CORRELATION OF TOTAL LEUCOCYTE COUNT, EOSINOPHIL, NEUTROPHIL AND LYMPHOCYTE COUNTS WITH PULMONARY FUNCTION IN COPD PATIENTS

Sureshbalaji R. A1, Prem Kumar S2, Lavanya R3, Krishna Kumar S4, Sivapriya D.V5, Ramaswamy C6, Shyamala Thirumeni7

1Tutor, Department of Physiology, Chennai Medical College Hospital and Research Centre, Irungalur, Tiruchirapalli. Tamilnadu.
2Assistant Professor, Saveetha Medical College Hospital and Research Centre, Thandalam, Chennai, Tamilnadu.
3Homoeo Consultant, SKY yothi Homoeo Clinic, Temple of Consciousness, Aliyar, Pollachi, Tamilnadu.
4Assistant Professor, Department of Physiology, Chennai Medical College Hospital and Research Centre, Irungalur, Tiruchirapalli.
5Associate Professor, Saveetha Medical College Hospital and Research Centre, Chennai, Tamilnadu.
6Professor, Department of Physiology, Saveetha Medical College and Hospital, Thandalam, Chennai, Tamilnadu.
7Professor, Department of Physiology, Saveetha Medical College and Hospital, Thandalam, Chennai, Tamilnadu.

ABSTRACT

BACKGROUND
Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in India. In COPD, there is an increased number of leucocytes, suggesting a role for the inflammatory response in the clinical progression of the disease.

The aim of the study is to evaluate the degree of pulmonary function impairment by pulmonary function tests and to correlate leucocyte counts with pulmonary function tests in COPD patients.

MATERIALS AND METHODS
This descriptive study was carried out among 30 COPD volunteer patients referred from Pulmonology Department of Saveetha Hospital. The pulmonary function test was done by using a computerised spirometer and complete blood cell count was also made for all the subjects. Pulmonary parameters (FEV1 and PEFR) recorded were correlated with blood parameters (TLC, neutrophils, eosinophils, lymphocytes).

RESULTS
There was strong negative significant correlation observed between FEV1 and TLC, absolute neutrophil count, absolute eosinophil count whereas there exists a weak negative significant correlation between FEV1 and absolute lymphocyte count (p<0.05).

CONCLUSION
The findings indicated that whenever the blood parameters (TLC, neutrophils, eosinophils and lymphocytes) increase there was a decrease in the pulmonary parameters in COPD patients.

KEYWORDS
COPD, Computerised Spirometer, FEV1.


BACKGROUND
Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in India and a major worldwide healthcare problem.1 Chronic obstructive pulmonary disease (COPD) is characterised by the progressive, partially reversible air flow limitation which occurs in association with a chronic inflammatory reaction triggered by smoking or other inhalation in predisposed subjects.2

Leucocytes are important cells which mediate various inflammatory responses. Inflammation has been identified as an important factor for disease exacerbation in obstructive lung disease. In this study, we used neutrophil and eosinophil counts as biomarkers for exacerbation in obstructive lung disease.2

It has been suggested that with severe COPD, there is an increased number of leucocytes, which is correlated with PFT suggesting a role for this inflammatory response in the clinical progression of the disease.3

Pulmonary Function Tests (PFTs) are very important in the medical evaluation of patients suffering from "shortness of breath", and they are effectively used for the diagnosis of pulmonary diseases, such as COPD (i.e. chronic obstructive pulmonary diseases). Measurement of Forced Vital Capacity (FVC) and Forced Expiratory Flow in the 1st second (FEV1) are very important for assessing the treatment of COPD.4

A number of studies have demonstrated the association between the reversibility of obstructive pulmonary function impairments and the number of leucocytes in peripheral blood. There is paucity of literature concerned with the relationship between markers of allergy, and airway inflammation and pulmonary function results in COPD.5 So this study has been designed to correlate total leucocyte count, eosinophil (anti-allergy), neutrophil (anti-inflammatory) and lymphocyte counts with pulmonary function in COPD patients.

MATERIALS AND METHODS

Study Design
Descriptive study.
Methodology
The present analytical study was carried out among 30 COPD volunteers (patients) referred from Pulmonology Department of Saveetha Hospital.

Inclusion Criteria
- Patients with COPD only.
- Age between 20-60 years.
- Both gender.
- Chronic bronchitis.
- Emphysema.
- Bronchiectasis.
- Bronchial asthma (with attack & follow-up).

Exclusion Criteria
Subjects with the following diseases were excluded
- Epilepsy.
- Infective diseases.
- Cardiac diseases.
- Mentally retarded subjects.

Procurement of Permissions
The protocol and the benefits of the study were explained to the subjects before carrying out the study on the subjects.

Ethical Clearance
The study proposal with the informed consent and the questionnaire were submitted to the ethical committee of our institution and was approved by the ethical committee.

Pulmonary Function Test
Instrument used for measuring FEV₁ - Computerised Spirometer, (RMS Helios 401, Recorders and Medicare Systems (P) Ltd).

Blood Analysis
- Total leucocyte count was done by manual method using haemocytometer.
- Differential leucocyte count was done by standard procedure of eosin and haematoxylin staining method.

Procedure
PFT Recording
- During recording, the patient was made to sit in high sitting position and asked to relax for 5 min.
- Soft nose clips were used to prevent air escaping through the nose.
- Sterile filter mouthpieces were used to prevent the spread of microorganisms

- The test was preceded by a period of quiet breathing in and out into the sensor through the mouth (tidal volume), or the rapid breath in (forced inspiratory part), which would come before the forced exhalation.
- The patient was asked to take a deep breath as forcefully as they can, and then exhale into the sensor as hard as possible preferably at least for 6 seconds.
- The manoeuvre is highly dependent on patient cooperation and effort.
- After the patients were educated about the procedure, the tests were carried out three times and best of the three was selected for analysis.
- The values obtained by the sensor were recorded.

Pulmonary Parameters
PEFR & FEV₁ values taken from computerised spirometric chart.

Blood Parameters
1. Total Leucocyte Count
   - Using WBC pipette, Turk’s fluid and Neubauer's counting chamber.
   - TLC (cells/mm³) = cells counted × 50.

2. Differential Leucocyte Count
   - Chart containing 100 counters were made.
   - Type of leucocytes was registered in each counter.
   - DLC was made by adding the registered counts.
   - Percentage of DLC was made.

   Absolute Count was made as follows
   i. Absolute neutrophil count = neutrophil % × TLC.
   ii. Absolute eosinophil count = eosinophil % × TLC.
   iii. Absolute lymphocyte count = lymphocyte % × TLC.

Statistical Analysis
Data obtained were represented as Mean ± SD. Correlation coefficient was calculated between lung function test variables (FEV₁) and blood examination results (Neutrophils, eosinophils & lymphocytes).

RESULTS
In this study, 30 patients who were diagnosed as COPD from the Pulmonology Department of Saveetha Hospital participated voluntarily. Pulmonary parameters (FEV₁ and PEFR) recorded were correlated with blood parameters (TLC, neutrophils, eosinophils, lymphocytes) and compared with "p-value". The results are given below.

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FEV₁ (%)</th>
<th>Blood Parameters (cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>38.5 ± 17.6</td>
<td>TLC 12,800 ± 1301.9</td>
<td>-0.80</td>
<td>p&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABS.NEU.CNT 73 692 ± 952.01</td>
<td>-7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABS.EOS.CNT 811 03 ± 228.72</td>
<td>-73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABS.LYM.CNT 3849 ± 516.03</td>
<td>-30</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Correlation between FEV₁ and Blood Parameters
Table 2. Correlation between PEFR and Blood Parameters

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>PEFR (L/min.)</th>
<th>Blood Parameters (cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
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</tr>
<tr>
<td>30</td>
<td>215 ± 49.39</td>
<td>TLC 12,800 ± 1301.9</td>
<td>-0.80</td>
<td>p&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABS.NEU.CNT 7369.2 ± 952.01</td>
<td>-73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ABS.EOS.CNT 811.03 ± 228.72</td>
<td>-60</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>ABS.LYM.CNT 3848 ± 516.03</td>
<td>-38</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Correlation between FEV₁ and Total Leucocyte Count (TLC)

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FEV₁ (%)</th>
<th>TLC (Cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
</thead>
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<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>38.5 ± 17.6</td>
<td>12,800 ± 1301.9</td>
<td>-0.80</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 4. Correlation between FEV₁ and Absolute Neutrophil Count

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FEV₁ (%)</th>
<th>ABS.NEU.CNT (cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>38.5 ± 17.6</td>
<td>7369.2 ± 952.01</td>
<td>-0.71</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 5. Correlation between FEV₁ and Absolute Eosinophil Count

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FEV₁ (%)</th>
<th>ABS.EOS.CNT (cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
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<tr>
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<td>Mean ± S.D.</td>
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<td></td>
<td></td>
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<tr>
<td>30</td>
<td>38.5 ± 17.6</td>
<td>811.03 ± 228.72</td>
<td>-0.73</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 6. Correlation between FEV₁ and Absolute Lymphocyte Count

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>FEV₁ (L/min.)</th>
<th>ABS.LYM.CNT (cells/mm³)</th>
<th>Correlation Coefficient</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>38.5 ± 17.6</td>
<td>3848 ± 516.03</td>
<td>-0.30</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Figure 1

The mean value of FEV₁ was 38.5 ± 17.6 and mean TLC value was 12,800 ± 1301.9. (Table: 3). When these two values were compared, the result showed that there was a strong negative significant correlation (-0.80) noted between FEV₁ and TLC (p<0.05).

Figure 2

The mean FEV₁ value was 38.5 ± 17.6 and mean absolute neutrophil count value was 7369.2 ± 952.01. (Table: 4). On comparing these two values, the result showed that there exists a strong negative significant correlation (-0.71) between FEV₁ and absolute neutrophil count (p<0.05).

Figure 3

The mean value of FEV₁ was 38.5 ± 17.6 and mean absolute eosinophil count value was 811.03 ± 228.72. (Table: 5) On comparing these two values, the result showed that there was a strong negative significant correlation (-0.73) noted between FEV₁ and absolute eosinophil count (p<0.05).

Figure 4

The mean value of FEV₁ was 38.5 ± 17.6 and mean absolute lymphocyte count value was 3848 ± 516.03 (Table 6). These two values were compared. The result showed that there was weak negative significant correlation (-0.30) observed between FEV₁ and absolute lymphocyte count (p<0.05).
DISCUSSION
COPD, the disease which affects the respiratory system, is normally diagnosed by certain pulmonary function tests. Among that measurement, Forced Vital Capacity (FVC), Forced Expiratory Flow in the 1st second (FEV1) are very important for assessing the treatment of COPD. FEV1 determines the severity of air flow obstruction.

It has been suggested that with severe COPD, there is an increase in number of leucocytes, which is correlated with PFT suggesting a role for leucocytes in this inflammatory response in the clinical progression of the disease.3 Leucocytes are important haemocytic cells which mediate various inflammatory responses. Inflammation has been identified as an important factor for disease exacerbation in obstructive lung disease.6

A significant increase in TLC with decrease in FEV1 is noticed in the study. Decrease in FEV1 indicates the severity of COPD. As the leucocytes have the important role in body’s defence mechanism, it is natural that their numbers increase with the severity of COPD. The present result also supports the abovementioned fact.

Further, to find out the relative role of individual leucocytes, the PFTs were compared with different leucocytes. There was a significant increase in absolute neutrophil count observed with decrease in FEV1 and PEFR. Neutrophils have been implicated in the pathogenesis of COPD, being recruited into the lung in response to cigarette smoke inhalation and being responsible for the release of protease and oxidant-producing enzymes that cause bronchitis and emphysema.7

An increase in the white blood cells that may be produced during an allergic reaction (eosinophil) may indicate that a condition such as asthma is causing the symptoms. In our results also, there was significantly high increase in absolute eosinophil count with decrease in FEV1.

On the contrary, the results of the lymphocytes showed a different correlation with PFTs. When the absolute lymphocyte count registered an increase, the PEFR showed a decrease and the FEV1 showed an increase. Lymphocytes are important effector and regulatory cells that participate actively in the inflammatory response of COPD especially for subjects who develop COPD from smoking.8 The inflammatory response of lymphocyte in COPD patients may be the cause for reduction in PEFR.

Thus, our study concludes that both pulmonary and blood parameters are showing a negative correlation. This inverse relationship if established may help the family physician to understand the severity of COPD and direct them to the referral centre for early diagnosis and treatment.

In short, the findings of this study indicate that the severity of COPD diagnosed with pulmonary function tests have negative correlation with the blood parameters i.e., whenever the blood parameters (TLC, neutrophils, eosinophils and lymphocytes) increase there was a decrease in the pulmonary test parameters in COPD patients.

CONCLUSION
- There was a strong negative significant correlation observed between FEV1 and TLC, absolute neutrophil count, absolute eosinophil count whereas there exists a weak negative significant correlation between FEV1 and absolute lymphocyte count.
- Further studies on these lines are required to prove this inverse relationship of blood parameters and pulmonary parameters.

REFERENCES