

SPUTUM NEUTROPHILS AS A BIOMARKER IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Introduction: COPD is a common, preventable and treatable disease with increased disease burden accounting for significant mortality. A hallmark feature of COPD is the increased numbers of pulmonary neutrophils that can secrete a wide range of pro-inflammatory cytokines and chemokines as well as proteases that play role in the development of emphysema. We aimed to find out a simple, accessible and economical biomarker to prevent morbidity & mortality associated with COPD. Induced sputum is a non-invasive method that allows evaluation of neutrophil numbers in the airway lumen. **Methods:** A longitudinal comparative study involving 50 COPD patients with age, sex, race matched individuals were categorized into treatment group (Group1) and control group (Group2) underwent sputum induction and analysis to look for sputum neutrophil and its relation to COPD exacerbation and management. Response to treatment was measured using spirometry (FEV₁, FEV₁/FVC). **Observation:** We found out that 76% of the COPD patients had >70 Sputum Neutrophil count which persisted among 28% of the COPD patients despite treatment with macrolide. Most of the group 1 patients improved with macrolide treatment in addition to usual bronchodilators as shown by improvement in pre and post FEV₁(p=0.001); compared to group 2 patients where there was no significant changes in pre and post FEV₁(p=0.516). Sputum Neutrophil was significantly associated with improvement in CAT Score (p=0.009) and reduction in exacerbation(p=0.002). **Conclusion:** The author strongly concludes that sputum neutrophil count could prove to be one of the Significant biomarker to predict and manage the exacerbation of COPD.

KEYWORDS: COPD, FEV₁, FEV₁/FVC.

INTRODUCTION

COPD is currently the fourth leading cause of death in the world but is projected to be the 3rd leading cause of death by 2020. More than 3 million people died of COPD in 2012 (up from 2.4 million deaths in 1990) accounting for 6% of all death globally. COPD affects 329 million people or nearly 5 percent of the global population. It typically occurs in people over the age of 40. More than 90% of these deaths occur in the developing world. The number of deaths is projected to increase further because of higher smoking rates and an aging population in many countries. In the European Union, the total direct costs of Respiratory diseases are estimated to be about 6% of the total health care budget, with COPD accounting for 56% (38.6 billion Euros) of the cost of respiratory diseases. In the United States, the estimated direct costs of COPD are \$32 billion and the indirect costs \$20.4 billion. It resulted in an estimated economic cost of \$2.1 trillion in 2010. d treatable. COPD is a major cause of chronic morbidity and mortality throughout the world. Many people suffer from this

disease for years, and die prematurely from it or its complications. COPD develops slowly.

Spirometry is used to confirm the diagnosis. Spirometry measures the amount of airflow obstruction present and is generally carried out after the use of a bronchodilator, a medication to open up the airways. Two main components are measured to make the diagnosis: the forced expiratory volume in one second (FEV₁), which is the greatest volume of air that can be breathed out in the first second of a breath, and the forced vital capacity (FVC), which is the greatest volume of air that can be breathed out in a single large breath. Normally, 75–80% of the FVC comes out in the first second and a FEV₁/FVC ratio of less than 70% in someone with symptoms of COPD defines a person as having the disease. Based on these measurements, spirometry would lead to over-diagnosis of COPD in the elderly. The Excellence criteria additionally require a FEV₁ of less than 80% of predicted.

However, there is a need for biomarkers that are reflective of the inflammatory mechanisms involved in disease pathogenesis. Such biomarkers may be useful for monitoring disease progression, evaluating the effects of therapeutic interventions or identifying disease sub-phenotypes with different clinical characteristics.

A hallmark feature of COPD is the increased numbers of pulmonary neutrophils that can secrete a wide range of pro-inflammatory cytokines and chemokines as well as proteases that play a role in the development of emphysema. Induced sputum is a non-invasive method that allows evaluation of neutrophil numbers in the airway lumen. The measurement of induced sputum neutrophils fulfils some of the ideal characteristics of a biomarker in COPD; neutrophils are thought to be mechanistically involved in disease pathophysiology, can be easily measured in the target organ using a non-invasive method, and are increased in patients with COPD compared to controls. There is a need to conduct large cohort studies to further explore the potential utility of this biomarker in COPD patients.

The use of sputum in research has improved our understanding of airway diseases in many ways because it is noninvasive (in the case of spontaneous sputum) or relatively noninvasive (with induced sputum), and cell counts in sputum have the qualities of excellent and highly reproducible measurements that are accurate and sensitive and identify the presence, type, and severity of airway inflammation. These measurements can be obtained repeatedly and in exacerbations, as well as in all severities of disease. Induced sputum, in particular, has been shown to be a highly effective method for determining the inflammatory processes in the airways (Gibson *et al* 1989; Pizzichini *et al* 1996; Pizzichini *et al* 1997; Jayaram *et al* 2000). Increasingly, sputum induction has been used in clinical and research settings to study airway inflammation in both asthma and COPD (Fahy *et al* 1993; Keatings and Barnes 1997; Wielders and Dekhuijzen 1997; Rutgers *et al* 2001).

Sputum analysis can also be used to determine the inflammatory response to inhaled glucocorticosteroids. Indeed, a single large dose (2400 µg) of inhaled budesonide was shown to result in a reduction in sputum eosinophil numbers 6 hours after administration (Gibson *et al* 2001).

In COPD, the most common sputum change is neutrophilia and increased products of neutrophil activation, including proteases, myeloperoxidase, and elastase (Chung 2001; Williams and Jose 2001; Kim and Nadel 2004; O'Donnell RA *et al* 2004). In cigarette smokers with COPD, the degree of neutrophilia is loosely related to the degree of chronic airway obstruction (Stanescu *et al* 1996). This suggests that sputum neutrophils or their products may be used as early markers of the manifestation of COPD.

AIMS AND OBJECTIVES

Objectives

1. To study prevalence of Sputum Eosinophilia and Neutrophilia in Chronic Obstructive Pulmonary Disease (COPD)
2. To study prevalence of Sputum Eosinophilia and Neutrophilia in Different COPD GOLD groups
3. To study prevalence of Sputum Eosinophilia and Neutrophilia in Response to COPD Treatment

MATERIALS AND METHOD

Patient Selection

We performed a longitudinal comparative study to evaluate sputum analysis in all diagnosed COPD patients who attended the Department of TB and respiratory diseases, SS Hospital, IMS, BHU.

If not previously documented/tested, all such patients were documented for COPD with post bronchodilator pulmonary function test confirmation (FEV1 / FVC < 0.7) with irreversible airway obstruction and were screened for other causes of breathlessness like exacerbation of Bronchial Asthma, Interstitial lung diseases, worsening of Dyspnea due to heart failure etc., by channeling through detailed history, thorough physical examination and a battery of relevant investigations. Data on demographic characteristics, sleep, medical history, medication use, and habits were obtained with the use of a modified standardized questionnaire.

Inclusion criteria

- Patients with COPD.
- Age 41 to 85 year.
- Patients as well as attendants willing to give informed consent.
- Patients ready to undergo necessary investigation.

Exclusion criteria

- Patients with multiple organ failure.
- Clinical features and spirometry suggestive of disease other than COPD.
- Haemodynamic instability.
- Those patients who are not giving consent.
- Women who are pregnant or currently lactating.
- Mixed and restrictive pattern of lung function in spirometry.
- Could not perform spirometry correctly.

The institutional ethics committee was asked to approve the protocol, informed consent was obtained from the participants.

Study Size

We did Sputum analysis of 50 COPD patients which were attend outdoor or indoor of our department. All patients included in study underwent evaluation as per pre-standardized protocol.

RESULTS AND OBSERVATION

A total of 50 COPD patients with age, sex, race, BMI matched were enrolled into the study, between 40 to 85 years of age group which were chosen from the OPD and ward of TB & Respiratory Diseases of Sir Sunderlal Hospital, BHU Varanasi.

In our study, out of 50 subjects in group I, 6 (24%) had Eosinophilia and 18 (72%) had Neutrophilia and in group II, 5 (20%) had Eosiniphilia and 20 (80%) had Neutrophilia. Earlier studies by Singh *et al.* (2010), total 359 subjects produced an evaluable sample at baseline, and 297 subjects after 1 year.

The researcher also found out that most of the patient in Group 1 fell into GOLD 3(n=12) and GOLD 2 (n=6) prior to initiation of treatment with macrolide compared to post treatment in GOLD 3 (n=10) and GOLD 2(n=15). However in Group 2, most of the patient fell into GOLD 3(n=11) and GOLD 2 (n=10) compared to post treatment in GOLD 3 (n=7) and GOLD 2 (n=13). Our findings were similar to Singh *et al.* where half of the subjects were GOLD stage 2, with the remaining subjects being GOLD stage 3 or 4. Another study conducted by Stanescu *et al.* (1996) suggested In cigarette smokers with COPD, the degree of neutrophilia is loosely related to the degree of chronic airway obstruction. This suggests that **sputum neutrophils or their products**

may be used as early markers of the manifestation of COPD

The researcher also showed that 22% (n=11 out of 50) of the COPD patients had $\geq 3\%$ Sputum eosinophil count and 78% (n=39 out of 50) had $< 3\%$ sputum Eosinophil. The lower sputum eosinophil may be due to treatment with ICS/oral steroid.

The researcher found out that 22% of the participants had sputum Eosinophil $\geq 3\%$ compared to most literatures which states that 40% of patients with COPD have eosinophilic airway inflammation.

The researcher also found out that **76% of the COPD patients had neutrophilic airway inflammation with sputum Neutrophil $> 70\%$** . According to Hoenderdos, and Condliffe *et al.* Neutrophilic inflammation predominates in the COPD airway wall and lumen, but, despite the presence of abundant innate immune cells, the progressive clinical course of the disease is punctuated by recurrent infection-driven exacerbations.

The Researcher also found out that increased sputum Neutrophil was associated with decline in lung function (FEV1) which is similar to the finding by Singh *et al.*, who concluded that Sputum neutrophil measurements in COPD are associated weakly with FEV₁ % predicted and health status.

Table 4-1a: Prevalence of Sputum Eosinophil in COPD Patients Prior to Treatment.

Eosinophil (PRE)	No. of patients	Percentage
$\geq 3\%$	11	22
$< 3\%$	39	78
Total	50	100

Table 4-1a, showed that 22% (n=11 out of 50) of the COPD patients had $\geq 3\%$ Sputum eosinophil count and 78% (n=39 out of 50) had $< 3\%$ sputum Eosinophil. The

lower sputum eosinophil may be due to treatment with ICS/oral steroid.

Table 4-1b: Prevalence of Sputum Neutrophil in COPD Patients Prior to Treatment.

Neutrophil (PRE)	No. of patients	Percentage
> 70	38	76
≤ 70	12	24
Total	50	100

Table 4-1b, showed that 76% (n=38 out of 50) of the COPD patients had > 70 Sputum Neutrophil count and 24% (n=12 out of 50) had ≤ 70 sputum Neutrophil.

Table 4-2a: Prevalence of Sputum Neutrophil in COPD Patients Prior to Treatment.

Gold Stage Pre	No. of patients	Percentage
≥ 80	3	6
50-79	16	32
30-49	23	46
< 30	8	16
Total	50	100

Table 4-2B: Prevalence of Sputum Neutrophil in COPD Patients Post Treatment.

Gold Stage Post	No. of patients	Percentage
≥80	1	2
50-79	28	56
30-49	17	34
<30	4	8
Total	50	100

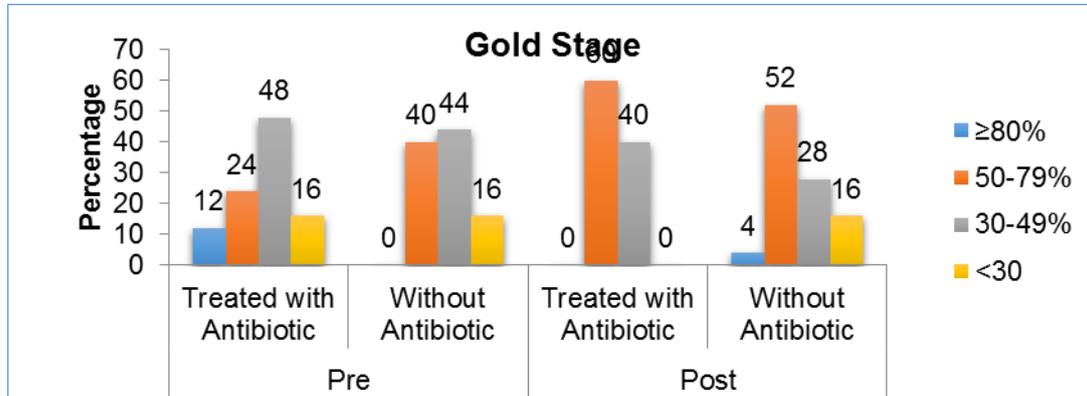
**Figure 4-2a: Prevalence of Sputum Neutrophil in COPD Patients Pre and post Treatment.**

Table 4-2a/4-2b and figure 4-2 showed maximum number of COPD patients in GOLD 3 (n=23 in pre treatment Vs n=17 after treatment) followed by GOLD

2 (n=16 prior to treatment vs n=28 after treatment), followed by GOLD 4 (n=8 in pre treatment vs n=4 post treatment).

Table 4-3a: Prevalence of Sputum Eosinophil in COPD Patients after Treatment.

Eosinophil Post	No. of patients	Percentage
≥3%	7	14
< 3%	43	86
Total	50	100

Table 4-3a, showed that 14% (n=7 out of 50) of the COPD patients had ≥ 3% Sputum eosinophil count and 86% (n=43 out of 50) had < 3% sputum Eosinophil. The

further decline in sputum eosinophil may be due to treatment with ICS/oral steroid.

Table 4-3b: Prevalence of Sputum Neutrophil in COPD Patients after Treatment.

Neutrophil Post	No. of patients	Percentage
>70	14	28
≤70	36	72
Total	50	100

Table 4-3b, showed that 28% (n=14 out of 50) of the COPD patients had > 70 Sputum Neutrophil count and 36% (n=36 out of 50) had ≤ 70 sputum Neutrophil.

There is a significant decrease in Neutrophil count after treatment with Macrolide.

Table 4-4: Comparison of Severity of COPD in Group 1 Vs Group 2.

Gold Stage	Group 1		Group 2	
	Treated with Antibiotic Pre	Treated with Antibiotic Post	Without Antibiotic Pre	Without Antibiotic Post
	No. (%)	No. (%)	No. (%)	No. (%)
≥80%	3 (12.0)	0 (0.0)	0 (0.0)	1 (4.0)
50-79%	6 (24.0)	15 (60.0)	10 (40.0)	13 (52.0)
30-49%	12 (48.0)	10 (40.0)	11 (44.0)	7 (28.0)
<30	4 (16.0)	0 (0.0)	4 (16.0)	4 (16.0)
Total	25 (100.0)	25 (100.0)	25 (100.0)	25 (100.0)
P-value	0.001		0.516	

Table 5-3: Summary of all findings of current study.

Variables	Mean±SD		p-value
	Treated with Antibiotic	Without Antibiotic	
Age	62.64±11.060	58.92±11.354	0.246
TLC	1.25E4±3860.928	2.00E4±43709.112	0.400
Neuter	82.24±9.697	78.44±10.697	0.194
Lymphocytes	10.12±5.925	15.36±12.278	0.061
Hb	12.216±2.2433	12.308±1.4323	0.864
FVC	2.405±1.0110	2.433±0.7789	0.913
FEV1 Pre	50.00±17.357	46.64±13.976	0.455
FEV1 Post	56.04±12.371	52.68±16.030	0.411
FVC1 FVC ratio	58.80±7.751	58.84±7.069	0.985
BMI	24.136±4.4631	22.616±4.8470	0.254
PaO2	96.96±66.977	98.72±42.651	0.912
PaCO2	65.648±22.1778	60.852±19.4685	0.420
PH	7.381±0.0752	7.367±0.0837	0.544
HCo 3	32.012±9.5260	30.704±7.0409	0.583
Neutro Pre	77.68±7.920	78.80±7.643	0.613
Neutro Post	57.36±8.010	68.20±9.862	0.000
Eosinophil Pre	1.88±2.489	1.60±2.784	0.709
Eosinophil post	2.00±0.645	1.92±0.759	0.690
CAT Pre	25.32±6.336	25.12±7.126	0.917
CAT Post	12.96±2.835	15.88±6.194	0.037

SUMMARY AND CONCLUSION

1. The author strongly concludes that sputum neutrophil count could prove to be one of the Significant biomarker to predict and manage the exacerbation of COPD.
2. The researcher found out that most of the patients with COPD (76%) had increased Sputum Neutrophil count.
3. COPD patients with increased Sputum Neutrophil count was associated with worsening of lung function and as well as CAT Score. Neutrophilic airways were less responsive to steroid compared to eosinophilic airway diseases.
4. After Treatment with macrolide in addition to LABA+LAMA+ICS, there was an improvement in lung function and sign and symptoms as revealed from CAT Score.
5. Sputum Neutrophil may be used as a biomarker for predicting future worsening and deteriorating lung function.
6. Sputum Eosinophil was increased in 22% of COPD patients and it could also be used as one of the biomarker for COPD progression, exacerbation.
7. The researcher also found out that number of exacerbation was significantly increased in patients with increased sputum Neutrophil and eosinophil count.
8. However, eosinophilic airway disease as evident from our study was associated with better lung function and improved responsiveness to therapy (ICS).
9. The Researcher also found positive correlation of Sputum Neutrophil with CAT Score in patients with

COPD i.e. CAT score improved with improvement in Sputum Neutrophil count.

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