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*Original Article*

# Elevated plasma cathepsin B and cystatin C levels in chronic obstructive pulmonary disease

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**Abstract:** Background: The aim of this study was to investigate differential changes in plasma levels of cathepsin B and its naturally inhibitory protein cystatin C in chronic obstructive pulmonary disease (COPD) patients during and 2 weeks as well as 8 weeks after acute exacerbation (AE). Materials and methods: Forty six COPD patients, including 44 male and 2 female, were included in this study. Plasma were collected in three different times, i.e., during, and 2 weeks as well as 8 weeks after AE. Plasma cathepsin B and cystatin C levels were measured in 46 adult patients with COPD and 18 healthy controls using a commercial enzyme-linked immunosorbent assay (ELISA). Results: The plasma levels of cathepsin B were significantly higher in COPD patients at 2 weeks and 8 weeks after AE when compared with those of healthy subjects. The plasma level of cystatin C showed significantly higher than the plasma levels of healthy subjects at time of AE, also 2 weeks and 8 weeks after AE. However, there was no significant difference between the time of AE and 2 or 8 weeks after AE. Conclusions: The persistently significant higher plasma levels of cystatin C in COPD patients not only on AE but also at 2 and 8 weeks after AE than those in healthy subjects might represent a chronic inflammatory status in COPD. Moreover, plasma level of cathepsin B significantly increased at 2 weeks after AE and which returned to be non-significant at 8 weeks after AE in COPD patients. These findings might hint cathepsin B is one of the acute phase reactive protein in AE of COPD.

**Keywords:** Cathepsin B, cystatin C, chronic obstructive pulmonary disease

## Introduction

Chronic obstructive pulmonary disease (COPD) is a disease of blocked airflow and no medication is available that provides full recovery. COPD is caused by abnormal inflammation of the lungs due to harmful micro-particles or gases, and the deterioration is progressive [1]. Characteristics of COPD include chronic systemic inflammation at the airways, lung parenchyma, and lung vessels. The number of macrophages, T cells (mostly CD8+) and neutrophils increases in various parts of the lungs. Activated inflammatory cells would release many mediators, such as CRP [2-4], interleukin-6 (IL-6) [2, 4, 5], interleukin-8 (IL-8) [6, 7] and tumor necrosis factor (TNF) [8, 9], which can damage lung structure and prolong neutrophil-caused inflammation.

Cathepsins are also called cysteine cathepsins because of a cysteine residual at the activation site [10]. Pathological studies have shown that cathepsin B is associated with rheumatoid arthritis [11] and cancers [12, 13]. Furthermore, cathepsin B can also be found in respiratory secretions [14]. Bronchial epithelial cells would secrete inactive cathepsin B, which is later activated by neutrophil elastases [14]. Together with neutrophil elastase, activated cathepsin B can hydrolyze the extracellular matrix, causing damage to tissues [15].

Cystatin C, a type of endogenous cysteine protease inhibitor, is a non-glycosylated basic protein. Clinically, cystatin C concentration is an ideal index for monitoring glomerular filtration rate (GFR) [16]. In addition, cystatin C is also related to modulating inflammatory responses

**Table 1.** Laboratory data of both controls and patients with chronic obstructive pulmonary disease (COPD)

| | Control (N=18) | COPD with AE (N=46) | *p* value |
|---|---|---|---|
| Gender | | | |
| Male | 10 (55.6%) | 44 (95.7%) | |
| Female | 8 (44.4%) | 2 (4.3%) | |
| Age | 65.17 ± 2.63 | 71.78 ± 1.31 | P=0.016 |
| FVC (%) | 85.12 ± 6.94 | 69.02 ± 2.47 | P=0.008 |
| FEV1 (%) | 99.18 ± 6.15 | 45.45 ± 1.94 | P<0.001 |
| CRP | 0.88 ± 0.28 | 4.32 ± 0.81 | P=0.007 |
| WBC (/mm$^3$) | 5852.22 ± 353.89 | 10306 ± 515.23 | P<0.001 |
| Neutrophils (/mm$^3$) | 3735.87 ± 325.22 | 7784.07 ± 521.59 | P<0.001 |
| Creatinine | 0.87 ± 0.07 | 1.12 ± 0.06 | P=0.032 |
| GFR | 86.24 ± 5.47 | 72.97 ± 4.21 | P=0.123 |

[17] and apoptosis [18]. Therefore, one can evaluate the progress or occurrence of diseases by monitoring concentration changes in cystatin C in the extracellular fluid or serum [19-22]. However, to the best of our knowledge, no study has investigated the prognostic value of cathepsin B and cystatin C in a cohort of patients with COPD. Therefore, we compared the plasma cathepsin B and cystatin C concentrations of COPD patients before and after acute exacerbation.

## Materials and methods

### *Subjects and specimen collection*

The experiment specimens were collected from patients admitted to the Emergency Department of Puli Christian Hospital. The investigators first screened the patients; those COPD patients with cancers, inflammatory diseases (e.g., rheumatoid arthritis, liver inflammation, gingivitis and respiratory inflammation), severe heart failure (e.g., NYHA class IV), and asthma or with no smoking history (less than 10 packs per year) were excluded. In addition, we also recruited 18 healthy subjects (10 males and 8 females) to be the control. After obtaining informed consent from all subjects, the investigators collected 3 mL lithium heparin-anticoagulant and 2 mL K2-EDTA-anticoagulant blood specimens from all of the 46 COPD subjects at AE, two weeks after AE, and eight weeks after AE for stability follow-up. But the blood samples from the eighteen healthy subjects were collected only once to be the control. The collected specimens were used for neutrophil count, the CRP test, and quantitative protein analysis for cathepsin B and cystatin C. After adding the blood, the tubes were slightly mixed to prevent coagulation and hemolysis. Tubes containing lithium heparin were centrifuged at 3500 rpm for ten minutes. Plasma at the upper layer was then collected and used for CRP, cathepsin B and cystatin C analyses. Tubes with K2 EDTA were not centrifuged but used for neutrophil count (whole blood). Before commencement of this study, approval was obtained from the Institutional Review Board of Chung Shan Medical University Hospital, and informed written consent to participate in the study was obtained from each person.

### *Measurements of plasma cathepsin B and cystatin C*

The enzyme-linked immunosorbent assay (ELISA) was used to measure the plasma levels of cathepsin B and cystatin C in the blood samples (R&D Systems, Abingdon, UK) [23]. For each plasma sample, 100 μL was directly transferred to the microtest strip wells of the ELISA plate and subsequently incubated for 2 hours at room temperature. After 3 washing steps, the detection antibody was added, and the reaction was incubated for 2 hours at room temperature. Antibody binding was detected with streptavidin-conjugated horseradish peroxidase and developed with a substrate solution. Next, the reaction was stopped, and optical density was determined with a microplate reader set at 450 nm. Cathepsin B and cystatin C levels were quantitated with a calibration curve using human cathepsin B and cystatin C standards.

### *Statistical analysis*

SPSS software was used for statistical analysis. An independent *t*-test was used to compare COPD patients with the healthy control in terms of cathepsin B, cystatin C, neutrophils and CRP. A paired *t*-test was used to compare COPD patients for test results obtained at AE, two weeks after AE, and eight weeks after AE for

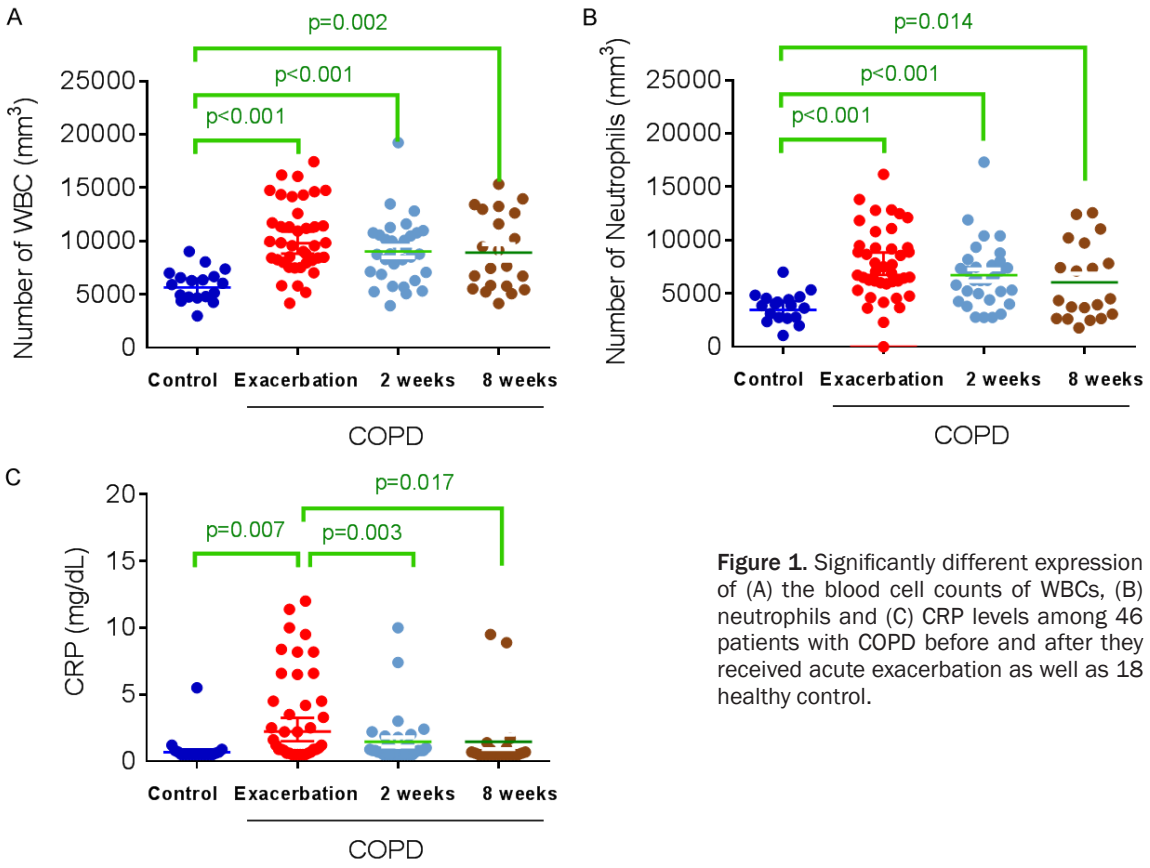


**Figure 1.** Significantly different expression of (A) the blood cell counts of WBCs, (B) neutrophils and (C) CRP levels among 46 patients with COPD before and after they received acute exacerbation as well as 18 healthy control.

stability follow-up. The acquired data were expressed as mean ± SE. Spearman's rank correlation was used for linear regression analysis to determine whether subjects' cathepsin B, cystatin C, neutrophils and CRP are correlated with the progress of COPD.

## Results

A summary of the demographic and clinical characteristics of the participants was presented in **Table 1**. The study included two different study groups, control subjects and COPD patients. The mean age of COPD patients in our data was 71.78 ± 1.31 and of healthy controls was 65.17 ± 2.63 (P=0.016). FVC (%) of the control group was 85.12 ± 6.94 and of the COPD group was 69.02 ± 2.47 (P=0.008). FEV1 (%) of the control group was 99.18 ± 6.15 and of the experimental group was 45.45 ± 1.94 (P<0.001). The blood cell counts of WBCs and neutrophils as well as CRP levels were significantly elevated in patients with COPD (WBCs, 10306/mm$^3$; neutrophils, 7784/mm$^3$ and CRP level, 4.32) compared with those controls (WBCs, 5852/mm$^3$; neutrophils, 3736/mm$^3$ and CRP level, 0.88) (P<0.001).

The blood cell counts of WBCs were significantly higher before treatment of COPD patients (10306 ± 515/mm$^3$) compared with those control (5852 ± 353/mm$^3$), two weeks after AE (9029 ± 533/mm$^3$), as well as eight weeks after AE for stability follow-up (8935 ± 775/mm$^3$) (**Figure 1A**). The similar results were found in the neutrophils counts as well as CRP levels (**Figure 1B** and **1C**).

**Figure 2A** presents the Cathepsin B levels in patients with COPD and the controls. The patients at 2 weeks and 8 weeks after AE exhibited significantly higher plasma Cathepsin B levels than the controls did (controls: 49.84 ± 4.75 ng/mL; patients at 2 weeks: 69.40 ± 6.06 ng/mL; P=0.048; patients at 8 weeks: 64.79 ± 4.81 ng/mL; P=0.034). **Figure 2B** presents the Cystatin C levels in patients with COPD and the controls. The COPD patients at AE, two weeks after AE, and eight weeks after AE follow-up exhibited significantly higher plasma Cystatin C

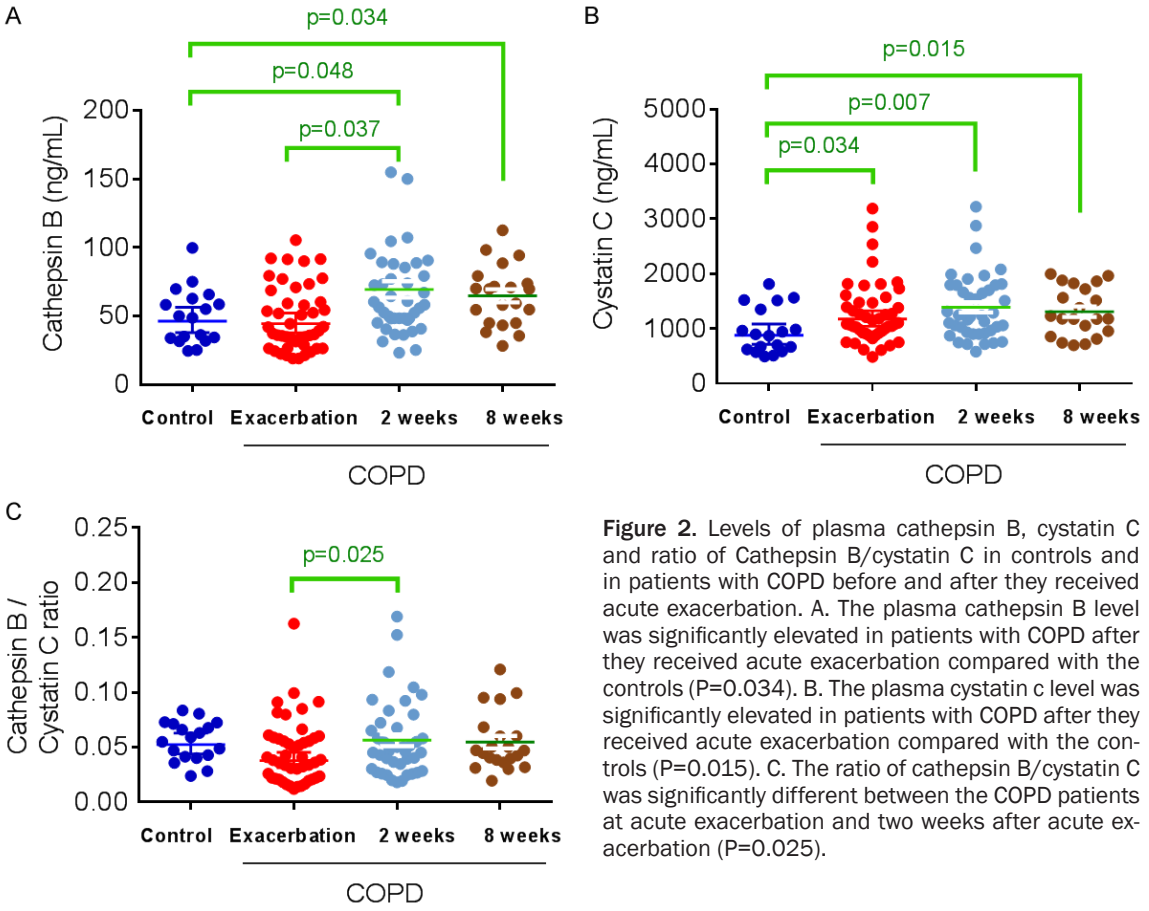


**Figure 2.** Levels of plasma cathepsin B, cystatin C and ratio of Cathepsin B/cystatin C in controls and in patients with COPD before and after they received acute exacerbation. A. The plasma cathepsin B level was significantly elevated in patients with COPD after they received acute exacerbation compared with the controls (P=0.034). B. The plasma cystatin c level was significantly elevated in patients with COPD after they received acute exacerbation compared with the controls (P=0.015). C. The ratio of cathepsin B/cystatin C was significantly different between the COPD patients at acute exacerbation and two weeks after acute exacerbation (P=0.025).

levels than the controls did (controls: 958 ± 98 ng/mL; AE: 1278 ± 84 ng/mL; P=0.034; patients at 2 weeks: 1390 ± 93 ng/mL; P=0.007; patients at 8 weeks: (1310 ± 96 ng/mL; P=0.015). The results of cathepsin B/cystatin C ratio were presented in **Figure 2C**. In COPD patients, the ratio of cathepsin B/cystatin C was significantly different between the COPD patients at AE (0.045 ± 0.004) and two weeks after AE (0.056 ± 0.005) (P=0.025).

## Discussion

Cathepsin B and cystatin C are both secreted proteins and clinically, they can be detected in the body fluids or blood specimens of humans. Some studies have shown that cathepsin B and cystatin C are closely associated with the occurrence of some inflammatory diseases [17, 20, 24-27].

Interestingly, it can be found in **Figure 2A** that there was a trend of increase in cathepsin B from at AE to two weeks post AE. This may be because COPD is a slowly progressing inflammatory response, and it is harder to see the response at the early onset of COPD. In contrast, there was a reversed increase of cathepsin B two weeks post AE (**Figure 2A**), which is the opposite of the finding that showed a drop in cathepsin B two weeks post AE in patients with pelvic inflammation [27]. In addition, in that pelvic inflammation study, cystatin C dropped before treatment but returned to normal after treatment. In this study, however, cystatin C in COPD patients was higher than in the control subjects (**Figure 2B**). The study of Chung et al., showed that cystatin C in patients with oral mucosal fibrosis was higher than in normal subjects [28]. In comparison, Chu et al. examined the association among cathepsin B, cystatin C and gout and showed an abnormal elevation of these two enzymes in chronic inflammation [26], suggesting that the higher concentration of cystatin C in COPD patients may be due to a compensatory mechanism in chronic inflammation, which explains why cystatin C in COPD patients is constantly higher than in the control subjects.

One limitation of this study is the lack of study subjects, and the fact that the expected number of subjects was not reached. As a result, the present findings may not be adequate for representing the entire experiment. Secondly, there was a significant drop in the number of subjects for the second and the third blood sampling compared to first time, and therefore, the investigators have to be more careful with recruiting and tracking study subjects. Third, because COPD is a systemic inflammatory disease, inflammatory symptoms at other parts of the body may also affect the results of the study. It is therefore necessary to be strict on the recruitment criteria. Lastly, it is important to recruit more people or to collect partial pulmonary specimens, such as tracheal fluid, to increase the accuracy and comprehensiveness of the results.

In conclusion, the persistently significant higher plasma levels of cystatin C in COPD patients not only on AE but also at 2 and 8 weeks after AE than those in healthy subjects might represent a chronic inflammatory status in COPD. Moreover, plasma level of cathepsin B significantly increased at 2 weeks after AE and returned to be non-significant at 8 weeks after AE in COPD patients. These findings might hint cathepsin B is one of the acute phase reactive protein in AE of COPD.

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## Disclosure of conflict of interest

None.

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