findings in peripheral blood, such as CRP and LDH levels, especially in the presence of a bacterial pathogen. More alkaline bronchoalveolar pH values are a clear indicator of more intense tissue damage. There is no correlation between bronchoalveolar pH and FEV₁ in patients with AECOPD and those with stable COPD. FEV₁ measurement does not adequately express the effect of COPD on the quality of life of patients. Therefore, measurement of local bronchoalveolar pH and proposed inflammatory mediators in bronchoalveolar fluid in some patients could be useful as an additional test for diagnosing the severity of AECOPD. Although somewhat invasive, analyses of lung pH and inflammatory bronchoalveolar and serum biomarkers represent a feasible and easy method for repeated assessment of systemic and local inflammation in AECOPD. Further research is required to fully determine the importance and influence of bacterial culture, bronchoalveolar pH, and

inflammatory biomarkers regarding clinical intervention and patient care in patients with AECOPD.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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