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ORIGINAL CONTRIBUTION

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Study of the Correlation between HRCT Semi-quantitative Scoring, Concentration of Alveolar Nitric Oxide, and Clinical-functional Parameters of Systemic Sclerosis-induced Interstitial Lung Disease

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Introduction: The correlation between alveolar nitric oxide (CANO) and the severity of interstitial lung disease (ILD) evaluated by high resolution computed tomography (HRCT) has not been well demonstrated. **Methods**: It was a perspective and observational study, including patients with diagnosed systemic sclerosis (SSc). They performed lung function testing (LFT), exhaled nitric oxide (NO) measurements, exercise testing, chest X-ray, and HRCT. Study patients were divided into SSc with ILD (SSc-ILD+) or without ILD (SSc-ILD-). SSC-ILD+ patients were revisited after 6 months and 12 months to complete the study. **Results**: Thirty-one control subjects and 74 patients with SSc (33 SSc-ILD- and 41 SSc-ILD+) were included. Forty-one SSc-ILD+ patients were followed-up at 6 months and 12 months. Lung functional parameters of patients with SSc-ILD+ were lower than that of SSc-ILD- patients. The level of CANO was significantly higher in SSc-ILD+ than SSc-ILD- patients (8.6 ± 2.5 vs 4.2 ± 1.3 ppb and P<0.01). Warrick and Goldin scores of patients with SSc-ILD+ were respectively 16.5 ± 5.2 and 12.7 ± 4.3. Warrick scores were reduced after 6 and 12 months of follow-up vs at inclusion (12.4 ± 4.3 and 9.1 ± 3.2 vs 16.5 ± 5.2; P<0.05, P<0.01, and P<0.05; respectively). ΔWarrick and ΔGoldin scores were significantly and inversely correlated with ΔFVC, ΔTLC, ΔTLCO, ΔVO_2 max; that was also correlated with ΔCANO (R= 0.783, P<0.01 and R= 0.719 and P<0.05). **Conclusion**: CANO is a relevant biomarker for the diagnosis of ILD in patients with SSc, especially in combination with HRCT.

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Abbreviations: CANO, alveolar nitric oxide; ILD, interstitial lung disease; HRCT, high resolution computed tomography; LFT, lung function testing; SSc, Systemic sclerosis; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; 6MWT, 6-minute walk test; 6MWD, 6-minute walk distance; VO_2max, Maximal Oxygen Consumption Test.

Keywords: Systemic sclerosis, interstitial lung disease, exhaled nitric oxide, CANO

Author Contributions: Conception and design of the work: HHD, QPH, TVM, and SDQ; Acquisition, interpretation, and analysis: HHD, QPH, TVM, and SDQ; Drafted the work: HHD, QPH, and SDQ; Review & editing: TVM and SDQ.

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INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune disease which is characterized by progressive fibrosis features in different tissues and organs. SSc induces an excessive deposition of extracellular matrix components in the skin, lung, kidney, gastrointestinal tract, or cardiovascular system [1]. Although skin sclerosis is a main symptom of SSc [2], lung involvement characterized by interstitial lung disease (ILD) is also more frequent in patients with SSc. The prevalence of ILD in SSC patients varied from 35% to 53% depending on limited or diffuse SSc [3]. ILD with lung fibrosis is one of the main causes of morbidity and mortality of patients with SSc.

In clinical practice, the main tool which has been used to diagnose ILD in patients with SSc is high-resolution computed tomography (HRCT) [4,5]. In SSc patients, the correlation between the severity of lung involvement measured by HRCT and lung functional testing has been demonstrated previously [6,7]. HRCT has been considered as the gold standard for the diagnosis of ILD in patients with SSc because HRCT is more sensitive than chest X-ray in the diagnosis of ILD, especially in the early stage of disease [8,9]. The common features on HRCT of ILD in SSc patients are diffuse parenchymal damage characterized by prominent ground-glass opacities and fine interstitial reticular images with lower lung predominance [10-12]. As the disease progresses, these lesions will be replaced by coarser interstitial reticulations, traction bronchiectasis and bronchiectasis, and honeycombing [13]. Thus, several scoring methods have been developed to quantify the severity of SSc patients with ILD [14-16]. These scoring systems have been usually correlated with some clinical parameters, prognostic features, disease progression, and treatment responsiveness.

Besides HRCT of the chest, other lung functional parameters including total lung capacity (TLC) and transfer factor of the lung for carbon monoxide (TLCO), as well as exercise testing are also used to estimate the severity of lung damage in SSc patients and follow-up patients [17]. Recently, the use of exhaled nitric oxide (NO) in the diagnosis of airway inflammation has been demonstrated. While the fractional concentration of NO (FENO) has been used as a relevant biomarker of proximal airway inflammation in patients with allergic rhinitis and asthma [18,19], the alveolar concentration of NO (CANO) has been used to evaluate distal airway and lung parenchymal inflammation [20-22]. The level of CANO has been increased in lung parenchymal inflammation and intermittent hypoxia-induced oxidative stress [23,24]. Therefore, the purposes of the present study were to compare different clinical and functional parameters between SSc patients with and without ILD and to demonstrate the correlations between the severity of lung damages on HRCT with other parameters such as CANO, TLC, TLCO, or exercise testing features.

METHODS

Subjects

Patients with diagnosed systemic sclerosis (SSc) who met the American College of Rheumatology/European League Against Rheumatism classification criteria [23] were enrolled at the Clinical Research Center of Lam Dong Medical College (Dalat, Vietnam) from May 2016 to May 2019 and signed Institutional Review Board-Approved (IRBA) consent forms. This study was approved by the Ethics Council of Lamdong Medical College (02.16/LMC-TTYSH-YD, approved in February 2016). The patients also had been informed that they could withdraw from the study without the impact on SSc management. Healthy people with the same age group were included as control subjects after signing IRBA.

All study subjects performed lung function testing (LFT), exhaled nitric oxide (NO) measurements, exercise testing, chest X-ray, blood tests, and high- resolution CT scan (HRCT; only for SSc patients). SSc patients were divided into SSc with interstitial lung disease (SSc-ILD+) or without ILD (SSc-ILD-) based on HRCT features. SSc-ILD- were treated and followed-up as conventional therapy. SSC-ILD+ patients were revisited after 6 months and 12 months during follow-up to complete the study.

Exclusion criteria: Study subjects having one of the following criteria were excluded from the present study: unable to perform laboratory testing necessary for the study, severe acute or chronic cardiovascular diseases (myocardial infarction, decompensated heart failure, or uncontrolled high blood pressure), diagnosed asthma or chronic obstructive pulmonary, medical history with tuberculosis, severe acute or chronic hepatitis or kidney failure, or SSC-ILD+ patients lost to follow-up during the study period.

Study design

It was a perspective and descriptive study. The study subjects were classified as healthy control subjects, SSc-ILD- patients, and SSc-ILD+ patients (Figure 1). SSc-ILD+ patients were revisited at 6 and 12 months to complete the study. All data of medical history, clinical examination, SSc treatment, HRCT, and laboratory tests were recorded for statistical analysis.

Laboratory Techniques

1. Exhaled nitric oxide (NO) measurements and Lung function testing (LFT): FENO and CANO measurements were done by Hypair $FeNO^{+}$ Machine (Me-

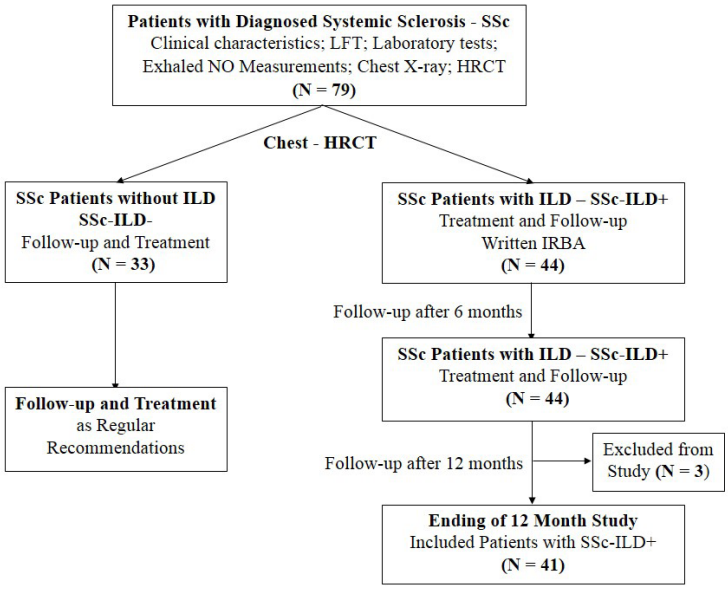

Figure 1. Flow chart of study design of included SSc patients with ILD. HRCT, high resolution computed tomography; ILD, interstitial lung disease; IRBA, Institutional Review Board-Approved; LFT, lung function testing; NO: nitric oxide; SSc, systemic sclerosis.

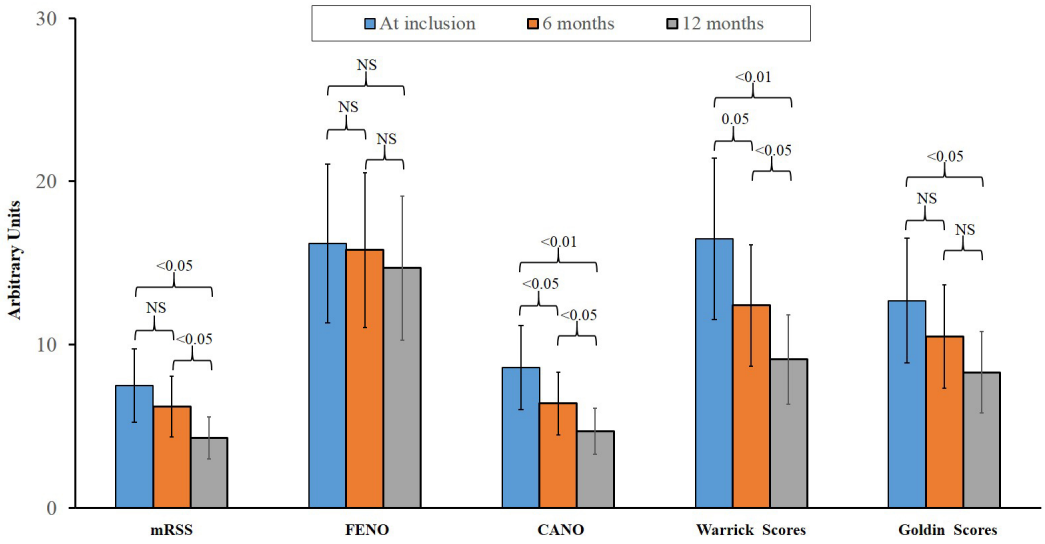

Figure 2. Modification of clinical and functional parameters of SSc-ILD+ patients after 6 and 12 months. CANO, concentration of alveolar nitric oxide; FENO, fractional exhaled nitric oxide; mRSS, modified Rodnan Skin Scores; NS: non-significant. Error bars represent Standard Deviation (SD).

disoft; Sorinnes, Belgium) according to manufacturer's instructions as described previously with expiratory air flows of 50 mL/sec for FENO and with multiple flows for CANO [24]. The mean value of two correct measurements was used for analysis. FENO, CANO levels were classified as recommended by the ATS (American Thoracic Society) / ERS (European Respiratory Society) and previous publications [25-27]. LFT was performed with Body Box 500 (Medisoft, Sorinnes, Belgium) for whole-body plethysmography. The measure of transfer factor of the lung for carbon monoxide (TLCO) was performed as per standard recommended guidelines of ERS with three levels of severity (mild: 60<TLCO<80%; moderate: 40<TLCO≤60%; severe: TLCO≤40%) [28].

Table 1. Comparison of the common characteristics of SSc patients vs control subjects.

Parameters	Control Subjects (N=31)	SSc Patients (N=74)	P
Age, *years*	52 ± 13	51 ± 11	NS
Female/Male, *ratio (N)*	0.93 (28/31)	0.97 (72/74)	NS
BMI, *kg/m²*	22.3 ± 4.5	21.8 ± 3.7	NS
Smoking status			
Never smoked, %(N)	96.7 (30)	98.6 (73)	NS
Former smokers, %(N)	3.3 (1)	1.4 (1)	NS
LFT			
FEV_1, %	89 ± 9	64 ± 17	0.00072
FVC, %	94 ± 7	72 ± 18	0.00081
FEV1/FVC	84 ± 8	78 ± 7	0.032
TLC, %	95 ± 12	68 ± 24	0.00053
RV, %	118 ± 15	84 ± 12	0.0021
TLCO, %	93 ± 7	67 ± 21	0.00068
Exhaled NO			
FENO, ppb	12.5 ± 6.4	15.6 ± 8.2	NS
CANO, ppb	3.5 ± 1.2	6.3 ± 3.6	0.0042
Exercise testing			
VO_2 max, %	91 ± 12	54 ± 17	0.0064
6MWT			
6MWD, meters	537 ± 85	328 ± 74	0.0071
DOD, %	2 ± 1	4 ± 3	0.045
Chest X-ray			
Normal, %(N)	100 (0)	44.5 (33)	0.00034
Abnormal, %(N)	0.0 (0)	55.5 (41)	NA
Blood tests			
Increased Leucocytes, %(N)	0.0 (0)	0.0 (0)	NA
Increased CRP, %(N)	0.0 (0)	95.9 (71)	NA
Increased Creatinine, %(N)	0.0 (0)	6.6 (5)	NA

BMI, body mass index; CANO, concentration of alveolar nitric oxide; CRP, C-reactive protein; DOD differentiation of oxygen desaturation; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FENO, fractional exhaled nitric oxide; LFT, lung function testing; RV, residual volume; SSc, systemic sclerosis; 6MWD: 6-minute walk distance; 6MWT, 6-minute walk test; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; VO_2 max, maximal oxygen consumption; NA: not applicable; NS: non-significant.

2. Six-minute Walk Test (6MWT) and Maximal Oxygen Consumption Test (VO_2 max): All study subjects performed the 6MWT and VO_2 max test at inclusion and after 6 and 12 months follow-up. The 6MWT was done as recommended by ATS [29]. The 6-minute walk distance (6MWD) and the differentiation of oxygen desaturation (DOD) were recorded for analysis. VO_2 max test was performed using an Ergo Card (Medisoft, Sorinnes, Belgium). It was based on the symptom-limited physical exercise test with ventilatory expired gas analysis using a cycle ergometer. Data for oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (VE), respiratory rate (RR), and workload were automatically and continuously collected during the exercise. The peak of oxygen consumption uptake (VO_2 max) was used to compare the exercise capacity of study subjects.

3. Scoring the severity of interstitial lung disease (ILD) in SSc patients with ILD (SSc-ILD) by HRCT: Semi-quantitative scoring methods described by Warrick and Goldin [5,30] were used to assess the severity and extent of disease. Different lung parenchymal abnormalities corresponding to severe disease were given increasingly high scores. The maximal scores were 30 for the Warrick method and 24 for the Goldin method. The latter was

Table 2. Clinical and functional characteristics of SSc-ILD- and SSc-ILD+.

Parameters	SSc-ILD+ Patients (N=41)	SSc-ILD- Patients (N=33)	P
Disease subtype and clinical manifestations			
Lim.SSc, %(N)	29.2 (12)	54.5 (18)	0.012
Dif.SSc, %(N)	70.8 (29)	45.5 (15)	0.018
Time of onset symptoms, *years*	17 ± 14	16 ± 12	NS
Raynaud phenomenon, *%(N)*	97.5 (40)	93.9 (31)	NS
History of digital ulcer, *%(N)*	26.8 (11)	24.2 (8)	NS
Skin involvement, *mRSS*	7.5 ± 2.6	4.4 ± 2.1	0.032
Muscle/Joint or tendon involvement, *%(N)*	60.9 (25)	57.5 (19)	NS
Respiratory symptoms			
Dry cough, %(N)	100 (41)	0.0 (0)	NA
Breathlessness, %(N)	78.0 (32)	0.0 (0)	NA
Auscultatory crackles, %(N)	95.1 (39)	0.0 (0)	NA
Treatments			
Corticosteroids, %(N)	92.6 (38)	81.8 (27)	NS
Cyclophosphamide, %(N)	60.9 (25)	57.5 (19)	NS
Azathioprine, %(N)	31.7 (13)	30.3 (10)	NS
Mycophenolate mofetil, %(N)	78.0 (32)	78.7 (26)	NS
CCBs, %(N)	75.6 (31)	81.8 (27)	NS
LFT			
FEV_1, %	53 ± 8	76 ± 9	0.0045
FVC, %	64 ± 9	81 ± 8	0.027
FEV1/FVC	77 ± 7	79 ± 8	NS
TLC, %	59 ± 15	78 ± 14	0.0071
RV, %	78 ± 11	92 ± 8	0.012
TLCO, %	44 ± 6	82 ± 7	0.0053
Exhaled NO			
FENO, ppb	16.2 ± 8.5	14.7 ± 7.4	NS
CANO, ppb	8.6 ± 2.5	4.2 ± 1.3	0.0072
Exercise testing			
VO_2 max, %	45 ± 9	62 ± 10	0.0064
6MWT			
6MWD, meters	302 ± 56	355 ± 61	0.044
DOD, %	5 ± 3	3 ± 2	NS
HRCT Scan			
Warrick scores	16.5 ± 5.2	0	NA
Goldin scores	12.7 ± 4.3	0	NA
Echocardiography			
FE, %	63 ± 5	69 ± 4	NS
mPAP, mmHg	21 ± 3	19 ± 4	NS
Blood tests			
ANA+, %(N)	100 (41)	100 (33)	NS
ACA+, %(N)	29.2 (12)	30.3 (10)	NS

ANA, antinuclear antibody; ACA, anti-centromere antibodies; CANO, concentration of alveolar nitric oxide; CCBs, calcium channel blockers; CRP, C-reactive protein; Dif.SSc, diffuse systemic sclerosis; DOD differentiation of oxygen desaturation; FEV_1, forced expiratory volume in one second; FVC: forced vital capacity; FENO, fractional exhaled nitric oxide; FE, fractional ejection; Lim.SSc, limited systemic sclerosis; LFT, lung function testing; mRSS, modified Rodnan skin scores; PAP, pulmonary arterial pressure; RV, residual volume; 6MWD: 6-minute walk distance; 6MWT, 6-minute walk test; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; VO_2 max, maximal oxygen consumption; NA: not applicable; NS: non-significant.

based on Kazerooni's method [31] to score three anatomical zones (upper, middle, and lower zones) in each lung. Each HRCT was scored by double-blinded radiologists with accurate agreements.

Statistical Analysis

Data were analyzed with IBM-SPSS 22.0 software (Chicago, IL, USA). Categorical variables were presented as numbers or percentages. Continuous variables were expressed as mean ± SD. Normal distribution was tested by using the Skewness-Kurtosis manner. Mann-Whitney U test was used for mean pair-comparison between two groups; Kruskal–Wallis test was used for pair comparison of more than two groups. The statistical significance was stated with $P<0.05$.

RESULTS

Characteristics of Study Subjects

From February 2016 to May 2019, 108 subjects were recruited for the present study, including 31 control subjects and 79 patients with SSc (33 SSc-ILD- patients and 44 SSc-ILD+ patients). Data from 41 SSc-ILD+ patients who completed the study protocol (follow-up visits at 6 and 12 months of the study) were used for analysis. Therefore, three SSc-ILD+ patients were excluded at the end of the study: one withdrawal and one unable to do exercise test at the 6th month, and one loss of follow-up (Figure 1).

There was not any significant difference between SSc patients and control subjects for mean age, female/male ratio, BMI, and smoking status (Table 1). SSc patients had declined lung function parameters compared with control subjects. There was no significant difference in FENO levels between SSc patients and control subjects (15.6 ± 8.2 vs 12.5 ± 6.4 ppb; $P>0.05$; Table 1). However, the level of CANO in patients with SSc was significantly higher than that of control subjects (6.3 ± 3.6 vs 3.5 ± 1.2 ppb; $P<0.01$; Table 1). There were significant differences between SSc patients and control subjects for VO_2 max, 6MWD, and DOD. There were 44.5% of SSc patients that had an abnormal chest X-ray and 95.9% of SSc patients had increased CRP (Table 1).

Comparison of Characteristics of Patients with SSc-ILD+ and SSc-ILD-

There were no significant differences between patients with SSc-ILD+ and SSc-ILD- for mean age, BMI, female/male ratio (Table 2). The percentage of SSc-ILD+ patients who had diffuse SSc (Dif.SSc) was significantly higher than that in SSc-ILD- patients (70.8% vs 45.5% and $P<0.05$; Table 2). There were no significant differences between SSc-ILD+ and SSc-ILD- patients for time of onset symptoms, Reynaud phenomenon, history of digital ulcer, and treatment (Table 2). Lung function parameters of patients with SSc-ILD+ were significantly lower than that in SSc-ILD- patients. Forced volume capacity (FVC) and total lung capacity (TLC) of SSc-ILD+ patients were significantly declined in comparison with which of SSc-ILD- patients (64 ± 9 vs 81 ± 8% and 59 ± 15 vs 78 ± 14%; $P<0.05$ and $P<0.01$; respectively; Table 2). TLCO of SSc-ILD+ patients was also significantly lower than that in SSc-ILD- patients (44 ± 6 vs 82 ± 7; $P<0.001$; Table 2). While the level of FENO was not significantly different, the level of CANO was significantly different between SSc-ILD+ vs SSc-ILD- patients (16.2 ± 8.5 vs 14.7 ± 7.4 ppb and $P>0.05$; 8.6 ± 2.5 vs 4.2 ± 1.3 ppb and $P<0.01$; respectively; Table 2). Patients with SSc-ILD+ also had lower levels of VO_2 max and 6MWD than that of patients with SSc-ILD- (Table 2). The Warrick scores and Goldin scores of patients with SSc-ILD+ were respectively 16.5 ± 5.2 and 12.7 ± 4.3 (Table 2).

Characteristics of SSc-ILD+ Patients after 6 and 12 month Follow-up

There were no significant differences after 6 and 12 months vs at inclusion for BMI, Reynaud phenomenon, muscle/Joint or tendon involvement, chronic respiratory symptoms, and SSc treatment of patients with SSc-ILD+ (Table 3). TLC and TLCO were significantly increased after 6 and 12 months vs at inclusion (64 ± 13 and 51 ± 5%, 67 ± 14 and 59 ± 7% vs 59 ± 15 and 44 ± 6%; $P<0.05$, $P<0.01$ and $P<0.05$, $P<0.01$; respectively; Table 3). While the level of FENO was not significantly changed, the level of CANO was significantly reduced after 6 and 12 month (6.4 ± 2.1 and 4.7 ± 1.5 ppb vs 8.6 ± 2.5; $P<0.05$, $P<0.01$, and $P<0.05$; respectively; Table 3 and Figure 2). The capacity of exercise (VO_2 max and 6MWD) of CCs-ILD+ patients was not significantly different after 6 and 12 months (Table 3). The Warrick scores were significantly diminished after 6 and 12 months during follow-up vs at inclusion (12.4 ± 4.3 and 9.1 ± 3.2 vs 16.5 ± 5.2; $P<<0.05$, $P<0.01$, and $P<0.05$; respectively; Table 3). However, Goldin scores were significantly reduced only after 12 months vs at inclusion (Table 3).

Table 3. Clinical and functional characteristics of SSc-ILD+ patients after 6 and 12 months.

Parameters	SSc-ILD+ Patients (N=41)			P
	At inclusion	6 months	12 months	
BMI, *kg/m²*	21.4 ± 4.4	21.1 ± 3.8	20.8 ± 3.5	NS#,##,###
Raynaud phenomenon, *%(N)*	97.5 (40)	93.9 (31)	93.9 (31)	NS#,##,###
Skin involvement, *mRSS*	7.5 ± 2.6	6.2 ± 2.1	4.3 ± 1.5	NS#, 0.032##, 0.041###
Muscle/Joint or tendon involvement, *%(N)*	60.9 (25)	63.4 (26)	60.9 (25)	NS#,##,###
Chronic respiratory symptoms				
Dry cough, %(N)	100 (41)	97.5 (40)	95.1 (39)	NS#,##,###
Breathlessness, %(N)	78.0 (32)	75.6 (31)	65.8 (27)	NS#,##,###
Auscultatory crackles, %(N)	95.1 (39)	95.1 (39)	92.6 (38)	NS#,##,###
Treatments				
Corticosteroids, %(N)	92.6 (38)	85.3 (35)	80.4 (33)	NS#,##,###
Cyclophosphamide, %(N)	60.9 (25)	68.2 (28)	73.1 (30)	NS#,##,###
Azathioprine, %(N)	31.7 (13)	29.2 (12)	26.8 (11)	NS#,##,###
Mycophenolate mofetil, %(N)	78.0 (32)	85.3 (35)	90.2 (37)	NS#,##,###
CCBs, %(N)	75.6 (31)	75.6 (31)	73.1 (30)	NS#,##,###
LFT				
FEV_1, %	53 ± 8	55 ± 9	57 ± 8	NS#,##,###
FVC, %	64 ± 9	67 ± 8	69 ± 10	NS#; 0.047##; NS###
FEV1/FVC	77 ± 7	76 ± 8	75 ± 8	NS#,##,###
TLC, %	59 ± 15	64 ± 13	67 ± 14	0.042#; 0.006##; NS###
RV, %	78 ± 11	78 ± 11	78 ± 11	NS#,##,###
TLCO, %	44 ± 6	51 ± 5	59 ± 7	0.035#; 0.008##; 0.041###
Exhaled NO				
FENO, ppb	16.2 ± 5.5	15.8 ± 5.2	14.7 ± 4.8	NS#,##,###
CANO, ppb	8.6 ± 2.5	6.4 ± 2.1	4.7 ± 1.5	0.027#; 0.003##; 0.022###
Exercise testing				
VO_2 max, %	45 ± 9	49 ± 8	57 ± 7	NS#; 0.013##; 0.032###
6MWT				
6MWD, *meters*	302 ± 56	325 ± 48	349 ± 43	NS#; NS##; 0.046###
DOD, %	5 ± 3	4 ± 3	3 ± 2	NS#,##,###
HRCT Scan				
Warrick scores	16.5 ± 5.2	12.4 ± 4.3	9.1 ± 3.2	0.046#; 0.006##; 0.045###
Goldin scores	12.7 ± 4.3	10.5 ± 3.6	8.3 ± 2.8	<NS#; 0.042##; NS###
Echocardiography				
FE, %	63 ± 5	62 ± 6	64 ± 7	NS#,##,###
mPAP, mmHg	21 ± 3	20 ± 4	21 ± 5	NS#,##,###

BMI, body mass index; CANO, concentration of alveolar nitric oxide; CCBs, calcium channel blockers; Dif.SSc, diffuse systemic sclerosis; DOD differentiation of oxygen desaturation; FEV_1, forced expiratory volume in one second; FVC: forced vital capacity; FENO, fractional exhaled nitric oxide; FE, fractional ejection; Lim.SSc, limited systemic sclerosis; LFT, lung function testing; mRSS, modified Rodnan skin scores; PAP, pulmonary arterial pressure; RV, residual volume; 6MWD: 6-minute walk distance; 6MWT, 6-minute walk test; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; VO_2 max, maximal oxygen consumption; NA: not applicable; NS: non – significant; #: at inclusion vs 6 months; ##: at inclusion vs 12 months; ###: 6 months vs 12 months.

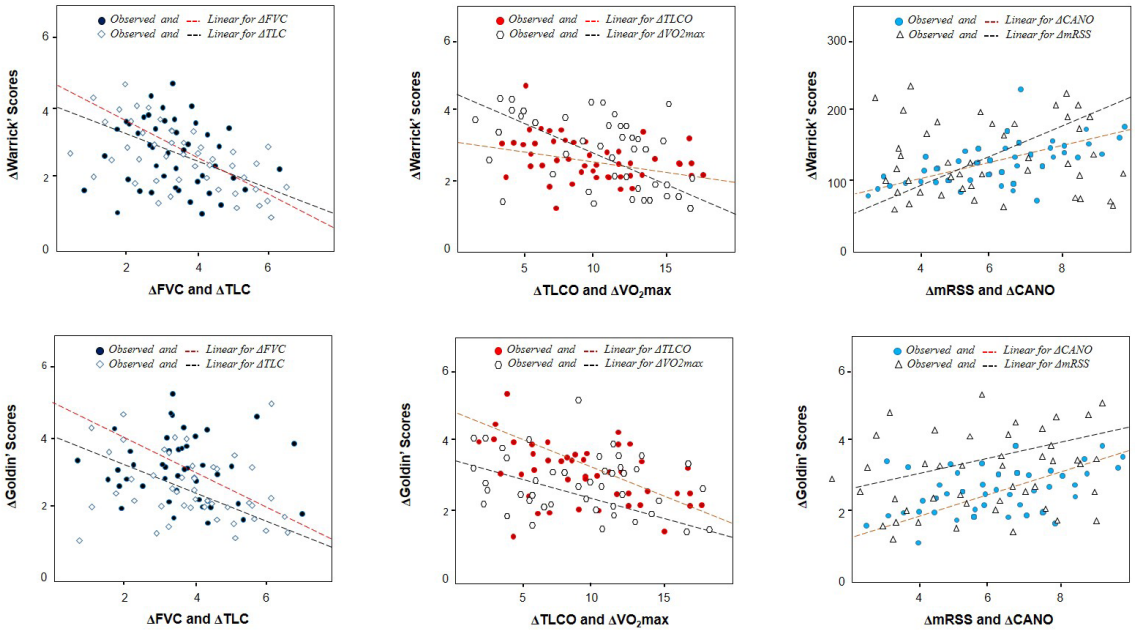

Figure 3. Correlation between ΔWarrick and ΔGoldin scores with other clinical and functional parameters. Δ: modified parameter after 12 months vs at inclusion; CANO, concentration of alveolar nitric oxide; FVC: forced vital capacity; mRSS, modified Rodnan Skin Scores; 6MWT, 6-minute walk distance; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; VO_2 max, maximal oxygen consumption.

Correlation Between the Modification of Warrick and Goldin Scores and other Clinical and Functional Parameters after 12 months in SSC-ILD+ Patients

There were significant correlations between the modification (Δ) of Warrick and Goldin scores and other clinical and functional parameters in SSC-ILD+ patients (Figure 3). ΔWarrick scores were significantly and inversely correlated with ΔFVC, ΔTLC, ΔTLCO, and ΔVO_2 max (R= -0.363, R= -0.417, R= -0.652, and R= -0.312; P<0.044, P< 0.05, P<0.05, P<0.01, and P<0.05; respectively; Table 4 and Figure 3); it was also significantly and tightly correlated with ΔCANO (R= 0.783 and P<0.01; Table 4 and Figure 3). There were also the significant correlations between ΔGoldin scores and ΔFVC, ΔTLC, ΔTLCO, and ΔVO_2 max (Table 4 and Figure 3). There was a tight correlation between ΔGoldin score and ΔCANO level (R= 0.719 and P<0.05; Table 4 and Figure 3).

DISCUSSION

The present study showed that 1) the level of CANO in patients with SSc-ILD+ was significantly higher than that in patients with SSc-ILD-; 2) the level of CANO was significantly reduced after 6 and 12 months of treatment and associated with lung function improvement in SSC-ILD+ patients; 3) there were significant correlations between ΔWarrick and Goldin scores with ΔCANO and other clinical and functional parameters in SSC-ILD+ patients.

The results of the present study showed that SSc patients had the declined lung functional parameters and lower exercise capacity than control subjects; especially, the level of CANO in patients with SSc was significantly higher than that of control subjects (P<0.01; Table 1). Tiev *et al*. also showed that the level of CANO was significantly increased in SSc patients as compared with controls; and among SSc patients, CANO level was significantly higher in patients with ILD compared with patients without ILD (7.5 vs 4.9 ppb) [20]. These results were similar to those of the present study (Table 2). Therefore, the increase of CANO level might be used to detect the extent of interstitial lung damage in patients with SSc. In addition, the cut-off level of CANO could be used to rule in or out SSc patients with ILD due to its high sensitivity for diagnosis value as suggested by the previous study [21].

Although the use of CANO level to detect early stage of ILD in patients with SSc is still controversial, the use of increased CANO level as a biomarker of early lung involvement to predict radiological changes of ILD in pa-

Table 4. Correlation between ΔWarrick and ΔGoldin scores with other clinical and functional parameters.

Parameters	ΔWarrick Scores		ΔGoldin Scores	
	R	P	R	P
ΔmRSS	0.078	0.672	0.142	0.432
ΔFVC	-0.363	0.044	-0.251	0.041
ΔTLC	-0.417	0.012	-0.312	0.034
ΔTLCO	-0.652	0.014	-0.678	0.011
ΔCANO	0.783	0.001	0.719	0.015
ΔVO2 max	-0.312	0.026	-0.448	0.032
Δ6 MWD	-0.067	0.164	-0.131	0.314

Δ: modified parameter after 12 months vs at inclusion; CANO, concentration of alveolar nitric oxide; FVC: forced vital capacity; mRSS, modified Rodnan Skin Scores; 6MWT, 6-minute walk distance; TLC, total lung capacity; TLCO, transfer factor of the lung for carbon monoxide; VO_2 max, maximal oxygen consumption.

tients with SSC has been demonstrated [32]. The results of the present study showed that lung function parameters (TLC and TLCO) were improved after 6 and 12 months of follow-up in SSC patients with ILD (Table 3). Interestingly, while the level of FENO was not significantly modified, the level of CANO was significantly reduced after 6 and 12 months of treatment. Moreover, Warrick scores and Goldin scores were also significantly reduced during follow-up vs at inclusion (Table 3). The present study showed that there were significant correlations between the modifications (Δ) of Warrick and Goldin scores and other clinical and functional parameters in SSC-ILD+ patients (Figure 3). The modification of these scores was significantly and inversely correlated with the modification of functional parameters such as ΔFVC, ΔTLC, ΔTLCO, and ΔVO_2 max (Table 4 and Figure 3). Especially, there was a tight and linear correlation between the reduction of CANO level and semi-quantitative scores measured in chest HRCT (Figure 3). These results suggest that CANO level may be used to follow-up the severity of lung involvement and to predict treatment responsiveness in SSc patients with ILD.

Thus, the use of exhaled nitric oxide (NO) in patients with SSc has been developed for more than twenty years and it has been considered currently as a potential biomarker to distinguish SSc patients who had or had not pulmonary arterial hypertension (PAH) and ILD [33,34]. The measure of CANO is a non-invasive and low cost than other conventional tests done during follow-up of patients with SSc. Also, the technique of eNO measurement is easy to perform and the result is ready to be interpreted simultaneously. Despite the use of exhaled NO has been used largely in the field of asthma and other airway diseases [23-26], the use of CANO in SSc patients with ILD is still controversial.

Limitations of the Study

Although the present study has given some relevant results concerning the correlation between CANO level and semi-quantitative scoring of ILD measured by HRCT, there are some limitations concerning a small number of the study population and the short-term follow-up. Therefore, more studies on the use of CANO measurement in patients with SSc are necessary.

CONCLUSION

Interstitial lung disease is common in patients with SSc. Patients with ILD have declined lung functional parameters and it might be improved during treatment. The use of chest HRCT is useful to measure the severity of lung damage in SSc patients with ILD. Moreover, CANO is a relevant biomarker to diagnose ILD in patients with SSc in combined with imagery features. Hence, more studies on the role of exhaled NO in combined with other biological or functional exploration and imagery techniques should be done in the future.

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