

# Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease

Tania Ahmed Shakoori<sup>a,\*</sup>, Don D. Sin<sup>b</sup>, Farkhanda Ghafoor<sup>c</sup>, Saira Bashir<sup>c</sup> and S. Nazim Hussain Bokhari<sup>a</sup>

<sup>a</sup>*Shaikh Zayed Medical Complex, (SZMC), Lahore, Pakistan*

<sup>b</sup>*The Providence Heart and Lung Institute, St. Paul's Hospital, The University of British Columbia, BC, Canada*

<sup>c</sup>*National Health Research Complex (NHRC), SZMC, Lahore, Pakistan*

**Abstract.** *Background:* There is a paucity of lung specific biomarkers to diagnose exacerbations of chronic obstructive pulmonary disease (COPD) and to track their progression. Surfactant protein D (SP-D) is a pulmonary collectin regulating the innate immunity of the lung and its serum expression is perturbed in COPD. However, it is not known whether serum levels change during exacerbations. We sought to determine whether serum SP-D levels are raised in COPD exacerbations.

*Objectives:* To determine whether or not patients with exacerbations have elevated serum SP-D levels compared with asymptomatic controls, stable disease.

*Study design:* case control study.

*Methods:* We measured serum SP-D levels from patients with stable COPD ( $n = 14$ ), patients experiencing acute exacerbations ( $n = 13$ ) and in control subjects ( $n = 54$ ) using a specific immunoassay and compared the levels using analysis of variance.

*Results:* Serum SP-D levels were significantly increased in patients who experienced an acute exacerbation ( $227 \pm 120$  ng/mL) compared to patients with stable disease ( $151 \pm 83$  ng/mL) or control subjects ( $128 \pm 65$  ng/mL;  $p = 0.003$ ). Serum SP-D levels were also found to be inversely related to various lung function parameters including FEV<sub>1</sub>/FVC% predicted.

*Conclusions:* Our study suggests that serum SP-D levels are increased in patients during exacerbations and may be a potential diagnostic biomarker for COPD exacerbations.

**Keywords:** Surfactant protein D, serum biomarker, COPD, exacerbations, diagnostic

## Abbreviations

|        |                                       |                       |  |
|--------|---------------------------------------|-----------------------|--|
| ANOVA: | analysis of variance                  | FEF <sub>2575</sub> : | Forced Expiratory Flow between 25% and 75% of expired forced vital capacity liters per second. |
| BALF:  | Bronchoalveolar Lavage Fluid          | FEV <sub>1</sub> :    | Forced expiratory volume in first second in liters   |
| BMI:   | Body mass index                       | FVC:                  | Forced vital capacity in liters  |
| CI:    | Confidence Interval                   | GOLD:                 | Global Initiative for Chronic Obstructive lung disease   |
| COPD:  | Chronic Obstructive Pulmonary disease | IRB:                  | Institutional Review Board   |
| ELISA: | Enzyme linked immunosorbent assay     | NHLBI:                | US National Heart, Lung, and Blood Institute.  |
|        |                                       | NHRC:                 | National Health Research Complex   |
|        |                                       | PEF:                  | Peak expiratory flow in liters per second  |
|        |                                       | SP-D:                 | Surfactant Protein D   |

\*Corresponding author: Dr. Tania Ahmed Shakoori. E-mail: drtaniashakoori@yahoo.com.

## 1. Introduction

Chronic obstructive pulmonary disease (COPD) “is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients” [1]. The current definition of COPD recognizes it as a systemic disease. This understanding of the disease has reinforced the search for a clinically usable serum biomarker for the disease. Such a biomarker may aid in the diagnosis, classification, staging, and prognostication of COPD patients. Surfactant protein D (SP-D) is one such molecule that has been interrogated as a possible biomarker in COPD [2]. SP-D is one of the four known pulmonary surfactant proteins in the lung, which are produced primarily by type II pneumocytes. Its principal functions are to regulate innate immunity of the lung [3] and assist in efferocytosis, which is necessary for regeneration of new alveolar cells and lung homeostasis [4,5]. Serum SP-D levels associate with lung function and with health status of patients with severe COPD [6]. However, it is not known whether serum SP-D levels are modified by exacerbations. The principal aim of this study was to compare SP-D levels between patients during exacerbations and those with stable disease. Because lungs become more permeable with injury and infection, causing increased translocation of lung-derived proteins across the lung-blood barrier, we hypothesized that patients with exacerbations would have increased serum SP-D levels compared with patients with stable disease.

## 2. Materials and methods

The study was approved by the ‘Ethical and Scientific Committee’ of the ‘Institutional Review Board’ of Shaikh Zayed Medical Complex (SZMC), Lahore, Pakistan (IRB reference number for the project is 1011). Smoker ( $n = 41$ ) and non smoker controls ( $n = 13$ ) were recruited from the general population of Lahore, Pakistan by advertising through posters at the major hospitals in the city and selected public places around SZMC. Cases ( $n = 27$ ) were chosen from the outpatient department and inpatient wards of SZMC among those who had respiratory symptoms of chronic cough and sputum production, a clinical diagnosis of COPD and demonstrated airflow obstruction on spirometry (Spirolab 2, SDI Diagnostics, 10 Hampden Drive, Easton, MA 02375). Airflow obstruction was defined by a forced expiratory volume in one second to forced

vital capacity (FEV<sub>1</sub>/FVC) ratio of < 70% following 200 µg of inhaled salbutamol administered through a spacer (Salbo, Getz Pharma, KIA, Karachi, Pakistan). The severity of COPD was classified based on FEV<sub>1</sub> as a percentage of predicted [1]. Control subjects included asymptomatic never smokers and current or ex-smokers who did not demonstrate any airflow limitation by spirometry. All subjects (cases and controls) were ethnically similar men (Punjabi Pakistanis). Smoking history was defined using pack-years. Exacerbations were defined as increase in any or all of the three major symptoms (dyspnea, sputum volume and sputum color) from day to day routine in accordance with the definition of Burge and Wedzicha [7]. Blood collection and spirometry were done at the same time in order to accurately correlate lung function with serum SP-D levels. For the hospitalized patients experiencing severe exacerbations, this was 7 to 10 days after the onset of exacerbation when patients were stable enough to perform the spirometric maneuver. In case of out-patients experiencing less severe exacerbations, spirometry and blood sampling were done when they first presented to the hospital. For each subject, venipuncture was performed and serum was extracted from 2cc of collected blood by allowing the blood to clot for at least 30 minutes and then centrifuging at 5000 rpm for 10–15 minutes at 4°C. The SP-D assay was performed using commercially available enzyme linked immunosorbent assay kits (BioVendor – Laboratorní medicína a.s. CT Park Modrice, Evropska 873 664 42 Modrice, Czech Republic) in the Immunoassay laboratory of National Health Research Complex (NHRC), SZMC, Lahore. Temperature conditions were strictly observed for carrying out the procedure as prescribed by the manufacturer. For precision of assay, calibrated pipettes/ multi channel from Gilson were used throughout the assay procedure and washing was performed with automatic plate washer. Informed consent was obtained from all study subjects.

## 3. Statistical analysis

All data were analyzed using SPSS for windows, version 11. SP-D data are expressed as mean ± SD in ng/ml unless otherwise specified. Serum SP-D levels were transformed to a natural logarithm to achieve normality of distribution and to mitigate the influence of extreme outliers. The average serum levels among the three groups (controls, stable COPD and exacerbators) were compared using analysis of variance and us-

Table 1  
Characteristics of the subjects in the study

| Characteristic                       | Controls  | Stable COPD                            | Exacerbators                           |
|--------------------------------------|---|--|--|
| Subjects                             | 54  | 14                                     | 13                                     |
| Age-yrs (mean $\pm$ SD)              | 36 $\pm$ 11   | 62 $\pm$ 11                            | 60 $\pm$ 13                            |
| Smoking status                       | Never smokers = 13<br>Current smokers = 35;<br>ex-smokers = 5 | Current smokers = 8;<br>ex-smokers = 6 | Current smokers = 9;<br>ex-smokers = 4 |
| Pack-years (mean+SD)                 | 16 $\pm$ 20   | 75 $\pm$ 41                            | 53 $\pm$ 23                            |
| BMI kg/m <sup>2</sup> *              | 26 $\pm$ 6  | 22 $\pm$ 4                             | 22 $\pm$ 5                             |
| FEV <sub>1</sub> /FVC (%)            | 87 $\pm$ 6  | 55 $\pm$ 13                            | 54 $\pm$ 10                            |
| FEV <sub>1</sub> %predicted          | 98 $\pm$ 13   | 50 $\pm$ 24                            | 45 $\pm$ 21                            |
| DRUG INTAKE**                        |   |  |  |
| Inhaled Short acting beta 2 agonists | None  | 8/14                                   | 11/13                                  |
| Inhaled Anti cholinergics            | None  | 8/14                                   | 11/13                                  |
| Inhaled steroids                     | None  | 8/14                                   | 11/13                                  |
| Oral steroids/ parenteral steroids   | None  | None                                   | 4/13                                   |
| Serum SP-D levels (ng/mL)            | 127 $\pm$ 65  | 151 $\pm$ 83                           | 227 $\pm$ 120                          |

\*BMI = Body Mass Index.

\*\*Drug intake at the time of sampling.

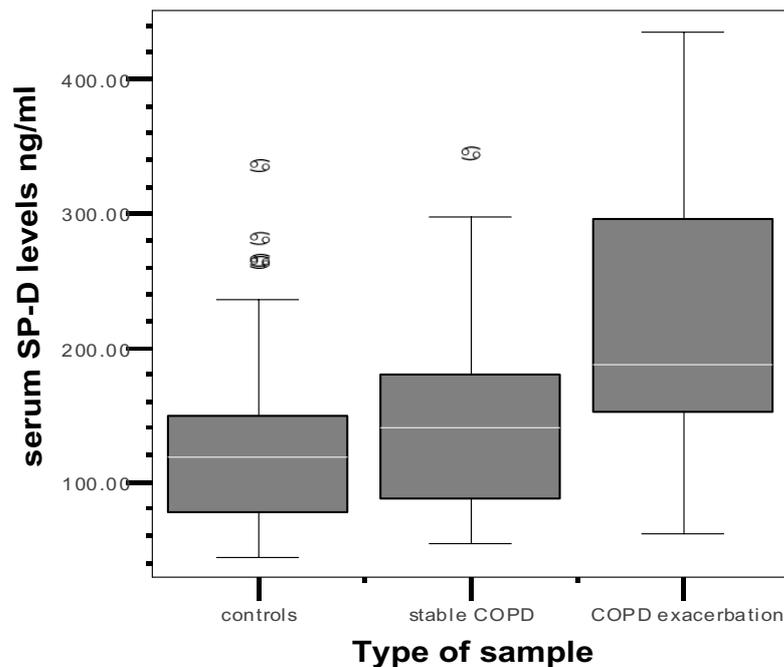


Fig. 1. Serum Surfactant protein levels in controls, stable COPD and COPD exacerbations  $p = 0.003$  (ANOVA).

ing controls as the referent group. Multiple regression modeling was performed to determine the independent relationship between exacerbation and ln-SP-D (natural logarithm of SP-D) with adjustments for covariates including age, body mass index, smoking status, pack years and systemic steroid use at the time of blood sampling. Lung function variables were also regressed against the natural log of SP-D while adjusting for age, BMI, and smoking status. As a sensitivity analysis, we repeated these analyses after excluding four subjects

who were taking systemic steroids at the time of sampling. A 'p' value of less than 0.05 was considered statistically significant.

#### 4. Results

All participants ( $n = 81$ ) in the study were men. Their baseline characteristics are shown in Table 1. As illustrated in Fig. 1, mean SP-D levels increased se-

Table 2  
The relationship between serum surfactant protein-d levels and various clinical and demographic factors

| Predictors   | Unadjusted coefficients |           | Adjusted coefficients | P value | 95% CI      |             |
|--|-------------------------|-----------|-----------------------|---------|-------------|-------------|
|  | Beta                    | Std error | Beta*                 |         | Lower bound | Upper bound |
| Age, for 1 year increment                          | 0.004                   | 0.007     | 0.111                 | 0.605   | -0.010      | 0.018       |
| Body mass Index, for 1 kg/m <sup>2</sup> increment | -0.010                  | 0.012     | -0.101                | 0.424   | -0.035      | 0.015       |
| Non smoker versus smokers                          | 0.100                   | 0.145     | 0.082                 | 0.492   | -0.190      | 0.390       |
| Smoking history, per 1 pack-year increment         | -0.006                  | 0.003     | -0.364                | 0.060   | -0.012      | 0.000       |
| Stable COPD  | 0.382                   | 0.235     | 0.279                 | 0.109   | -0.087      | 0.852       |
| COPD exacerbation                                  | 0.553                   | 0.238     | 0.404                 | 0.023   | 0.078       | 1.029       |
| Systemic steroids at the time of sampling          | 0.239                   | 0.317     | 0.105                 | 0.453   | -0.395      | 0.874       |

Model summary:  $R = 0.481$ ,  $R^2 = 0.232$ , Adjusted  $R^2 = 0.139$ .

\*Beta represents the multiple linear regression coefficient for serum surfactant D (in natural logarithm; ng/ml) after adjustments for age, BMI, smoking status, pack years, use of systemic steroids, and disease status ( $n = 81$ ).

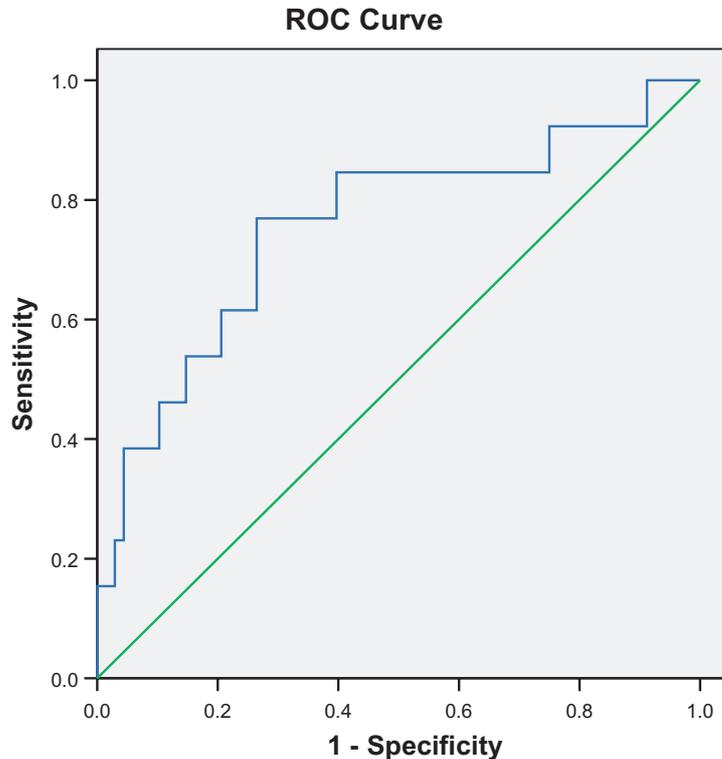


Fig. 2. ROC curve with all subjects included. (AUC = 0.76).

quentially from controls ( $128 \pm 65$  ng/mL; mean  $\pm$  SD) to stable COPD ( $151 \pm 83$  ng/mL) and COPD exacerbations ( $227 \pm 120$  ng/mL), respectively ( $p = 0.003$ ). These data were driven largely by differences in serum SP-D levels between controls and patients with COPD exacerbations ( $p = 0.001$  for pairwise comparison). Multiple regression analysis revealed exacerbation to be the only significant predictor of serum SP-D

levels at  $p = 0.023$  (see Table 2). Smoking history also showed some association, ( $p = 0.06$ ). When we regressed various lung functions against ln-SP-D with adjustments for age, BMI and smoking status, we found FEV<sub>1</sub>/FVC % predicted ( $p = 0.018$ ), PEF% predicted ( $p = 0.013$ ) and FEF<sub>2575</sub>% predicted ( $p = 0.003$ ) to be inversely and significantly associated with serum SP-D levels (see Table 3). Figure 2 shows the association of

Table 3  
The relationship between lung function parameters and serum surfactant protein-D levels

| Lung function                    | Adjusted Beta coefficient* | 95 % CI          | P value |
|----------------------------------|----------------------------|------------------|---------|
| FEV1% predicted                  | -0.004                     | -0.011 to -0.002 | 0.192   |
| FEV <sub>1</sub> /FVC %predicted | -0.007                     | -0.013 to -0.001 | 0.018   |
| PEF % predicted                  | -0.008                     | -0.014 to -0.002 | 0.013   |
| FEF <sub>2575</sub> % predicted  | -0.007                     | -0.012 to -0.003 | 0.003   |

\*Beta represents the multiple linear regression coefficient for serum surfactant D (in natural logarithm; ng/ml) after adjustments for age, BMI, smoking status and pack years ( $n = 81$ ).

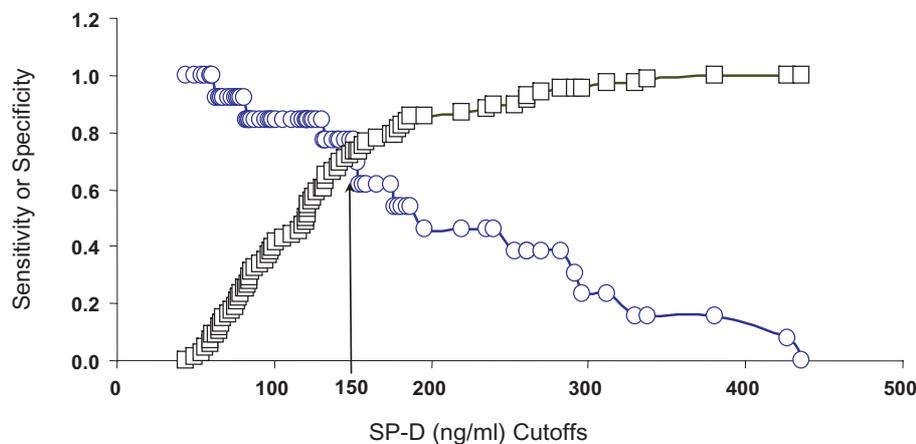


Fig. 3. The sensitivity and specificity of predicting exacerbation for various cutoff values of serum surfactant protein-D. The circles represent sensitivity while the squares represent specificity. As the SP-D values increase, sensitivity decreases, while the specificity of the assay increases. The best cutoff is the value at which there is intersection of the specificity and sensitivity curves. In this study, we observed such intersection at SP-D value of 150 ng/ml.

#### lnSP-D with FEV<sub>1</sub>/FVC % predicted.

As systemic steroids can confound the relationship between lung function and serum SP-D levels, we repeated the multiple regression analysis after excluding four patients who were taking systemic steroids at the time of blood collection. In this analysis ( $n = 77$ ), we again found that exacerbation status was most significantly associated with serum SP-D levels (beta coefficient, 0.57;  $p = 0.018$ ). Moreover, with the exclusion of steroid-treated patients, FEV<sub>1</sub>%predicted became significantly associated with serum SP-D levels (beta coefficient, -0.005;  $p = 0.049$ ). FEV<sub>1</sub>/FVC % predicted was also significantly related to serum SP-D levels (beta coefficient, -0.009;  $p = 0.003$ ).

A receiver operating characteristics curve was generated to evaluate the ability of SP-D levels to discriminate between patients with and without exacerbation using all subjects in this study. The total area under the curve was 0.76 (95% CI, 0.597, 0.916;  $p = 0.003$ ). The most optimal cutoff of sensitivity and 1-specificity was at SP-D level of 150 mg/L at which point sensi-

tivity and specificity were 77% and 74% respectively (see Figs 2 and 3). When only COPD patients were included in the analysis, total area under the curve was 0.68 (95% CI, 0.48, 0.89;  $p = 0.096$ ) and the optimum cutoff of sensitivity and 1-specificity was at SP-D level of 153 mg/L at which point sensitivity and specificity were 77% and 63% respectively.

## 5. Discussion

The most important finding in the present study was that serum SP-D levels were increased in patients who were experiencing an exacerbation. Compared to control subjects, the levels in patients with exacerbations were nearly 75% higher. Why SP-D levels would increase during exacerbations is unknown. SP-D is primarily involved in regulating innate immunity in the lungs. SP-D facilitates the clearance of inhaled pathogens before they have a chance to cause significant tissue damage [8]. SP-D also plays a major role

in the removal of apoptotic cells (efferocytosis) and by doing so, it may assist in maintaining lung homeostasis [9]. It is thus plausible that during acute exacerbations, local lung expression of SP-D may rise to fight off infectious stimuli in the airways and to orchestrate the down-stream inflammatory response. Serum levels may in turn reflect the increased lung expression. Because we did not measure lung expression of SP-D during exacerbations, additional experiments will be needed to validate this hypothesis. An alternate explanation is that the increased serum expression of SP-D may reflect increased extra-pulmonary production of SP-D.

Bronchoalveolar lavage fluid (BALF) levels of SP-D are modified by various pulmonary conditions including asthma [10] and cystic fibrosis [11] and by smoking. Previous studies indicate that smokers have reduced SP-D expression in BALF compared to non-smokers [12, 13]. The effect of smoking on serum SP-D levels is less well known. In one study by Mutti et al. [14], serum SP-D levels were slightly increased in smokers compared to non smoker controls but the differences failed to reach statistical significance. These data are similar to the findings in the present study. The effects of reduced lung function and SP-D in BALF and serum have been recently studied. The totality of evidence indicates that SP-D expression in BALF is decreased but serum levels are increased in COPD [6, 12–16].

Recently, Sin et al. [6] showed that serum SP-D levels were inversely correlated to FEV<sub>1</sub> in patients with advanced COPD, which are consistent with the present findings where we found a significant inverse relationship between serum SP-D and various lung functions. In a large study of patients with and without COPD, Lomas et al. reported that COPD patients have higher serum SP-D levels compared to healthy smoking controls [17]. Similar to these studies, we found that serum SP-D levels were elevated in patients with stable COPD but the difference did not reach statistical significance probably owing to a smaller sample size. We extend the findings of the previous study by demonstrating that elevated SP-D levels were associated with exacerbations.

The serum levels of SP-D are influenced by four factors: pulmonary and extra-pulmonary synthesis, breakdown, and translocation into the systemic circulation [2]. However, during exacerbations, the extra-pulmonary synthesis of SP-D is thought to be trivial compared with pulmonary expression [15]. If metabolism is assumed to be constant (regardless of COPD severity or during exacerbations), then pul-

monary synthesis and alveolar-capillary permeability are the major determinants of serum levels during exacerbations. In experimental models, both factors are perturbed with acute lung injury [15, 18–20]. Genetic factors also influence serum SP-D levels. Single nucleotide polymorphism (SNP) rs721917 causes the substitution of amino acid threonine (Thr) in place of methionine (Met) at residue number 11 in the amino terminal domain of the mature protein and individuals who have this genotype demonstrate reduced serum expression of SP-D [21, 22]. Regardless of the mechanism, the present study suggests that SP-D is a promising biomarker during acute exacerbations.

There are several important limitations to our study. Firstly, we did not have serum in the same subjects before, during and after exacerbations. Thus, we cannot rule out the possibility that patients with exacerbations had elevated basal serum levels of SP-D and that the exacerbations themselves did not modify SP-D levels. Secondly, the control subjects were younger than those with COPD. We adjusted for the differences in the age by multivariate modeling and still found a difference in the SP-D levels, indicating that the differences could not be explained away by age. Thirdly, the patients with COPD were on a variety of inhalers and drugs that could modify serum expression of SP-D. However, anti-COPD drugs such as inhaled steroids tend to decrease (not increase) serum SP-D levels [16], which may have led to underestimation of the SP-D levels during exacerbations. Similarly blood sampling several days into exacerbation may have also led to underestimation of serum SP-D levels, but this allowed us to obtain concurrent pulmonary function tests so we could accurately correlate serum SP-D with lung function. All our subjects were men and have the same ethnicity. This is because gender and ethnicity affect serum levels of SP-D [21, 22] and we did not want these factors influencing the results of our study.

Another surfactant associated protein, SP-A, has also been implicated in the pathophysiology of COPD and is a possible biomarker for COPD [23, 24]. Future studies will be needed to determine which of the lung-specific proteins (if any) will be the most optimal serum biomarker in COPD exacerbations.

Several candidate molecules are being investigated as possible biomarkers of COPD exacerbations. These include C-reactive protein [25], serum amyloid A [26], procalcitonin [27], and copeptin [28]. However, none of these proteins are lung-specific; they are largely derived from extra-pulmonary sources, which reduces their specificity and negative predictive value. SP-D on

the other hand is derived largely from type II pneumocytes, making it a very promising biomarker for exacerbation. Nonetheless, additional studies are required to further establish its role in COPD exacerbation and its utility as a diagnostic marker for exacerbations of COPD.

## 6. Conclusion

Surfactant protein D is significantly raised in patients experiencing COPD exacerbations, raising the possibility that it may be used as a biomarker for exacerbation. Additional studies will be needed to determine whether SP-D levels decrease upon resolution of the exacerbation episode and whether systemic corticosteroids can modify its expression during exacerbations.

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Sampling was done at: Pulmonology Department, Shaikh Zayed Medical Complex Lahore, Pakistan.

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ELISA was done at: Immunoassay lab, National Health Research Complex, SZMC, Lahore, Pakistan.

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### Conflict of Interest Declaration

None of the authors have any conflicts of interest to declare.

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