Comparison and Correlation of Oxidative and Antioxidant Markers with Clinical Outcomes in Patients With Interstitial Lung Disease And Healthy Individuals

Comparação e Correlação de Marcadores Oxidantes e Antioxidantes com Desfechos Clínicos em Pacientes com Doença Pulmonar Intersticial e Indivíduos Saudáveis

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Abstract

Within the spectrum of interstitial lung diseases (ILD), idiopathic interstitial pneumonias (IIP) and connective tissue diseases (CTD) are notably prevalent. The potential differences in oxidative stress biomarkers across various ILD diagnoses have not been fully elucidated. Objective: This study aims to compare oxidative stress biomarkers among different ILD diagnoses, specifically IIP and CTD, and to examine the correlation of these markers with clinical outcome variables. The study assessed body composition, lung function, exercise capacity, and peripheral muscle strength. The oxidative stress biomarkers evaluated included Paraoxonase 1 (PON-1), Nitric Oxide (NO), Protein Oxidation (AOPP), Hydroperoxides (LOOH), Sulfhydryl (SH), Superoxide Dismutase (SOD), Catalase (CAT), Total Glutathione (TG), Reduced Glutathione (GSH), and Oxidized Glutathione (GSSG). The study included healthy individuals (n=39; 60±9 years old; BMI 27±4 kg/m²; DLCO 77±12% predicted), patients with IIP (n=22; 64±10 years old; BMI 28±5 kg/m²; DLCO 39±21% predicted), and patients with CTD (n=29; 58±10 years old; BMI 27±6 kg/m²; DLCO 51±20% predicted). Significant differences were observed between healthy controls and patients with ILD in terms of lung function, 6-minute walk test (6MWT), muscle strength (MS), and the biomarkers GSH, SH, and LOOH ($p<0.05$). In intergroup comparisons, only the LOOH marker showed significant differences (IIP 201440±9160 vs. CTD 156140±1137; $p=0.02$). No significant differences were found between IIP and CTD for lung function, 6MWT, MS, and most oxidative stress biomarkers. Both patient groups demonstrated a correlation between antioxidant levels and quadriceps muscle strength.

Keywords: Interstitial Lung Diseases. Clinical outcomes. Biomarkers.

Resumo

Dentre o grupo de doenças pulmonares intersticiais (DPI), as pneumonias intersticiais idiopáticas (PII) e as doenças do tecido conjuntivo (DTC) apresentam alta prevalência. Porém, ainda não se sabe se há diferenças de biomarcadores de estresse oxidativo entre os diferentes diagnósticos das DPI. Comparar os biomarcadores de estresse oxidativos entre os diferentes diagnósticos das DPI (i.e., PII e DTC) e correlacionar os marcadores com variáveis de desfechos clínicos. Foram realizadas as avaliações de composição corporal, função pulmonar, capacidade de exercício e força muscular periférica. Os biomarcadores analisados foram: Paraoxonase 1 (PON-1), Óxido Nítrico (NO), Oxidação de Proteínas (AOPP), Hidroperóxidos (LOOH), Sulfidrila (SH), Superóxido Dismutase (SOD), Catalase (CAT), Glutationa Total (GT), Glutationa Reduzida (GSH) e Glutationa Oxidada (GSSG). Foram incluídos saudáveis (n=39; 60±9 anos; índice de massa corpórea IMC 27±4 kg/m2; difusão de monóxido de carbono DLCO 77±12% predito); pacientes com PII (n=22; 64±10 anos; IMC 28±5 kg/m2; DLCO 39±21% predito); e pacientes com DTC (n=29; idade 58±10 anos; IMC 27±6 kg/m2; DLCO 51±20% predito). Houve diferença entre controle e DPI para a função pulmonar, TC6min, FM e para os marcadores: GSH, SH e LOOH ($p<0.05$). Na análise intergrupo, apenas o marcador LOOH apresentou diferenças (PII 201440±9160url vs DTC 156140±1137url; $p=0.02$). Não existem diferenças entre PII e DTC para a função pulmonar, TC6min, FM e para maioria de biomarcadores de estresse oxidativo. Ambos os grupos apresentaram correlação dos antioxidantes com FM de quadríceps.

Palavras-chave: Doenças Pulmonares Intersticiais. Desfechos clínicos. Biomarcadores.

1 Introduction

Interstitial Lung Diseases (ILDs) encompass a diverse group of over 300 diseases that share characteristics like chronic inflammation and fibrosis within the lung tissue, leading to the deterioration of functional alveolar-capillary units and a decrease in the diffusion capacity for carbon monoxide (DLCO)1,2. This also results in reduced vital and total lung capacities, as well as diminished distensibility and size of the alveoli3,4. Within this group, idiopathic interstitial pneumonias (IIP) and interstitial pneumonia associated with connective tissue diseases (CTD) are notably prevalent. Generally, an ILD diagnosis linked to CTD suggests a more favorable prognosis, whereas patients with IIP tend to experience more severe gas exchange impairments, which are

associated with a poorer prognosis1,5. In patients with chronic lung diseases, chronic inflammation and oxidative stress play crucial roles in disease pathogenesis. Increased oxidative stress arises from a heightened presence of oxidants and elevated levels of reactive oxygen species (ROS) produced by inflammatory, immune, and airway epithelial cells. This leads to cellular imbalance and an inflammatory response due to the disruption of oxidative equilibrium6-7. Consequently, the antioxidant system is essential for preventing or mitigating the damage caused by the harmful effects of free radicals in pathological processes8-9. The antioxidant system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione-related enzymes (total glutathione [TG], reduced glutathione [GSH], and oxidized glutathione [GSSG]). The oxidative system consists of markers like advanced oxidation protein products (AOPP) and lipid hydroperoxides (LOOH)9-11.

Some studies suggest that an imbalance between oxidants and antioxidants in the airways plays a crucial role in the pathogenesis of pulmonary fibrosis12,13. It is believed that unidentified fibrotic stimuli disrupt the balance between oxidant production and antioxidant defenses, leading to an accumulation of free radicals10. Moreover, oxidants may contribute to the progression of pulmonary fibrosis by influencing cytokine production and genes associated with fibroblast growth factors12.

Considering this, research on lung diseases indicates altered levels of oxidant and antioxidant markers10. The expression of these biomarkers can exacerbate inflammation, playing a significant role in the pathogenesis of fibrosing diseases14. Consequently, the primary objective of the current study was to compare the levels of oxidative stress biomarkers across different ILD diagnoses, specifically IIP and interstitial pneumonia associated with CTD. The secondary objectives were to correlate these biomarkers with clinical outcome variables. It was hypothesized that there are no biomolecular and functional differences among the various diagnoses within ILD.

2 Materials and Methods

2.1 Ethnic aspects and type of study

The study involved human participants and was conducted in compliance with Resolution 466/2012 of the National Health Council. It received approval from the Institution's Research Ethics Committee. This was a cross-sectional study, and all participants signed an informed consent form (Appendix A). Individuals with ILD were recruited from the Specialties Outpatient Clinic associated with the University Hospital of the State University of Londrina - PR. The study included participants of both genders, aged 40 to 75 years, who had a diagnosis of interstitial lung disease based on internationally accepted criteria, including idiopathic pulmonary fibrosis, sarcoidosis, collagenosis, occupational lung disease, hypersensitive pneumonitis, and other forms of idiopathic pulmonary pneumonia. Participants had a diagnosis duration of no more than two years, had maintained clinical stability for the last four weeks, and lacked any comorbidities that might interfere with test performance. The healthy group comprised apparently healthy adults with normal spirometry results of both genders, aged 40 to 75 years, who also had no comorbidities that could affect test performance. Patients were excluded from the study if they had severe or unstable heart disease identified during the initial cardiopulmonary exercise test, lacked the cognitive ability to perform the tests, developed lung cancer, or were placed on a waiting list for a lung transplant.

2.2 Assessments

2.2.1 Body composition

Body composition was evaluated using bioelectrical impedance analysis (Biodynamics® USA). This assessment provided calculations for the percentage of body fat and lean mass. Additionally, body mass index (BMI) and the circumference of the quadriceps femoris of the dominant lower limb were determined.

2.2.2 Lung function

Lung function was assessed using whole-body plethysmography (Vmax, CareFusion®) in accordance with international guidelines3-5. The variables analyzed included forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and carbon monoxide diffusion capacity (DLCO). The results were compared with normative data for the Brazilian population15-17.

2.2.3 Exercise capacity

Exercise capacity was evaluated using the 6-minute walk test (6MWT) and the cardiopulmonary exercise test (CPET). For the 6MWT, the results were compared against normative data18. For the CPET, a lower limb cycle ergometer was utilized following international guidelines19. The test protocol included 3 minutes of rest, followed by 3 minutes of unloaded pedaling, and then an incremental exercise phase, with increases of 10 Watts per minute for patients and 20 Watts per minute for controls.

2.2.4 Physical activity in daily life

To analyze physical activity in daily life, specifically the number of steps, individuals were instructed to wear an activity monitor (Actigraph, wGT3x-BT, Actigraph®, USA) on their waist for six consecutive days, 24 hours a day. This monitor has been validated as a reliable method for assessing physical activity in daily life in patients with respiratory

diseases20.

2.2.5 Peripheral muscle strength:

Peripheral muscle strength was evaluated through measurements of handgrip and quadriceps strength. Handgrip strength of the dominant limb was assessed using a portable dynamometer (Jamar– Medical Iberica)21. Quadriceps strength was measured by maximum voluntary isometric contraction (MVIC) of the dominant limb using a load cell (EMG System®, Brazil) attached to a multistation device (CRW 1000, CRW, Brazil)22.

2.2.6 Oxidative stress through blood samples

A total of 20 mL of blood was collected by venipuncture from all volunteers who had fasted for at least 8 and no more than 12 hours. This included 10 mL of blood in vacuum tubes (vacutainer®) containing EDTA, 5 mL in tubes containing fluoride for glucose measurement, and 5 mL in tubes without anticoagulant for biochemical parameter analysis. The tests were performed in triplicate, and the intra-assay coefficient of variation was less than 10%9,23,24.

To assess oxidant levels, the following markers were analyzed: Paraoxonase 1 (PON-1), Nitric Oxide Metabolites (NO), Protein Oxidation (AOPP), Hydroperoxides (LOOH), and Sulfhydryl (SH). For antioxidant evaluation, the markers used were Superoxide Dismutase (SOD), Catalase (CAT), Total Glutathione (TG), Reduced Glutathione (GSH), and Oxidized Glutathione (GSSG).

2.3 Statistical analysis

Statistical analysis was conducted using SAS University Edition software (SAS Institute, USA), with a significance level set at $p<0.05$. Data normality was assessed using the Shapiro-Wilk test. Continuous variables were reported as either mean and standard deviation (for normal distributions) or median and interquartile range (for asymmetric distributions). To compare outcomes between groups, the ANOVA test was employed for parametric data, followed by either Tukey's post-hoc test or the Kruskal-Wallis test with Dunn's post-hoc test for nonparametric data. Spearman's coefficient was used to evaluate correlations.

3 Results and Discussion

A total of 90 individuals participated in this study, comprising 39 healthy participants (healthy group), 22 patients with idiopathic interstitial pneumonia (IIP), and 29 patients with connective tissue disease (CTD). The characteristics of the study participants and the results of the evaluations conducted are detailed in Table 1. Patients with interstitial lung disease (ILD), including both IIP and CTD, exhibited reduced lung function, diminished peripheral quadriceps muscle strength, and impaired exercise capacity compared to the healthy group.

Table 1 – Sample characterization and comparison of clinical outcomes analyzed between groups

Variables	Healthy Group (n=39)	Group A IIP (n=22)	Group B CTD (n=29)	p-value
Age (years)	60±9	67 [56-71]	59 [47-66]	0.0870
Sex, male (%)	49% [b]	68% [b]	14%	0.0005*
BMI (kg/m²)	27±4	28±5	27±6	0.7911
Lean Mass (%)	75 [73-77]	76 [74-77]	73 [72-76]	0.1149
Body Fat (%)	30±8 [b]	33±7	35±7	0.0255*
Thigh Circumference (cm)	52 [48-54] [a]	48 [44-52]	51 [49-55]	0.0397*
Lung Function:				
FVC (L)	3.24 [2.74-4.03] [a,b]	2.34 [2.16-2.76]	2.44 [1.78-2.72]	<.0001*
FVC (% predicted)	99±12 [a,b]	68±18	72±20	<.0001*
FEV_1 (L)	2.56 [2.30-3.11] [a,b]	1.99 [1.77-2.33]	1.98 [1.39-2.27]	<.0001*
FEV_1 (% predicted)	98±13 [a,b]	72±18	75±20	<.0001*
DLCO (mL/mmHg/min)	23±6 [a,b]	11±6	13±6	<.0001*
DLCO (% predicted)	77±12 [a,b]	39±21	52±20	<.0001*
Peripheral Muscle Strength:				
Quadriceps (kgf)	42 [35-53] [a,b]	32 [26-49]	29 [24-34]	<.0001*
Handgrip Strength (kgf)	30 [25-36] [b]	30 [21-32]	23 [19-27]	0.0219*
Exercise Capacity:				
6MWT (meters)	569±75 [a,b]	440±104	457±103	<.0001*
6MWT (% predicted)	105±12 [a,b]	81±19	86±17	<.0001*
CPET (Maximum Watts)	100 [80-140] [a,b]	40 [20-60]	60 [25-75]	<.0001*
Steps Counts (steps/day)	6881 [5103-8501] [a,b]	4402 [2846-6252]	5175 [4094-6635]	<.0001*

a vs IIP (idiopathic interstitial pneumonia); b vs CTD (connective tissue diseases); data are described as mean ± standard deviation or median [interquartile range]. BMI: body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; DLCO: diffusing capacity for carbon monoxide; 6MWT: six-minute walk test; N: newton; Kgf: kilogram-force.
Source: research data.

When comparing the biomarkers between the groups (Table 2), it was possible toobserve that there were no differences for most of the markers analyzed. The oxidantmarker (SH) showed a difference between the healthy group and the CTD group($p=0.0151$). And there were also differences in the oxidant marker (LOOH) between thehealthy group versus IIP, and between IIP versus CTD ($p=0.0012$).

Table 2 – Levels of biomarkers (oxidants and antioxidants) and comparison of markers between groups.

Biomarkers	Healthy Group (n=39)	Group A IIP (n=22)	Group B CTD (n=29)	p-value
Oxidants:				
PON-1	204±58	196±52	201±54	0.7939
NO	6 [4-7]	5 [4-8]	5 [4-7]	0.8747
AOPP	108 [74-186]	120 [84-129]	106 [84-163]	0.7496
SH	300±55 [b]	269±67	259±57	0.0151*
LOOH	13092 [95530 – 14345] [a]	18170 [13981-24529] [b]	10923 [91847-19634]	0.0012*
Antioxidants:				
SOD	59 [51-70]	58 [51-67]	57 [45-64]	0.7724
CAT	77 [59-92]	67 [58-85]	77 [65-95]	0.3665
GT	6 [5-7]	6 [5-7]	7 [6-8]	0.0724
GSH	5 [5-6]	5 [5-6]	5 [5-6]	0.4890
GSSG	0.4 [0.2-0.5]	0.4 [0.2-0.6]	0.6 [0.4-0.8]	0.0520

a vs IIP (Idiopathic Interstitial Pneumonias); b vs CTD (Connective Tissue Diseases); PON-1: Paraoxonase; NO: Nitric Oxide Metabolites; AOPP: Protein Oxidation; SH: Sulfhydryl; LOOH: Hydroperoxides. SOD: Superoxide Dismutase; CAT: Catalase; GT: Total Glutathione; GSH: Reduced Glutathione; GSSG: Oxidized Glutathione.
Source: research data.

In the IIP group, significant correlations were found between

peripheral quadriceps muscle strength and antioxidant markers, specifically Catalase (CAT) (r=-0.50; p=0.01), Total Glutathione (GT) (r=-0.50; p=0.01), and Oxidized Glutathione (GSSG) (r=-0.53; p=0.01). Additionally, a correlation was noted between thigh circumference and Reduced Glutathione (GSH) (r=-0.66; p=0.02) (Table 3).

Table 3 – Correlation of the PII group with clinical variables

Idiopathic Interstitial Pneumonias (IIP)									
	Age	**BMI**	**% Lean Mass**	**% Body Fat**	**Thigh Circumference**	**Maximum Load (TCPE)**	**Quadriceps**	**Hand**	**Steps**
Antioxidants:									
SOD	r=0.27 p=0.41	r=0.12 p=0.70	r=0.15 p=0.65	r=-0.15 p=0.65	r=0.50 p=0.66	r=-0.44 p=0.26	r=-0.06 p=0.85	r=0.10 p=0.74	r=0.07 p=0.83
CAT	r=0.12 p=0.58	r=0.04 p=0.82	r=-0.13 p=0.54	r=0.12 p=0.57	r=0.20 p=0.53	r=-0.37 p=0.15	**r=-0.50 p=0.01***	r=0.00 p=0.96	r=-0.23 p=0.28
GT	r=0.10 p=0.63	r=-0.11 p=0.62	r=0.12 p=0.58	r=0.17 p=0.44	r=-0.48 p=0.12	r=-0.11 p=0.66	**r=-0.50 p=0.01***	r=-0.29 p=0.19	r=-0.12 p=0.59
GSH	r=0.12 p=0.57	r=-0.09 p=0.68	r=0.19 p=0.38	r=0.14 p=0.50	**r=-0.66 p=0.02***	r=0.06 p=0.79	r=-0.38 p=0.08	r=-0.21 p=0.35	r=0.09 p=0.66
GSSG	r=0.30 p=0.16	r=-0.28 p=0.19	r=0.20 p=0.34	r=0.00 p=0.97	r=-0.37 p=0.25	r=-0.38 p=0.14	**r=-0.53 p=0.01***	r=-0.22 p=0.35	r=-0.35 p=0.10
Oxidants:									
PON	r=0.09 p=0.67	r=0.13 p=0.53	r=-0.03 p=0.87	r=0.06 p=0.77	r=0.01 p=0.96	r=-0.12 p=0.62	r=-0.00 p=0.97	r=-0.05 p=0.81	r=0.12 p=0.56
NO	r=-0.45 p=0.08	r=0.20 p=0.46	r=-0.10 p=0.72	r=0.34 p=0.21	r=0.28 p=0.49	r=0.05 p=0.86	r=-0.25 p=0.36	r=-0.27 p=0.32	r=-0.02 p=0.92
AOPP	r=0.01 p=0.94	r=-0.07 p=0.72	r=-0.17 p=0.43	r=-0.02 p=0.90	r=-0.06 p=0.84	r=-0.08 p=0.73	r=0.19 p=0.40	r=0.07 p=0.76	r=0.07 p=0.74
LOOH	r=0.33 p=0.12	r=-0.41 p=0.05	r=0.28 p=0.19	r=-0.13 p=0.55	r=-0.33 p=0.30	r=-0.37 p=0.13	r=-0.30 p=0.18	r=0.13 p=0.58	r=-0.32 p=0.14
SH	r=-0.18 p=0.50	r=0.34 p=0.21	r=-0.10 p=0.69	r=0.12 p=0.64	r=0.41 p=0.30	r=0.00 p=0.98	r=-0.15 p=0.58	r=-0.15 p=0.57	r=-0.30 p=0.26

PON-1: Paraoxonase; NO: Nitric Oxide Metabolites; AOPP: Protein Oxidation; SH: Sulfhydryl; LOOH: Hydroperoxides. SOD: Superoxide Dismutase; CAT: Catalase; GT: Total Glutathione; GSH: Reduced Glutathione; GSSG: Oxidized Glutathione.
Source: research data.

In the correlation analysis for the CTD group, significant associations were observed for several markers with the following variables: age (Superoxide Dismutase [SOD] r=0.71; p=0.04; Hydroperoxides [LOOH] r=0.44; p=0.01), body mass index (SH r=0.41; p=0.05; LOOH r=-0.39; p=0.03), percentage of lean mass (Paraoxonase 1 [PON] r=-0.50; p=0.001; LOOH r=0.42; p=0.02), percentage of body fat (CAT r=-0.55; p=0.001), maximum CPET load (PON r=0.49; p=0.02; SOD r=0.81; p=0.04), and peripheral quadriceps muscle strength (SOD r=0.81; p=0.02) (Table 4). No significant correlations were found for the remaining variables.

Table 4 – Correlation of the CTD group with clinical variables.

Connective Tissue Diseases (CTD)									
	Age	**BMI**	**% Lean Mass**	**% Body Fat**	**Thigh Circumference**	**Maximum Load (TCPE)**	**Quadriceps**	**Handgrip**	**Steps**
Antioxidants:									
SOD	**r=0.71 p=0.04**	r=-0.59 p=0.11	r=-0.25 p=0.58	r=-0.35 p=0.43	r=-0.02 p=0.95	**r=0.81 p=0.04***	**r=0.81 p=0.02***	r=0.69 p=0.12	r=-0.11 p=0.77

CAT	r=-0.07 p=0.69	r=-0.29 p=0.12	r=-0.08 p=0.66	**r=-0.55** **p=0.001**	r=-0.30 p=0.15	r=0.22 p=0.33	r=0.16 p=0.41	r=0.00 p=0.96	r=0.30 p=0.11
GT	r=-0.07 p=0.68	r=-0.26 p=0.16	r=-0.08 p=0.68	r=-0.30 p=0.12	r=0.12 p=0.57	r=0.21 p=0.36	r=0.28 p=0.14	r=0.14 p=0.47	r=0.05 p=0.76
GSH	r=-0.00 p=0.96	r=-0.19 p=0.31	r=0.12 p=0.54	r=-0.30 p=0.12	r=0.11 p=0.61	r=0.02 p=0.90	r=0.24 p=0.20	r=0.01 p=0.95	r=0.12 p=0.53
GSSG	r=-0.22 p=0.24	r=-0.10 p=0.57	r=-0.33 p=0.08	r=0.01 p=0.93	r=0.10 p=0.62	r=-0.01 p=0.95	r=-0.03 p=0.85	r=0.11 p=0.58	r=-0.20 p=0.28
Oxidants:									
PON	r=-0.27 p=0.16	r=0.05 p=0.78	**r=-0.50**	r=-0.10 p=0.59	r=-0.32 p=0.14	**r=0.49** **p=0.02***	r=0.30 p=0.12	r=0.22 p=0.28	r=0.13 p=0.48
NO	r=-0.00 p=0.99	r=-0.05 p=0.81	r=-0.01 p=0.95	r=-0.33 p=0.15	r=0.07 p=0.75	r=0.08 p=0.76	r=-0.14 p=0.54	r=0.24 p=0.31	r=0.17 p=0.44
AOPP	r=0.13 p=0.51	r=0.30 p=0.12	r=-0.09 p=0.65	r=-0.02 p=0.91	r=0.29 p=0.18	r=0.42 p=0.06	r=0.08 p=0.68	r=0.07 p=0.72	r=0.14 p=0.46
LOOH	**r=0.44** **p=0.01***	**r=-0.39** **p=0.03***	**r=0.42** **p=0.02***	r=-0.13 p=0.51	r=-0.05 p=0.81	r=0.09 p=0.68	r=0.15 p=0.43	r=0.21 p=0.28	r=-0.02 p=0.89
SH	r=-0.07 p=0.75	**r=0.41** **p=0.05***	r=-0.41 p=0.06	r=0.33 p=0.13	r=0.19 p=0.41	r=0.08 p=0.74	r=-0.04 p=0.85	r=-0.14 p=0.55	r=-0.32 p=0.14

PON-1: Paraoxonase; NO: Nitric Oxide Metabolites; AOPP: Protein Oxidation; SH: Sulfhydryl; LOOH: Hydroperoxides. SOD: Superoxide Dismutase; CAT: Catalase; GT: Total Glutathione; GSH: Reduced Glutathione; GSSG: Oxidized Glutathione.

Source: research data.

The hypothesis of the present study was accepted, as there were no clinical or functional differences, nor significant differences observed in most of the biomarkers analyzed when comparing the IIP group with the CTD group. However, the LOOH marker (oxidant) did show significant differences between the different diagnoses. In the IIP group, correlations were found between antioxidant biomarkers and both thigh circumference and quadriceps muscle strength. In contrast, for the CTD group, significant relationships were identified between the biomarkers (both oxidants and antioxidants) and various variables, including age, body composition, quadriceps muscle strength, and exercise capacity as assessed by cardiopulmonary exercise testing.

Given the critical importance of maintaining redox homeostasis in muscle fibers, muscle cells (myocytes) have developed a network of antioxidant defense mechanisms to reduce the risk of oxidative damage during periods of increased reactive oxygen species (ROS) production. Free radicals can impair muscle strength, gradually affecting the overall function of the musculoskeletal system25. In the present study, correlations were observed in both groups between quadriceps muscle strength and specific antioxidant markers, suggesting that greater muscle strength is associated with enhanced antioxidant production.

The respiratory system, particularly the lungs, comprises both enzymatic and non-enzymatic antioxidant systems. According to Regan et al., in patients with lung diseases, serum activity of the Superoxide Dismutase (SOD) marker is significantly associated with the degree of airflow obstruction26. Since SOD and Catalase (CAT) are sensitive to the effects of increased oxidative stress in the airways, their enzymatic activity can be adversely affected, leading to modifications that result in a loss of function. For instance, SOD oxidation has been observed in the airways of patients with asthma and correlates with the severity of the disease27.

In contrast, the findings of this study indicated that the SOD marker did not exhibit significant differences between the analyzed groups. However, a notable relationship was identified between SOD levels in the CTD group and both muscle strength and exercise capacity. Furthermore, as noted by Kaarteenaho and Lappi-Blanco, analyzing biomarkers is essential, particularly in idiopathic interstitial pneumonia (IIP), as they can serve as valuable tools for differential diagnosis and may help identify predictors of disease progression and response to treatment. Early diagnosis of idiopathic pulmonary fibrosis (IPF) is particularly important

to mitigate disease progression28,30.

Regarding the limitations of this study, it is important to recognize that patients exhibit varying degrees of lung parenchyma involvement, which renders this population a complex target for study. Additionally, oxidative stress is a multifaceted phenomenon with significant physiological and pathophysiological implications that can be influenced by various factors, including medication use, presence of comorbidities, diet, exercise, and overall lifestyle. Moreover, there is still a limited understanding of when oxidative stress is primarily an epiphenomenon versus a key factor related to disease25. Due to this gap in knowledge, along with the absence of standardized biomarkers that correlate with clinical or functional outcomes, further research is necessary to analyze and characterize systemic levels of blood biomarkers in interstitial lung disease (ILD).

4 Conclusion

Patients with idiopathic interstitial pneumonia (IIP) and connective tissue disease (CTD) do not exhibit significant differences in lung function, exercise capacity, muscle strength, or most oxidative stress biomarkers. In the IIP group, moderate correlations were found between certain biomarkers and quadriceps muscle strength as well as thigh circumference measurements. Conversely, the CTD group demonstrated correlations with body composition, quadriceps muscle strength, and exercise capacity.

Research on the expression of these biomarkers in patients with interstitial lung disease (ILD) remains limited. The complexities associated with continuous oxidative modifications pose significant challenges in translating these findings into high-yield and cost-effective clinical diagnostics.

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