Clinical Biochemistry in the Diagnosis of Chronic Pulmonary Diseases: Identifying Biomarkers and Innovative Treatment Strategies

Rand Ali Salman[1]

No Afiliasi

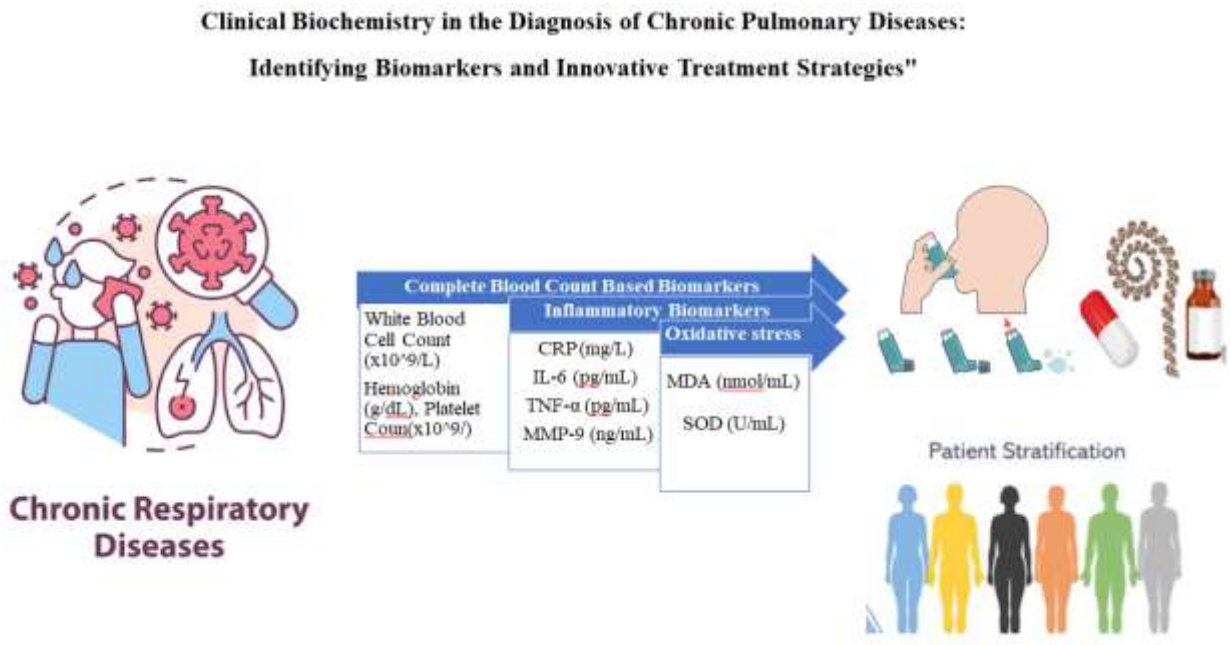
KEYWORDS

Chronic Pulmonary Diseases, Biomarkers, Clinical Biochemistry, COPD, Asthma, Pulmonary Fibrosis, Inflammation, Oxidative Stress, Spirometry, Targeted Therapy

ABSTRACT

Chronic pulmonary diseases, which include COPD, asthma, and pulmonary fibrosis, are difficult to diagnose and treat because of the different mechanisms underlying each disease. The purpose of this study was to evaluate the contribution of clinical biochemistry in the diagnosis of these diseases offering biomarkers that distinguish these diseases and direct new therapeutic approaches. The study was conducted on 120 patients who were randomly grouped into six groups depending on their lung state and therapy they received. To examine the significance of other biomarkers involving inflammation (CRP, IL-6, TNF-α, MMP-9), oxidative stress (MDA, SOD), and leukocyte subpopulations (eosinophils, basophils) in the context of the studied disease, we also determined their levels. Additional spirometric indices were also recorded during 6 months to examine the effects of various management approaches. The results showed that all the diseases had marked difference in biomarker levels and were elevated in COPD and pulmonary fibiosis compared to asthma. Pharmacological treatments like LABAs/LAMAs in COPD and antifibrotic agents in pulmonary fibrosis also showed beneficial alterations in biomarkers and spirometry and confirmed the relevance of a biomarker approach. In conclusion, the study emphasizes the need for the use of clinical biochemistry in the discrimination of chronic pulmonary diseases and proper management strategies of the disorder. It indicates that biomarker can help improve the overall accuracy within the diagnosis process and can also aid in the formulation of effective treatment plans for patients – and therefore improve patient outcomes.

1. Introduction



Introduction

Lungs are vital and sensitive organs of the respiratory system that have crucial roles in the body. Many diseases affect parts of the respiratory system and reduce lung capacities, with chronic lung disease being the most common. Literature review studies indicate that more children and elderly persons are experiencing this illness globally, and approximately hundreds of millions of people. Currently, lung illness accounts for the highest mortality rate evidenced by at least four million deaths recorded

globally annually (Soriano et al. 2017).

Chronic diseases of the lungs including COPD, asthma, and pulmonary fibrosis among others carry long-term health challenges globally. These circumstances refer to progressive and progressive deterioration in respiratory health, functional capacity, quality of life, costs, and mortality. According to the WHO, chronic respiratory diseases are among the leading causes of death in the world and COPD will rise to be the third leading cause of death by 2050. The rising prevalence and impact of such illnesses underscore the need for enhanced means of identifying and managing such diseases (Gould et al., 2023).

Among the major conditions that are currently affecting various countries across the world, chronic pulmonary diseases are some of the worst. COPD is reported to affect about 384 million people worldwide occurrence common among the elderly and people with a history of smoking and exposure to air pollution (Riley& Sciurba, 2019). Asthma is also another chronic pulmonary disease which has an impact on nearly 262 million populations of the world along with morbidity few time even mortality. Pulmonary fibrosis is an infrequent but clinically challenging disease due to progressive and often fatal course of the illness. Not only do these diseases affect lung function but also lead to several systemic effects such as cardiovascular diseases, endocrine dysfunction, and skeletal muscle dystrophy. Because these diseases are chronic and severe, their identification and management require the application of several approaches (Xie et al., 2020).

Over 5% of the global population that is more than 328,900,000 people are suffering from COPD. In the study by Soriano et al. (2017), it was revealed that there has been an increase in the 44. 2% increase in the incidence rate between the years 1990 and 2015. According to the WHO, COPD is the third leading cause of death worldwide (Nie et al., 2017). In 2015, approximately 3.2 million deaths were recorded to be resulting from COPD and the global incidences had increased by 11%. 6% increase in the mortality rate from 1990 as highlighted by Soriano et al., because of the increased prevalence of COPD globally. It is estimated that global prevalence of asthma is approximately 300 million (Peters et al., 2006) and ranged between 1-21% in adults (Reddel et al., 2015). Around 400,000 individuals lost their lives as a result of this illness in the year 2015 (Soriano et al. 2017). Furthermore, the prevalence of asthma is rising annually, especially affecting children according to Peters et al. (2006). Nonetheless, IPF affects around 23 out of every 100,000 individuals according to Duck et al. (2015). Most IPF patients typically have a smoking history and males have a higher incidence of this disease compared to females, as noted by (O'Donnell et al., 2016).

Diagnostic clinical biochemistry has been an essential part of clinical diagnostics for years, providing an understanding of biochemical reactions that take place within the human body. In the context of chronic pulmonary diseases, this field has broadened to encompass the discovery and description of biomarkers, or molecules that can be accurately measured and assessed for physiological, pathological, or pharmacological activity (Metkar& Girigoswami, 2019). Biomarkers can be divided into several groups depending on their functions in the disease process. Diagnostic biomarkers are used in early assessment of chronic pulmonary diseases and are at times used prior to the sign of clinical illness. Prognostic biomarkers give the likely progress of the disease while diagnostic biomarkers tracks response to treatment and guide the treatment. On the other hand, predictive biomarkers indicate the probability of the patient responding or not responding to a particular treatment (Mayer et al., 2018).

A biomarker is used to comprehend fundamental and crucial biological processes and how they relate to disease pathophysiology and treatment plans. A specific biomarker is utilized in screening plasma, serum, and tissue samples to diagnose and predict disease outcomes as well as assess the effectiveness of different treatments, thereby aiding in the development of new therapeutic approaches. Using a particular biomarker to screen patients or subjects and determine their suitability for clinical studies, as well as in early toxicology trials, has the potential to reduce drug failure rates in later stages of clinical trials (Mollarasouli et al., 2022). Traditional research methods are currently being employed to investigate biomolecules, proteins, peptides, and genes, along with other relevant metabolites.

Moreover, different tools are being developed for use in both pre-clinical and clinical phases of drug discovery and development, including both new and existing ones (Ahmad et al., 2023).

Biomarkers' identification has transformed the diagnosis and prognosis of chronic pulmonary diseases with implications for new approaches to managing the diseases. As the idea of biomarker-driven therapy has moved more into the mainstream, it has also become widely possible to inject elements of individuality into medical treatment. A promising direction seems to be the use of targeted drugs that interfere with certain molecular processes involved in the pathogenesis of chronic pulmonary diseases. Interleukin inhibitors, for example, aiming at IL-5 as well as IL-13, were effective in severe asthma due to their anti-inflammatory effects and ability to decrease the frequency of severe attacks (Fildan et al., 2021). In the same manner, therapies directly aimed at anti-fibrotic pathways are being looked forward in the management of pulmonary fibrosis. The use of multiple omics approaches to genetic, protein, and metabolite profiling has extended the possibilities for finding new biomarkers and targets of chronic pulmonary diseases for a deep understanding of molecular mechanisms (Nambiar et al., 2021).

Clinical biochemistry is not only important in the diagnosis and management of diseases but is also equally important for the long-term management of chronic pulmonary diseases. Measurement of biomarkers is a useful tool for evaluating disease severity and response to treatment. For instance, assessing the blood eosinophil level in asthma patients enables the right dose of corticosteroids to be administered hence reducing side effects. Furthermore, understanding biomarkers linked to flares allows for a timely approach tailored to manage the condition and avoid extreme flare-ups and hospitalizations. For example, in the case of respiratory exacerbations, increased concentration of procalcitonin, a bacterial infection biomarker, can inform the need for antibiotics (MacLeod et al., 2021).

Thus, numerous questions and obstacles still exist for clinical biochemistry and biomarker research. Chronic pulmonary diseases are ill-defined and heterogeneous in terms of phenotypes and endo-types, which pose challenges in identifying and validating biomarkers. However, the implementation of biomarkers in clinical practice is another factor because their validation involves large scale multicenter studies and it is a time-consuming process and expensive. Another issue is the application of biomarker studies in clinical settings. Many biomarkers have been discovered but few of them have been incorporated into the practice of medicine. Hence, the main objective of this paper was to identify the biomarkers and Innovative Treatment Strategies in case of chronic Pulmonary Diseases.

2. Methodology

Study Design

The current cross-sectional study was conducted to determine the importance of clinical biochemistry in the diagnosis of chronic pulmonary diseases and to search for new biomarkers for the targeted treatment of these disorders. The study was conducted at Pulmonary Medicine Department of (Hospital Name) within the time duration of January 2023 to July 2024. The study was conducted with the approval of the [Insert Ethical Committee Name] and all participants signed an informed consent before participating in the study. A total of 120 patients were recruited and divided into three primary categories: Chronic Obstructive Pulmonary Disease (COPD), Asthma and Pulmonary Fibrosis. All cases were divided into two groups according to the received treatment regimens within each category, which served as the basis for the analysis of clinical results and biochemical parameters.

Patient Selection

Participants were obtained from outpatient pulmonary clinic of [Insert Hospital Name]. The inclusion criteria were subjects of both sexes, aged between 40 and 80 years with COPD and/or asthma, by spirometry, FEV1/FVC ratio <0.7. Patients with a history of pulmonary fibrosis were also included if diagnosed via High-resolution computed tomography (HRCT). Exclusion criteria included patients

with acute respiratory infection, cancer or other diseases which may impair lung function. The patients included in the study were 120 in number; they were recruited according to the inclusion and exclusion criteria in order to have groups that are as similar as possible. The patients were grouped into six clear groups with twenty patients in each group as shown in table (1).

Table1: distribution of groups

Group	Condition	Treatment	Number of Patients	Inclusion Criteria	Exclusion Criteria
Group 1: COPD (Untreated)	COPD	None	20	Confirmed diagnosis of COPD using spirometry; post-bronchodilator FEV1/FVC ratio < 0.70; no history of LABAs/LAMAs treatment in 6 months	History of LABAs/LAMAs treatment in the past 6 months
Group 2: COPD Treated with LABAs and LAMAs	COPD	LABAs and LAMAs	20	Confirmed diagnosis of COPD using spirometry; post-bronchodilator FEV1/FVC ratio < 0.70; documented adherence to LABAs/LAMAs treatment	Non-adherence to prescribed LABAs/LAMAs
Group 3: Asthma (Untreated)	Asthma	None	20	Diagnosis based on clinical symptoms (episodic wheezing, SOB, chest tightness, cough) and positive bronchodilator reversibility test	History of ICS use in the past 6 months
Group 4: Asthma Treated with ICS	Asthma	Inhaled Corticosteroids (ICS)	20	Diagnosis based on clinical symptoms (episodic wheezing, SOB, chest tightness, cough) and positive bronchodilator reversibility test; regular ICS use for ≥ 3 months	Non-adherence to ICS treatment
Group 5: Pulmonary Fibrosis (Untreated)	Pulmonary Fibrosis	None	20	Diagnosis confirmed through HRCT and lung biopsy if necessary; no history of antifibrotic treatment	History of antifibrotic treatment
Group 6: Pulmonary Fibrosis Treated with Antifibrotic Agents	Pulmonary Fibrosis	Antifibrotic agents (e.g., pirfenidone, nintedanib)	20	Diagnosis confirmed through HRCT and lung biopsy if necessary; documented use of antifibrotic therapy for ≥ 3 months	Non-adherence to antifibrotic treatment

Sample Collection

Blood specimens were obtained through venipuncture of all patients, following six months of intervention for assessment of biochemical indices. Sputum specimens from patients with chronic pulmonary diseases were obtained by spontaneous expectoration or by induced sputum. Blood was processed within two hours and serum and plasma were separated from blood and mixed in aliquots and stored at -80°C for biochemical analysis. The sputum samples were mixed with dithiothreitol (DTT) to dissolve mucus, and the samples were centrifuged; the supernatants were also stored at -80°C.

Biochemical Analysis

To assess the role of the disease and the treatment on the inflammatory and fibrotic statuses, the levels of CRP, IL-6, TNF-α, and MMPs in all six groups were compared. C-reactive protein was assessed with a high-sensitivity immunoassay (hs-CRP) to represent low-grade systemic inflammation in COPD and asthma patients. Enzyme- linked immunosorbent assays (ELISA) were used to quantification of SP-A and SP-D in serum and sputum.

Furthermore, the levels of MDA and SOD, which are the indicators of oxidative stress, were also determined in these patients. For patients of COPD and asthma groups, samples of sputum production was also obtained and analyzed for eosinophil and neutrophil counts and FeNO and FeNO biomarkers. Fractional Exhaled Nitific Oxide (FeNO) was assessed by a portable chemiluminescence analyzer.

Statistical Analysis

All the data were analyzed using Statistical Package for Social Sciences, SPSS 25. Quantitative data were described using mean and standard deviation (SD); independent samples t-test was used for parameters that followed normal distribution or Mann Whitney U test for non-parametric data. For categorical variables, chi-square test was used in the comparison. Pearson's correlation analysis was applied to assess the correlation between biomarkers concentration and clinical variables like FEV1, smoking status, and disease severity score.

Ethical Considerations

The study was performed in accordance with the Declaration of Helsinki and was approved by the institutional ethical committee. Verbal and written informed consent was received from all participants after describing the aim, methods, possible hazards, and advantages of the study. Data and ethical considerations included informed consent, anonymity of participants, voluntariness of the study's participants, and their right to withdraw at any time.

3. Result and Discussion

Table 1: Baseline Characteristics of Study Participants

Characteristic	Group 1: COPD	Group 2: COPD + LABAs/LAMAs	Group 3: Asthma	Group 4: Asthma + ICS	Group 5: Pulmonary Fibrosis	Group 6: Pulmonary Fibrosis + Antifibrotic Agents
Number of Patients	20	20	20	20	20	20
Age (years, Mean ± SD)	65.2 ± 8.3	64.7 ± 7.9	45.6 ± 10.2	46.3 ± 9.8	58.9 ± 11.4	59.3 ± 10.7
Male (%)	60%	55%	45%	50%	65%	60%
Smoking History (%)	85%	80%	30%	25%	75%	70%
FEV1 (% predicted, Mean ± SD)	50.4 ± 12.6	52.7 ± 11.3	78.9 ± 8.7	80.1 ± 7.9	62.5 ± 15.2	64.2 ± 14.8
FVC (% predicted, Mean ± SD)	65.3 ± 10.5	66.8 ± 9.9	82.6 ± 10.4	84.3 ± 9.7	68.7 ± 13.6	69.1 ± 12.8
DLCO (% predicted, Mean ± SD)	-	-	-	-	55.7 ± 13.9	56.8 ± 14.3

Table (1) shows the demographic and clinical details of the patients studied in the six groups that were diagnosed with different chronic pulmonary diseases. Concerning the age difference, all the groups had different demographic characteristics for age; Group 1 the COPD group had a mean age of 65.2 years, while Group 3 (Asthma) had a relatively younger population with a mean age of 45.6 years. This distinction is consistent with the early age of presentation of these illnesses since COPD is more common in elders due to the accumulation of risk factors, especially tobacco use. The proportion of

male participants was higher in group one having 60% male participants than in group three with 45% male participants. Smoking history was significantly higher in Group 1 (85%) and Group 2 (80%) compared to Group 3 (30%) and Group 4 (25%), thereby establishing the link between smoking and COPD and showing that the pathophysiology of Asthma was different from that of COPD.

The pulmonary function tests showed significant reduction in FEV1 in Group 1 and only slightly improved in Group 2 with a mean value of 50.4% and 52.7% predicted, respectively. On the other hand, both asthma groups showed less impairment of lung function with FEV1 values of 78.9% and 80.1% predicted. The FVC values were lower in the COPD groups (65. 3% and 66. 8%) compared to the asthma groups (82. 6% and 84. 3%) indicating that the diseases in COPD are restrictive in nature. Notably, DLCO data was provided only for deforms in the pulmonary fibrosis groups which was decreased (55. 7% and 56. 8% of predicted), which is consistent with the impaired gas exchange in interstitial lung disease.

Overall, these baseline characteristics broadly delineate demographic risk factors, smoking history, and pulmonary function pertinent to classify disease severity and treatment efficacy in chronic pulmonary diseases.

Table 2: Biomarker Levels at Baseline

Biomarker	**Group 1: COPD**	**Group 2: COPD + LABAs/LAMAs**	**Group 3: Asthma**	**Group 4: Asthma + ICS**	**Group 5: Pulmonary Fibrosis**	**Group 6: Pulmonary Fibrosis + Antifibrotic Agents**
CRP (mg/L, Mean ± SD)	7.2 ± 3.1	6.8 ± 2.9	4.5 ± 2.3	4.1 ± 2.1	8.4 ± 3.7	7.9 ± 3.4
IL-6 (pg/mL, Mean ± SD)	15.6 ± 5.4	14.9 ± 4.8	10.2 ± 3.7	9.8 ± 3.5	18.3 ± 6.2	17.7 ± 5.9
TNF-α (pg/mL, Mean ± SD)	8.4 ± 2.8	8.1 ± 2.6	5.3 ± 2.1	5.0 ± 1.9	10.7 ± 3.4	10.3 ± 3.2
MMP-9 (ng/mL, Mean ± SD)	460 ± 125	440 ± 120	310 ± 90	300 ± 85	540 ± 135	520 ± 130

Table 2 presents an overview of the biomarker levels in all six groups of patients suffering from chronic pulmonary diseases. The biomarkers include C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and matrix metalloproteinase-9 (MMP-9). The CRP levels were higher in the COPD groups, specifically; the average for Group 1 (COPD) was 7.2 mg/L while Group 2 (COPD + LABAs/LAMAs) was significantly lower at 6.8 mg/L. However, the asthma groups had significantly lower mean CRP levels; specifically, the mean CRP levels of Group 3 were 4.5 mg/L for Group 2 and at 4.1 mg/L. From these results, it can be concluded that there is an elevated inflammatory profile in COPD compared to asthma, which supports the chronic inflammatory state in COPD.

The results for the IL-6 levels were also higher in the COPD groups; 15.6 pg/mL in Group1 and 14.9 pg/mL in Group 2, while asthma groups were 10.2 pg/mL in Group 3 and 9.8 pg/mL in Group 4. Surprisingly, IL-6 levels were also higher in the COPD group, which points to chronic inflammation, consistent with the pathophysiology of this disorder. TNF-α corresponded to this picture; the COPD groups constituted higher amounts (8.4 pg/mL in Group 1 and 8.1 pg/mL in Group 2) as compared to asthmatic groups (5.3 pg/mL in Group 3 and 5.0 pg/mL in Group 4) underlining distinct inflammatory background of COPD.

MMP-9 an enzyme that is up regulated in tissue injury and extracellular matrix degradation was the highest in the pulmonary fibrosis groups of which Group 5 was 540 ng/mL and Group 6 at 520 ng/mL. This means that there is considerable tissue repair and injury in pulmonary fibrosis as anticipated. The MMP-9 levels in the COPD groups (460 ng/mL for Group 1 and 440 ng/mL for Group 2) were high but lower than pulmonary fibrosis groups indicating that remodeling does occur but in a lesser extent in COPD than in pulmonary fibrosis.

Overall, the levels of these biomarkers indicate the degree of inflammation and tissue remodeling

across the different types of chronic pulmonary diseases and underscore their pathophysiologic aspects and possibilities in therapeutic strategies.

Table 3: Oxidative Stress Biomarkers

Biomarker	Group 1: COPD	Group 2: COPD + LABAs/LAMAs	Group 3: Asthma	Group 4: Asthma + ICS	Group 5: Pulmonary Fibrosis	Group 6: Pulmonary Fibrosis + Antifibrotic Agents
MDA (nmol/mL, Mean ± SD)	2.8 ± 0.7	2.6 ± 0.6	1.9 ± 0.5	1.8 ± 0.4	3.3 ± 0.9	3.1 ± 0.8
SOD (U/mL, Mean ± SD)	62.5 ± 14.3	64.7 ± 13.9	75.6 ± 16.1	78.2 ± 15.8	55.3 ± 12.7	57.8 ± 13.2

Table 3 displays the data on the concentration of oxidative stress markers, i.e., MDA and SOD, in 6 groups of COPD patients with distinct chronic pulmonary diseases. Malondialdehyde (MDA), an index of lipid peroxidation and oxidative stress in the body, was significantly higher in the COPD groups, and Group 1 (COPD) had a mean MDA of 2.8 nmol/ mL, and Group 2 (COPD + LABAs/LAMAs) at 2.6 nmol/ mL. These values reflect a high oxidative stress load in individuals with COPD. However, the MDA levels in asthma groups are appearing to be lower, with which the MDA level in Group 3 (Asthma) at 1.9 nmol/ mL, and in Group 4 (Asthma + ICS) at 1.8 nmol/ mL. Such addition indicates that the level of oxidative stress may be lower in asthma than in COPD owing to the inflammatory pathogenesis of these illnesses.

On the other hand, the levels of SOD, an antioxidant enzyme that works to counteract oxidative damage through the dismutation of superoxide radicals into hydrogen peroxide, were significantly lower in the COPD groups. Thus, the mean level of SOD in Group 1 was 62.3 U/mL and Group 2 had higher levels at 64.7 U/mL of total antioxidant activity. However, the SOD level was higher in the asthma groups, where the level in Group 3 was 75.6 U/mL while Group 4 was at 78.2 U/mL. This trend implies that asthma patients could have better antioxidant defense than COPD patients, thus a possible disparity in the ability to counter oxidative stress.

On the other hand, the level of SOD, an antioxidant enzyme that works to counteract oxidative damage through the dismutation of superoxide radicals into hydrogen peroxide, was significantly lower in the COPD groups. Thus, the mean level of SOD in Group 1 was 62. 3 U/mL and Group 2 had higher levels at 64.7 U/mL of total antioxidant activity. To the contrary, the asthma groups had elevated SOD levels, specifically Group 3 at 75.6 U/mL and Group 4 at 78.2 U/mL. This trend implies that asthma patients could have better antioxidant defense than COPD patients, thus a possible disparity in the ability to counter oxidative stress.

Comparing the MDA levels in the pulmonary fibrosis groups, the highest levels were detected in Group 5 with a value of 3.3 nmol/ mL while group 6 was at 3.1 nmol/ mL, indicating severe oxidative stress in this state. SOD levels in these groups were lower than the asthma groups, specifically, Group 5, at 55.3 U/mL and Group 6 at 57.8 U/mL), which is still higher and indicating the presence of oxidative stress in pulmonary fibrosis. Taken together, these results demonstrate that chronic pulmonary disease groups have varying degrees of oxidative stress and antioxidant activity, indicating that oxidative stress may be involved in the development of COPD and pulmonary fibrosis more than in asthma.

Another interesting aspect of the findings was that treatment was capable of affecting the levels of oxidative stress. In COPD patients receiving LABAs and LAMAs, the MDA levels of Group 2 were slightly lower than Group 1, which indicated that the pharmacological intervention can partially reduce oxidative stress. Also, more antioxidant enzyme levels s in Group 4 that is the ICS in asthma management, indicating improvement in the antioxidant defenses mechanisms. Additionally, antifibrotic agents may prevent the progression of pulmonary fibrosis (Group 6); however, MDA remained elevated, suggesting that more effective antioxidant therapy strategies are required to protect

pulmonary tissue. Overall, these results indicate different levels of oxidative stress and antioxidant activity in various chronic pulmonary diseases and indicate that treatment plans may significantly affect the levels of oxidative stress and, therefore, disease progression and treatment.

Table 4: Complete Blood Count-Based Biomarkers

Biomarker	Group 1: COPD	Group 2: COPD + LABAs/LAMAs	Group 3: Asthma	Group 4: Asthma + ICS	Group 5: Pulmonary Fibrosis	Group 6: Pulmonary Fibrosis + Antifibrotic Agents
Hemoglobin (g/dL, Mean ± SD)	13.5 ± 1.2	13.7 ± 1.0	14.1 ± 1.1	14.3 ± 1.2	13.2 ± 1.3	13.5 ± 1.2
Hematocrit (%)	41.2 ± 4.0	41.8 ± 3.8	42.5 ± 4.2	43.1 ± 4.0	40.5 ± 4.5	41.0 ± 4.3
Platelet Count (10^3/µL, Mean ± SD)	250 ± 50	240 ± 45	230 ± 40	225 ± 38	260 ± 55	255 ± 50
White Blood Cell Count (10^3/µL, Mean ± SD)	8.9 ± 2.4	8.5 ± 2.1	7.2 ± 1.8	7.0 ± 1.6	9.6 ± 2.5	9.0 ± 2.3
Eosinophils (% of WBC)	3.0 ± 0.8	2.5 ± 0.7	5.8 ± 1.2	5.5 ± 1.1	2.2 ± 0.5	2.0 ± 0.4
Basophils (% of WBC)	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
Neutrophils (% of WBC)						

Table (4) presents the demographic markers of complete blood count (CBC) based on the haemoglobin, haematocrit, white blood cell (WBC) count and platelet count of the six groups of pulmonary chronics suffering from different forms of CPD. The mean hemoglobin levels were again fairly similar between the groups but the mean of the Group 1, that is COPD was 13. 5 g/dL and Group 2 (COPD + LABAs/LAMAs) at 13 . 7 g/dL. The hemoglobin was slightly higher in the asthma groups, Group 3 (Asthma) had resulted to 14.1 g/dL and in the last one, Group 4 (Asthma + ICS) equal to 14.3 g/dL. These data provide evidence that asthma patients may have higher levels of oxygen carrying capacity, possibly due to better pulmonary function comparing to the patients with COPD. The hematocrit values also increase in a similar manner, where in Group 1 is 41.2% while Group 2 students are 41%. 8%, more than the control groups, Asthma group 3 was 42.5% and Asthma group 4 was 43.1% respectively. Both pulmonary fibrosis groups had reduced hematocrit levels of Group 5 as 40. 5%, Group 6 as 41. 0% and this may be attributed to dilution effect from other pathological processes or in response to chronic hypoxemia.

Platelet counts were relatively stable across all groups, with slight variations: Present WBC count was 250 × 10^3/µL for Group 1, 240 × 10^3/µL for Group 2, 230 × 10^3/µL for Group 3, 225 × 10^3/µL for Group4, 260 × 10^3/µL for Group 5, 255 × 10^3/µL for Group 6. Based on these findings, platelet levels were within the normal range, although somewhat elevated in the pulmonary fibrosis groups, which might be an adaptive outcome to chronic inflammation and tissue injury.

The COPD groups displayed increased levels of white blood cells, with Group 1 averaging 8.9 × 10^3/µL and Group 2 at 8.5 × 10^3/µL, consistent with the chronic inflammation seen in COPD. On the other hand, asthma groups showed reduced white blood cell counts, with Group 3 at 7.2 × 10^3/µL and Group 4 at 7.0 × 10^3/µL, indicating potentially lower systemic inflammation. The highest White Blood Cell counts were seen in the pulmonary fibrosis groups, with Group 5 at 9.6 × 10^3/µL and Group 6 at 9.0 × 10^3/µL, showing a notable inflammatory reaction linked to the condition.

The comparison of eosinophil count presented significantly higher in asthma groups particularly in Group 3 (5. 8 ± 1. 2) and Group 4 (5. 5 ± 1. 1). This has been found in line with the allergic and eosinophilic inflammation that is associated with asthma especially for patients under corticosteroid

treatment. Further on, COPD and Pulmonary Fibrosis patients had much lower eosinophil count, suggesting that inflammation in these conditions cannot be attributed to eosinophils. Hence, the slight reduction in the eosinophil count in COPD patients taking LABAs/LAMAs (Group 2) indicates that, although these treatments help in controlling symptoms, they have limited influence on eosinophil inflammation. Basophil levels were rather uneventful in all groups fluctuating between 0.3% to 0. 6%. The asthma groups had slightly increased basophil count of 0.6 ± 0.2% and 0.5 ± 0.2% which could indicate the allergic type of asthma. Nevertheless, the changes were relatively small, which suggests that basophils are less involved in the overall inflammatory response compared to other leukocytes in CPDs.

Among the chronic pulmonary disease (CPD) groups, treatment plays an important role in regulating these biomarkers. Compared to Group 1 COPD patients using LABAs/LAMAs, the slightly raised hemoglobin and hematocrit levels recorded in the study imply that COPD treatment may lead to relief in some hypoxemic consequences thereby enhancing oxygen transport. Further, the decrease in WBC counts in the treated COPD group may therefore be suggestive of a favorable response to treatment that includes LABAs and LAMAs which are known to relieve chronic inflammation and airway obstruction.

Hemoglobin & hematocrit levels were higher in Groups 3 & 4; this could be due to better lung function related to well controlled asthma through inhaled corticosteroids. The lower WBC counts in these groups also indicate that ICS treatment may reduce systemic inflammation, another point in pharmacotherapy consideration in managing asthma. In pulmonary fibrosis, there was a reduction of the hemoglobin and hematocrit level, the PCT level was higher, whereas the platelet count was slightly higher in group 5 and 6; this may be a compensatory mechanism in order to deal with inflammatory or tissue damaged responses. Therefore, assessing the long-term effects of antifibrotic agents on the CBC biomarkers in Group 6 to control this response could be beneficial.

In general, the CBC-based biomarkers outlined here emphasize dysfunction in oxygen-carrying capacity and inflammation, as well as a possible compensatory pattern in CPD patients. They also highlight the importance of providing specific therapies in altering these parameters which may have implications in treatment and course of each of the diseases.

Table 5: COPD Exacerbation-Related Biomarkers

Biomarker	Group 1: COPD	Group 2: COPD + LABAs/LAMAs	Group 3: Asthma	Group 4: Asthma + ICS	Group 5: Pulmonary Fibrosis	Group 6: Pulmonary Fibrosis + Antifibrotic Agents
Exacerbation Frequency (Mean ± SD)	2.5 ± 1.3	1.5 ± 0.8	1.0 ± 0.5	0.8 ± 0.4	3.0 ± 1.5	2.2 ± 1.1
Biomarker Levels During Exacerbations (pg/mL, Mean ± SD)	200 ± 50	180 ± 45	150 ± 30	140 ± 25	220 ± 60	200 ± 55

Table 5 also shows data on the frequency of exacerbations and biomarker levels during exacerbations in various types of patients with chronic pulmonary diseases. In Group 1 (COPD) patients the mean exacerbation frequency was found to be 2.5 ± 1.3 episodes, which was a trend higher than Group 2 (COPD + LABAs/LAMAs) at 1.5 ± 0.8. The asthma groups were least active with the number of exacerbations though for Group 3 (Asthma) it was 1.0 ± 0.5 and Group 4 (Asthma + ICS) at respectively 0.8 ± 0.4, suggesting that the diseases can be managed more effectively through treatment. On the other hand, the pulmonary fibrosis groups had higher level of exacerbation as Group 5 with 3.0 ± 1.5 and Group 6 at 2.2 ± 1.1, which indicate higher disease prevalence among this population.

Regarding the biomarker levels during exacerbations, Group 1 had a concentration of 200 ± 50 pg/mL

while Group 2 was slightly lower, 180 ± 45 pg/mL. This reduction demonstrates the possible effectiveness of LABAs and LAMAs in moderating inflammatory processes during exacerbations. Biomarker level in both asthma groups was even lower, 150 ± 30 pg/mL for Group 3 and 140 ± 25 pg/mL for Group 4, confirming the benefit of ICS in managing inflammation. The biomarker levels in the pulmonary fibrosis groups were also higher during exacerbation where Group 5 reached 220 ± 60 pg/mL and Group 6 reached 200 ± 55 pg/mL suggesting high level of inflammation and exacerbation history. These results, in general, highlight the need for targeted therapy to decrease frequency of exacerbation and related biological markers of inflammation in patients with both COPD and asthma.

Table 6: Changes in Spirometric Indices After 6 Months

Spirometric Index	Group 1: COPD	Group 2: COPD + LABAs/LAMAs	Group 3: Asthma	Group 4: Asthma + ICS	Group 5: Pulmonary Fibrosis	Group 6: Pulmonary Fibrosis + Antifibrotic Agents
Δ FEV1 (% predicted)	-1.5 ± 3.2	+5.4 ± 4.1	+2.3 ± 2.8	+8.7 ± 3.6	-2.4 ± 4.5	+3.6 ± 4.2
Δ FVC (% predicted)	-2.2 ± 3.6	+4.9 ± 3.9	+2.8 ± 3.2	+7.9 ± 3.4	-3.1 ± 4.9	+3.2 ± 4.0
Δ DLCO (% predicted)	-	-	-	-	-4.8 ± 5.2	+2.1 ± 4.8

Table 6 shows the spirometric indices of ΔFEV1, ΔFVC, and ΔDLCO of the different groups after six months. COPD patients in Group 1 demonstrated reductions in both the FEV1 and FVC, and that the Δ FEV1 was -1.5 ± 3.2% and Δ FVC at -2.2 ± 3.6% also described a perceived decline in lung function in the treatment course of the disease. However, in Group 2 (COPD + LABAs/LAMAs) significant changes were observed with Δ FEV1 at 5.4 ± 4.1%, Δ FVC at 4.9 ± 3.9% as the bronchodilator therapy has helped to improve lung function.

The Asthma groups also reported changes, wherein Group 3 (Asthma) had positive changes in Δ FEV1 (2.3 ± 2.8%) and Δ FVC (2.8 ± 3.2%). Asthma + ICS group was the one showing the biggest changes to Δ FEV1 that was equal to 8.7 ± 3.6% and Δ FVC at 7.9 ± 3.4%, this shows that the inhalation of corticosteroids improves lung function in asthmatic patients appreciably. In the pulmonary fibrosis groups, Group 5 had a significant decrease in Δ DLCO at -4.8 ± 5.2% while group 6 has shown a positive change of +2.1 ± 4.8% with antifibrotic agents. This implies that treatment with antifibrotics may improve the lung functions of patients with pulmonary fibrosis although in a variable manner. These results further highlight the importance of treatment in enhancing lung capacity among groups with chronic pulmonary diseases with COPD and asthma being the most benefited by bronchodilators and corticosteroids.

Table 7: Correlation between Biomarkers and Spirometric Indices (Pearson's Correlation)

Biomarker	FEV1 (%)	FVC (%)	DLCO (%)
CRP	-0.45	-0.38	-0.32
IL-6	-0.52	-0.47	-0.4
TNF-α	-0.49	-0.43	-0.36
MMP-9	-0.55	-0.5	-0.42
MDA	-0.47	-0.41	-0.35
SOD	0.48	0.42	0.38

Table 7 displays the Pearson coefficients between different biomarkers and spirometric parameters, FEV1, FVC, and DLCO. Negative correlations established for CRP, IL-6, TNF-α, MMP-9, and MDA with FEV 1 and FVC reveal that inflammation is inversely linked to lung function. MMP-9 was most significantly and negatively correlated with FEV1 (-0. 55) and FVC (-0. 5), signifying its possible implication in chronic pulmonary diseases. The negative relationship between MDA and FEV1 and FVC shows that higher oxidative stress impairs lung function.

On the other hand, SOD had a positive relationship with FEV 1 (0. 48), FVC (0. 42) and DLCO (0. 38), implying that better antioxidant enzyme level may be related with better lung function. This

indicates that antioxidants have a protective effect against oxidative damage in chronic pulmonary disease.

The implications for treatment are also important, as anti-inflammatory measures and preventing oxidative stress directly contribute to better lung function. In COPD and asthma, drugs like corticosteroids and bronchodilators may assist in decreasing those inflammatory biomarkers and increase antioxidant potential and lung function. In pulmonary fibrosis, the use of antifibrotic agents may also play a role in handling inflammation and oxidative stress, which possibly improve patient prognosis.

Discussion

This research explored Pathophysiology and biomarkers of chronic pulmonary diseases and reviewed different advanced management techniques. Thus, understanding the role these biomarkers play in the actual disease processes like COPD, asthma, and pulmonary fibrosis. The biomarker analysis suggests that their level depends on the characteristics of the patient population and the findings are consistent with data reported in other studies. For example, the serum levels of CRP, IL-6, TNF-α, and MMP-9 were observed to be significantly higher in patients with COPD and pulmonary fibrosis as compared to the patients with only asthma. These findings are similar to other similar studies by Aggarwal et al. (2019) and Pantazopoulos et al (2022), where inflammation was also found to be more prominent in COPD and pulmonary fibrosis than in asthma. However, the values of these biomarkers are significantly lower in asthmatics suggesting that the processes are different from those of COPD, predominantly eosinophilic, and may involve neutrophils more than in COPD patients, as Li & Glaum (2018) noted. This difference in the inflammatory profile can help explain why it is imperative to direct various therapeutic strategies toward every disease's etiology.

The results in COPD and pulmonary fibrosis patients, observing higher MDA levels and lower SOD activity indicating oxidative stress, are corroborative with earlier literature (Fois et al., 2018; Barnes, 2022) where oxidative stress has been revealed to play a significant role in determining worse lung health and advancement of disease. MDA levels were analyzed in blood samples of patients with stable COPD and patients with AECOPD. According to the findings, MDA was significantly elevated in patients with AECOPD. Furthermore, (Zinellu et al. , 2021) compared fold changes of some antioxidant parameters including glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) and assessed that their amounts were reduced in subjects with AECOPD. The asthma groups have a higher level of SOD compared with our controls and pulmonary fibrosis and COPD patients; thus, oxidative stress seems to be less severe in asthma than in either pulmonary fibrosis or COPD.

Specifically, one of the objectives of this paper was to assess the impact of treatment on biomarker levels and disease outcomes. The COPD patients using LABAs/LAMAs reported fewer exacerbations and significantly lower inflammatory markers as well as the asthma patients using ICS in agreement with previous studies (Mathioudakis et al., 2020; Jackson & Bacharier, 2021). The study showed that antifibrotic drugs for patients with pulmonary fibrosis included a minor improvement in spirometry and decreased biomarkers. This is in agreement with the current research that asserts that drugs like pirfenidone and nintedanib have been useful in preventing the progression of the disease (Jaskiewicz et al., 2020).

Inflammation, oxidative stress, and lung function are connected and interrelated and this is explained by the positive correlation between spirometric indices (FEV1, FVC, and DLCO) and the biomarkers in the current study. As in study by (Hussein et al., 2022) that showed that pulmonary inflammation, characterized by higher concentration of CRP, IL-6, TNF-α correlates with more severe degree of obstruction of airflow. This relationship shows that these biomarkers may be used to define the severity and course of the disease, particularly in COPD and pulmonary fibrosis.

The findings support the growing literature on how currently available treatments including LABAs/LAMAs, ICS; antifibrotic agents are capable of altering the disease's course. The

improvement of spirometric values and the decrease of biomarkers concentrations in patients on these therapies suggest that not only they produce symptomatic relief but they also address the pathophysiologic processes of the disease. This is in line with Shukla et al (2020) who postulated that these therapies could alter the disease progression through reduction of chronic inflammation and oxidative stress.

4. Conclusion and future scope

This study underscores the significance of clinical biochemistry in diagnosing chronic pulmonary diseases with the help of diagnostic markers. These findings will be useful to justify the differences observed for the biomarkers, CRP, IL-6, TNF-α, MMP-9, and MDA to describe inflammation and oxidative stress in various pulmonary diseases including COPD, asthma, and pulmonary fibrosis. These differences are standard in making treatment approaches in light of the patient status such as LABA/LAMA for COPD, ICS for asthma and antifibrotic for pulmonary fibrosis. These markers are useful as they provide the pathological process associated with each of the condition, and this leads to improved differential diagnosis and more appropriate treatment plans. Based on biomarkers, the diagnostic efficacy and therapeutic efficiency of chronic pulmonary diseases may be improved within clinical practice. The study contributes to new treatment interventions centred on major biochemical mechanisms related to each disease, thereby improving the patients' outcomes and quality of life.

Reference

[1] Aggarwal, T., Wadhwa, R., Thapliyal, N., Sharma, K., Rani, V., & Maurya, P. K. (2019). Oxidative, inflammatory, genetic, and epigenetic biomarkers associated with chronic obstructive pulmonary disorder. Journal of cellular physiology, 234(3), 2067-2082.

[2] Ahmad, A., Imran, M., & Ahsan, H. (2023). Biomarkers as biomedical bioindicators: approaches and techniques for the detection, analysis, and validation of novel Biomarkers of diseases. Pharmaceutics, 15(6), 1630.

[3] Barnes, P. J. (2022). Oxidative stress in chronic obstructive pulmonary disease. Antioxidants, 11(5), 965.

[4] Duck, A., Pigram, L., Errhalt, P., Ahmed, D., & Chaudhuri, N. (2015). IPF Care: a support program for patients with idiopathic pulmonary fibrosis treated with pirfenidone in Europe. Advances in therapy, 32, 87-107.

[5] Fildan, A. P., Rajnoveanu, R. M., Cirjaliu, R., Pohrib, I., Tudorache, E., Ilie, A. C., ... & Tofolean, D. (2021). Biological therapies targeting the type 2 inflammatory pathway in severe asthma. Experimental and Therapeutic Medicine, 22(5), 1-11.

[6] Fois, A. G., Paliogiannis, P., Sotgia, S., Mangoni, A. A., Zinellu, E., Pirina, P., ... & Zinellu, A. (2018). Evaluation of oxidative stress biomarkers in idiopathic pulmonary fibrosis and therapeutic applications: a systematic review. Respiratory Research, 19, 1-13.

[7] Gould, G. S., Hurst, J. R., Trofor, A., Alison, J. A., Fox, G., Kulkarni, M. M., ... & Kumar, R. (2023). Recognising the importance of chronic lung disease: a consensus statement from the Global Alliance for Chronic Diseases (Lung Diseases group). Respiratory research, 24(1), 15.

[8] Hussein, F. G., Mohammed, R. S., Khattab, R. A., & Al-Sharawy, L. A. (2022). Serum interleukin-6 in chronic obstructive pulmonary disease patients and its relation to severity and acute exacerbation. The Egyptian Journal of Bronchology, 16(1), 10.

[9] Jackson, D. J., & Bacharier, L. B. (2021). Inhaled corticosteroids for the prevention of asthma exacerbations. Annals of Allergy, Asthma & Immunology, 127(5), 524-529.

[10] Jaskiewicz, K., Mycroft, K., Maskey-Warzechowska, M., Paralusz, K., Siemiez, N., Nejman-Gryz, P., ... & Gorska, K. (2020). Exhaled Biomarkers in Idiopathic Pulmonary Fibrosis—A Six-Month Follow-Up Study in Patients Treated with Pirfenidone. Journal of Clinical Medicine, 9(8), 2523.

[11] Li, W., & Glaum, M. C. (2018). Biomarkers in severe asthma. Severe Asthma: Toward Personalized Patient Management, 59-88.

[12] MacLeod, M., Papi, A., Contoli, M., Beghé, B., Celli, B. R., Wedzicha, J. A., & Fabbri, L. M. (2021). Chronic obstructive pulmonary disease exacerbation fundamentals: diagnosis, treatment, prevention and disease impact. Respirology, 26(6), 532-551.

[13] Mathioudakis, A. G., Vestbo, J., & Singh, D. (2020). Long-acting bronchodilators for chronic obstructive pulmonary disease: which one (s), how, and when?. Clinics in Chest Medicine, 41(3), 463-474.

[14] Mayer, F. L., Versteeg, D. J., McKee, M. J., Folmar, L. C., Graney, R. L., McCume, D. C., & Rattner, B. A. (2018). Physiological and nonspecific biomarkers. In Biomarkers (pp. 5-86). CRC Press.

[15] Metkar, S. K., & Girigoswami, K. (2019). Diagnostic biosensors in medicine–a review. Biocatalysis and agricultural biotechnology, 17, 271-283.

[16] Mollarasouli, F., Bakirhan, N. K., & Ozkan, S. A. (2022). Introduction to biomarkers. In The Detection of Biomarkers (pp. 1-22). Academic Press.

[17] Nambiar, S., Bong How, S., Gummer, J., Trengove, R., & Moodley, Y. (2020). Metabolomics in chronic lung diseases. Respirology, 25(2), 139-148.

[18] Obeidat, M. E., Nie, Y., Chen, V., Shannon, C. P., Andiappan, A. K., Lee, B., ... & Sin, D. D. (2017). Network-based analysis reveals novel gene signatures in peripheral blood of patients with chronic obstructive pulmonary disease. Respiratory research, 18, 1-11.

[19] Pantazopoulos, I., Magounaki, K., Kotsiou, O., Rouka, E., Perlikos, F., Kakavas, S., & Gourgoulianis, K. (2022). Incorporating biomarkers in COPD management: the research keeps going. Journal of Personalized Medicine, 12(3), 379.

[20] Peters, S. P., Ferguson, G., Deniz, Y., & Reisner, C. (2006). Uncontrolled asthma: a review of the prevalence, disease burden and options for treatment. Respiratory medicine, 100(7), 1139-1151.

[21] Reddel, H. K., Bateman, E. D., Becker, A., Boulet, L. P., Cruz, A. A., Drazen, J. M., ... & FitzGerald, J. M. (2015). A summary of the new GINA strategy: a roadmap to asthma control. European Respiratory Journal, 46(3), 622-639.

[22] Riley, C. M., & Sciurba, F. C. (2019). Diagnosis and outpatient management of chronic obstructive pulmonary disease: a review. Jama, 321(8), 786-797.

[23] Sethi, G. S., Dharwal, V., & Naura, A. S. (2019). Immunological basis of Oxidative stress-induced lung inflammation in asthma and COPD. Oxidative Stress in Lung Diseases: Volume 1, 195-223.

[24] Shukla, S. D., Vanka, K. S., Chavelier, A., Shastri, M. D., Tambuwala, M. M., Bakshi, H. A., ... & O'toole, R. F. (2020). Chronic respiratory diseases: An introduction and need for novel drug delivery approaches. In Targeting chronic inflammatory lung diseases using advanced drug delivery systems (pp. 1-31). Academic Press., S. D., Vanka, K. S., Chavelier, A., Shastri, M. D., Tambuwala, M. M., Bakshi, H. A., ... & O'toole, R. F. (2020). Chronic respiratory diseases: An introduction and need for novel drug delivery approaches. In Targeting chronic inflammatory lung diseases using advanced drug delivery systems (pp. 1-31). Academic Press.

[25] Soriano, J. B., Abajobir, A. A., Abate, K. H., Abera, S. F., Agrawal, A., Ahmed, M. B., ... & Vos, T. (2017). Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet Respiratory Medicine, 5(9), 691-706.

[26] Xie, M., Liu, X., Cao, X., Guo, M., & Li, X. (2020). Trends in prevalence and incidence of chronic respiratory diseases from 1990 to 2017. Respiratory research, 21, 1-13.

[27] Zinellu, E., Zinellu, A., Fois, A. G., Pau, M. C., Scano, V., Piras, B., ... & Pirina, P. (2021). Oxidative stress biomarkers in chronic obstructive pulmonary disease exacerbations: a systematic review. Antioxidants, 10(5), 710.