

This is the peer reviewed version of the following article:

High serum levels of silica nanoparticles in systemic sclerosis patients with occupational exposure: Possible pathogenetic role in disease phenotypes / Ferri, C., Artoni, E., Sighinolfi, G.L., Luppi, F., Zelent, G., Colaci, M., Giuggioli, D.. - In: SEMINARS IN ARTHRITIS AND RHEUMATISM. - ISSN 0049-0172. - 48:3(2018), pp. 475-481. [10.1016/j.semarthrit.2018.06.009]

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

03/06/2026 21:53

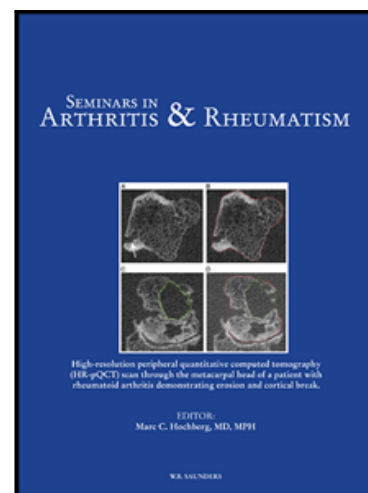
(Article begins on next page)

Accepted Manuscript

High serum levels of silica nanoparticles in systemic sclerosis patients with occupational exposure: possible pathogenetic role in disease phenotypes

Clodoveo Ferri MD , Erica Artoni BS , Gian Luca Sighinolfi BS , Fabrizio Luppi MD , Gabriele Zelent MD , Michele Colaci MD , Dilia Giuggioli MD

PII: S0049-0172(17)30700-X
DOI: [10.1016/j.semarthrit.2018.06.009](https://doi.org/10.1016/j.semarthrit.2018.06.009)
Reference: YSARH 51368



To appear in: *Current Problems in Pediatric and Adolescent Health Care*

Received date: 19 October 2017
Revised date: 31 May 2018
Accepted date: 18 June 2018

Please cite this article as: Clodoveo Ferri MD , Erica Artoni BS , Gian Luca Sighinolfi BS , Fabrizio Luppi MD , Gabriele Zelent MD , Michele Colaci MD , Dilia Giuggioli MD , High serum levels of silica nanoparticles in systemic sclerosis patients with occupational exposure: possible pathogenetic role in disease phenotypes , *The End-to-end Journal* (2018), doi: [10.1016/j.semarthrit.2018.06.009](https://doi.org/10.1016/j.semarthrit.2018.06.009)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

High serum levels of silica nanoparticles in systemic sclerosis patients with occupational exposure: possible pathogenetic role in disease phenotypes.

Clodoveo Ferri, MD, Erica Artoni, BS, Gian Luca Sighinolfi, BS, Fabrizio Luppi, MD*, Gabriele Zelent*, MD, Michele Colaci, MD, and Dilia Giuggioli, MD.

Chair and Rheumatology Unit, Medical School, University of Modena and Reggio Emilia, Azienda Ospedaliero Universitaria, Modena, Italy.

*Department of Neuroscience, Biomedical and Metabolic Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Keywords: systemic sclerosis, scleroderma, silica, occupational exposure, etiopathogenesis, microparticles, nanoparticles, interstitial lung fibrosis.

Word count

Abstract: 250

Text: 2,967

Corresponding author:

Clodoveo Ferri, MD
UOC di Reumatologia, Dpt of Internal Medicine,
University of Modena and Reggio E.
Azienda Ospedaliero-Universitaria

Via del Pozzo, 71
41100 Modena Italy
Tel +39-059-4224053 Fax +39-059-4224178
E-mail : clferri@unimore.it

Bullet points:

- Epidemiological studies suggested that systemic sclerosis (SSc) can be associated to occupational/environmental triggering factors among which the silica dust exposure
- The present study first demonstrated significantly higher serum silica levels (s-Si) in SSc patients with previous occupational exposure compared to non-exposed subjects and healthy controls
- Patients with elevated s-Si showed statistically higher percentages of diffuse cutaneous SSc variant, myositis, and/or lung fibrosis compared to those without; moreover, s-Si correlated with the severity of lung fibrosis scoring at high resolution computed tomography
- Silica dust exposure with high s-Si might be included among numerous etiopathogenetic –genetic, infectious, occupational/environmental- co-factors responsible for different SSc clinical phenotypes

Abstract

Background Systemic sclerosis (SSc) is an autoimmune systemic disease characterized by diffuse fibrosis of skin and visceral organs due to different genetic, infectious, and/or environmental/occupational causative factors, including the inhalation of silica dust.

Objectives To investigate serum trace elements including silicon (s-Si) levels in SSc patients living in a restricted geographical area with high density of worksites with silica exposure hazard.

Methods This case-control study included 80 SSc patients (M:F 10:70; aged 58.4 ± 11.9 SD years, mean disease duration 10.1 ± 7.8 SD) and 50 age-/sex-matched healthy control subjects consecutively investigated at our University-based Rheumatology Unit.

Patients and controls were evaluated for environmental/occupational exposure categories (structured questionnaire), morphological characterization of serum micro-/nanoparticles (Environmental Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy microanalysis), and quantitative assessment of trace elements (inductively coupled plasma atomic emission spectroscopy).

Results Among various categories, only occupational exposure to silica dust was recorded in a significant proportion of SSc patients compared to controls (55% vs 11%; $p < .0001$). Qualitative analysis showed serum silica micro- and nanoparticles in all exposed patients. Quantitative evaluation evidenced significantly higher s-Si levels in SSc patients versus controls ($p < .0001$); in addition, higher s-Si levels were detected in patients with occupational exposure ($p < .0001$), diffuse cutaneous SSc ($p = .0047$), myositis ($p = .0304$), and/or lung fibrosis ($p = .0004$) compared to those without; notably, the severity of lung fibrosis scoring positively correlated with s-Si levels ($p < .0001$).

Conclusions The study first demonstrated high s-Si levels in exposed SSc patients; this element might represent a pathogenetic co-factor of more severe clinical phenotypes, mainly diffuse scleroderma with lung fibrosis.

Pre-clinical Trial registration: [UFP2015, University of Modena and Reggio Emilia Ethics Committee approved].

Funding Source: none.

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by immune-system dysfunction, diffuse microangiopathy, and multiple organ fibrotic involvement (1-3). The etiology of SSc remains still obscure; probably it may recognize different predisposing/causative -genetic, infectious, and/or environmental- factors (1-4). A variable combination of these factors may lead to complex, multistep pathogenetic process leading to different clinical phenotypes that characterize the scleroderma spectrum (1-4). During the last decades numerous environmental/occupational 'toxic' agents have been suggested as potential triggering factors of SSc (4), among which the silica exposure (5-7). Several clinico-epidemiological observations pointed out that the inhalation of silica-containing dust may trigger, in genetically predisposed individuals, various autoimmune disorders, among which the SSc (5-8). This peculiar association is termed 'Erasmus syndrome' following the description of a series of South African male miners in 1957 (9), although Byrom Bramwell had suspected in 1914 the possible link of SSc with distinct occupational factors in a small series of five stone-masons, one coal-miner, and one coppersmith (10). Besides epidemiological and occupational studies on the association between silica exposure and SSc (5-8), laboratory investigations in both animals' models and humans are mostly focusing on the pathophysiology of silicosis (7, 11-13). In this respect, silica particles seem to be able to yield multiple immune-system alterations and cytokine production responsible for inflammasome activation and/or fibrotic lesions (11). Similarly, it is supposable that in genetically predisposed individuals silica particles might contribute to the development of SSc, and in particular to specific clinical variants.

The present study aimed to investigate the presence of silica micro- and nanoparticles and serum silicon (s-Si) levels in SSc patients from a restricted geographical area with high density of worksites with silica exposure hazard and its possible correlation with scleroderma clinical phenotypes.

Methods

Eighty SSc patients (10 males, 70 females; mean age 58.4 ± 11.9 SD years, mean disease duration 10.1 ± 7.8 SD) and 50 age- and sex-matched healthy control subjects were consecutively recruited for this case-control study at our university-based Rheumatology Unit between June 2015 and May 2017. Control subjects were selected among outpatients referred to our Unit

because of transient symptoms due to degenerative, non-inflammatory rheumatic disorders. As inclusion criteria we decided to recruit only patients and controls living in the same geographical area of the Italian province of Modena, characterized by high density of industries with high risk of silica dust exposure (Tab.1); all subjects gave their written consents. The study was approved by the local Ethical Committee (protocol n. UFP2015).

SSc patients fulfilled the 2013 ACR/EULAR criteria for SSc and were classified according to the extent of skin involvement in limited and diffuse SSc [14]; while, control subjects were systematically screened in order to exclude possible autoimmune systemic disorders by means of anamnestic and clinical evaluation, and routine laboratory investigations.

Study design

All study subjects were interviewed using a structured questionnaire based on a previous model administered by a not blinded interviewer [15]. After informed consent, clinic-epidemiological data were collected and fresh blood samples were obtained from all subjects to evaluate the presence of micro- and nanoparticles and inorganic trace elements.

Exposure assessment

The possible exposure to micro- and nanoparticles was assessed on the basis of information obtained by the structured questionnaire. Four major sources of particles pollution were categorized: occupational exposure, environmental exposure, smoking habits and prosthesis implants. Occupational exposure category included patients with daily exposure to inhaled dust, vapors, or aerosols during work for at least 5 years (16). The sub-categories concerning the possible occupational exposure to respirable crystalline silica were classified using the current OSHA guidelines (16; see also Tab. 1). Environmental exposure category encompassed patients exposed to air pollution and airborne particles. Patients who lived at a small (< 4 km) distance from airports, highways, incinerators or other sources of exhaust powders were included in this category. Information on smoking habits included whether the patients did or did not smoke, the average number of cigarettes or cigars smoked daily and the number of years of active smoking. Previous studies have demonstrated that hip joint prostheses are subject to wear with consequent particles debris release that may cause an inflammatory reaction [17]. Taking into account these data we also investigated both patients and controls with regards to dental or orthopedic (hip or femur) implants, pacemaker and coronary stents .

Blood samples collection

Patients blood samples were collected in trace elements free polystyrene tubes (Vacutest Kima, Pd, Italy) and immediately kept at 37°C for 2 hours. Serum was later separated by centrifuging samples at 37°C for 10 minutes at 3000 rpm. Serum samples were stored at -80°C and later

subjected to qualitative and quantitative analysis to evaluate respectively the presence of micro- and nanoparticles and inorganic trace elements.

Qualitative analysis of serum samples and elemental microanalysis

The morphological characterization of inorganic particles was carried out by Environmental Scanning Electron Microscopy - ESEM (Quanta 200, Fei company, Holland). The chemical identification of the particles was carried out by EDS: energy-Dispersive X-ray Spectroscopy (Oxford Instruments, Manheim, Germany). Serum samples were put on carbon slips and analyzed by ESEM at 25kV in low vacuum conditions without any further treatment. To assess the number of positive areas of the sample containing micro- and nanoparticles, the method by Fassina et al (18) was adopted.

Quantitative analysis of inorganic serum trace elements

The total quantitative assessment of trace elements present in serum samples was performed by Inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Thermo iCAP 6000, Fisher Scientific, USA) applying the trace element detection guidelines of the Italian Istituto Superiore di Sanità (19).

The elements measured were: Al (aluminum), Cr (chromium), Cu (copper), Fe (iron), Mg (Magnesium), Mn (manganese), Si (silicon), Ti (Titanium), Zn (zinc). ICP-AES optimization was performed using the wavelengths specified by the ISS protocols considering the detection limits for each element. Specific wavelengths for each element were selected to avoid the interference of the other elements (20). Serum samples were diluted 1: 5 with deionized water (ultralow metals and silica content) and later subjected to a digestion process using 1% nitric acid to solubilize the organic part. The results obtained from the trace elements analysis were compared with the data obtained by electron microscopy to evaluate the differences between quantitative and qualitative data.

Patients' clinical assessment

Scleroderma cutaneous and visceral organ involvement, including pulmonary, cardiac, renal, and gastrointestinal alterations, as well as routine blood chemistry, urinalysis, and immunological alterations were evaluated according to previously described methodologies (21). The following serological markers were detected by means of standard techniques: anti-nuclear (ANA), anti-centromere (ACA), anti-nucleolar (ANoA), and anti-extractable nuclear antigen (ENA) antibodies; these latter included anti-Scl70, -Sm, -RNP, -SSB, -SSA, -PCNA, -SL, and Jo1 [21].

All patients underwent echocardiography with pulmonary arterial pressure estimation, barium esophagus X-ray, nailfold videocapillaroscopy, and abdominal and thyroid ultrasound

examination. On the basis of anamnestic, physical, and instrumental findings, organ involvement was defined as follows: 'heart involvement': presence of arrhythmias and/or right/left heart failure; 'kidney involvement': renal function deficiency (creatinine-based approximation of the glomerular filtration rate at least < 50 mg/ml/min); 'gastrointestinal involvement': presence of dyspepsia, motility dysfunctions, and/or signs of small intestinal bacterial overgrowth.

Interstitial lung involvement was deeply investigated by means of typical clinico-radiological, and functional manifestations; namely, all patients underwent spirometric and DLCO tests, and high resolution computed tomography (HRCT) of the full thorax, using a 32-slice scanner (Lightspeed VCT - GE Healthcare). CT examination was performed using a single apnoea (full inspiration; supine decubitus). Scanning were performed on average using the following parameters: 120 kVp, 100 mAs, rotation time 0.5 s, feed/rotation 18 mm bone plus filter and collimation 0.75 mm with 1-mm reconstruction. Two chest radiologists estimated the presence and the extent of lung abnormalities, performing a blind and independent evaluation of all the CT scans and therefore a three scans at pre-established levels were used: the origin of the great vessels, the tracheal carina and the right inferior pulmonary vein. The radiologists were not aware of the patient's lung functional and laboratory data. Thin-section CT images were analyzed for the presence of the different interstitial lung diseases (ILDs) pattern, including ground-glass attenuation, reticulation, honeycombing, consolidation and nodules. Lastly, the evaluation of fine reticulation and fibrosis extension was done for each patient, considering the entire lung using a four-point scale (0=no involvement; 1=1%–25% involvement; 2=26%–50%; 3=51%–75%; and 4=76%–100%) (22).

These data were used to calculate inter-observer agreement and, in case of discrepancies, a consensus reading was performed to obtain only one visual score for the disease extent and only one visual score for the radiological pattern. The presence of nodules was specifically evaluated for excluding the radiological diagnosis of silicosis (23).

Statistical analysis of data

Using Fisher's exact probability test, we estimated odds ratios (ORs; 95% Confidence Interval) to evaluate the association between occupational/environmental exposure, smoking habits and prosthesis implants presence in subjects and SSc patients. For qualitative variables, the frequency distribution was calculated using Image-Pro Plus software. The quantitative variables are reported in terms of mean and standard deviation (SD) and analyzed by Student's test (t-

test) and Analysis of Variance (ANOVA) with Bonferroni post-test using GraphPad Prism 5 software.

Results

The main characteristics of 80 SSc patients included in the study are shown in the Tab. 2; overall, demographic and clinico-serological SSc features were comparable to those observed in our larger patients series previously described (21). The assessment of possible exposure to micro- and nanoparticles by structured questionnaire revealed occupational exposure, mainly in the setting of ceramic industries (Tab. 1), in over half SSc patients (43/80; 54%) and in 6/50 (12%) control subjects (OR 8,52, 3.264 to 22.25, $p < .0001$; Tab. 2). Other exposure categories, namely environmental exposure, smoking habits, and prosthesis implants were seldom recorded in both patients and controls without significant differences.

Tab. 1. Occupational exposure categories in SSc patients and controls.

The time period of occupational exposure to silica dust lasted medially 16.4 ± 10.8 SD years without correlation with s-Si levels. In all cases the exposure to silica dust preceded the disease onset, while 21/43 patients were still exposed to silica at the time of the present study. These subjects showed significantly higher s-Si levels if compared with the remaining 22 patients with past history of silica exposure ($p .012$).

On the whole, patients with anamnestic exposure to silica dust showed a statistically higher prevalence of some disease manifestations compared to those without; namely, diffuse cutaneous SSc (35% vs 11%; $p = .0169$), myositis (16% vs 0%; $p = .0134$), and/or lung fibrosis at HRCT (86% vs 38%; $p < .0001$).

In all exposed patients serum qualitative analysis by ESEM showed the presence of silica nano- and microparticles of widely variable dimensions (from 30 nm to 4 μ m) (Fig. 1).

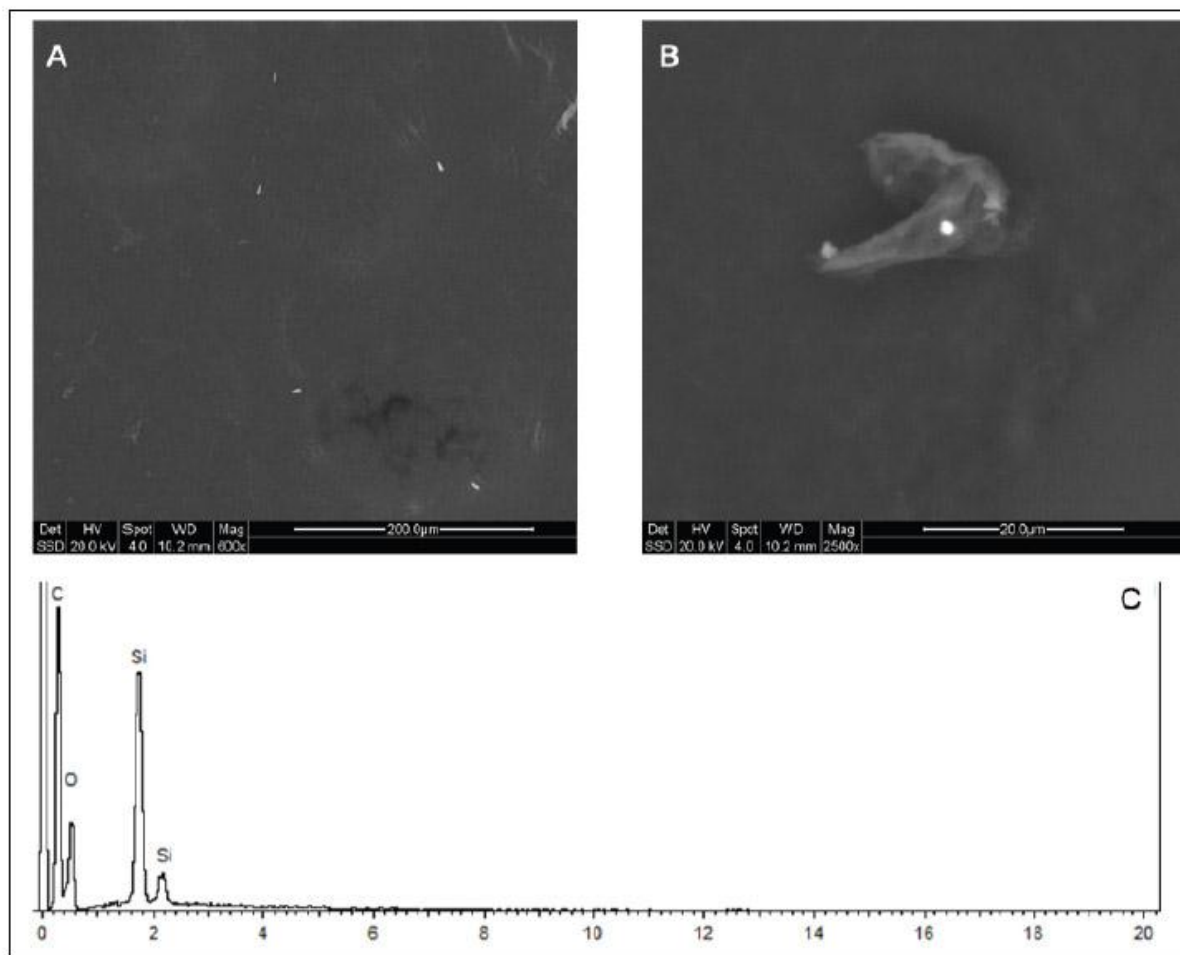


Fig. 1. ESEM analysis of serum sample from an SSc patient: image (A) with magnification of 600x and (B) with magnification 2500x showing a cluster of nanoparticles. The chemical analysis of this cluster (C) by means of EDS shows the presence of Si element.

In addition, the chemical characterization by EDS revealed a complex composition of particles found, i.e. Al, Cr, Cu, Fe, Mg, Mn, Si, Ti, Zn. However, quantitative determination of these elements confirmed high levels of only s-Si compared to controls ($p < .0001$; Fig. 2). More interestingly, patients with occupational exposure had significantly higher values of s-Si compared to non-exposed individuals ($p < .0001$; Fig. 2).

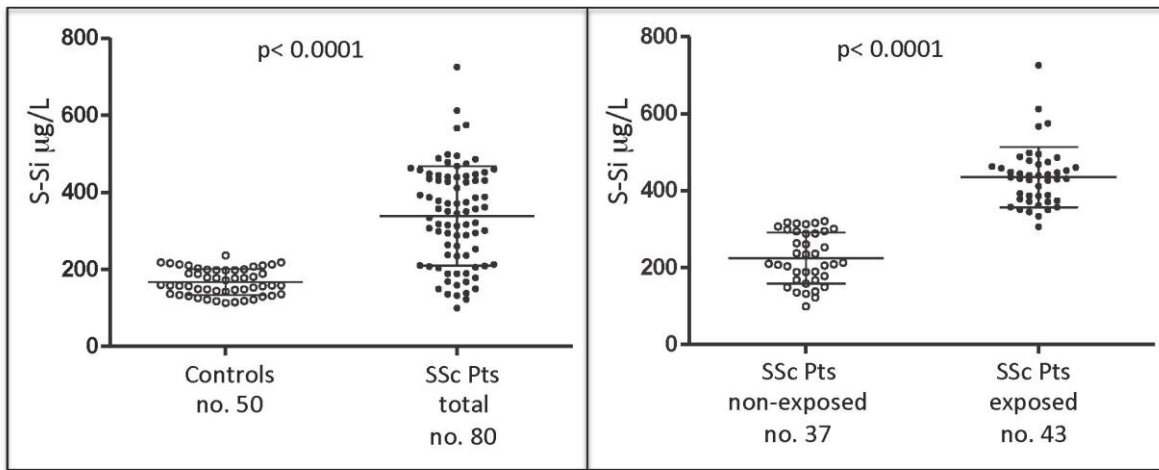


Fig. 2. Comparison between silica levels in SSc patients and control subjects (left), and between silica-exposed and non-exposed SSc patients (right). The results are reported as mean \pm SD.

Significantly higher s-Si levels were detected in patients with some important scleroderma features compared to those without (Tab. 2); namely, diffuse cutaneous SSc subset ($p=0.0406$), myositis ($p=0.0447$), lung fibrosis ($p<0.0001$), ground glass opacities ($p=0.003$), and honeycombing ($p=0.0496$) at HRCT. On the whole, from mild to moderate fibrosis at HRCT was observed in the majority of silica-exposed patients (32/43; 74%), while mild fibrosis (scoring 1) was detected in 10/37 (27%) of non-exposed patients ($p<0.0001$). The relationship of lung fibrosis with silica exposure was reinforced by the significant correlation between the severity of fibrosis detected at HRCT (scoring 0-3) and s-Si levels (Fig. 3).

Silica serum levels and scleroderma lung fibrosis at HRCT

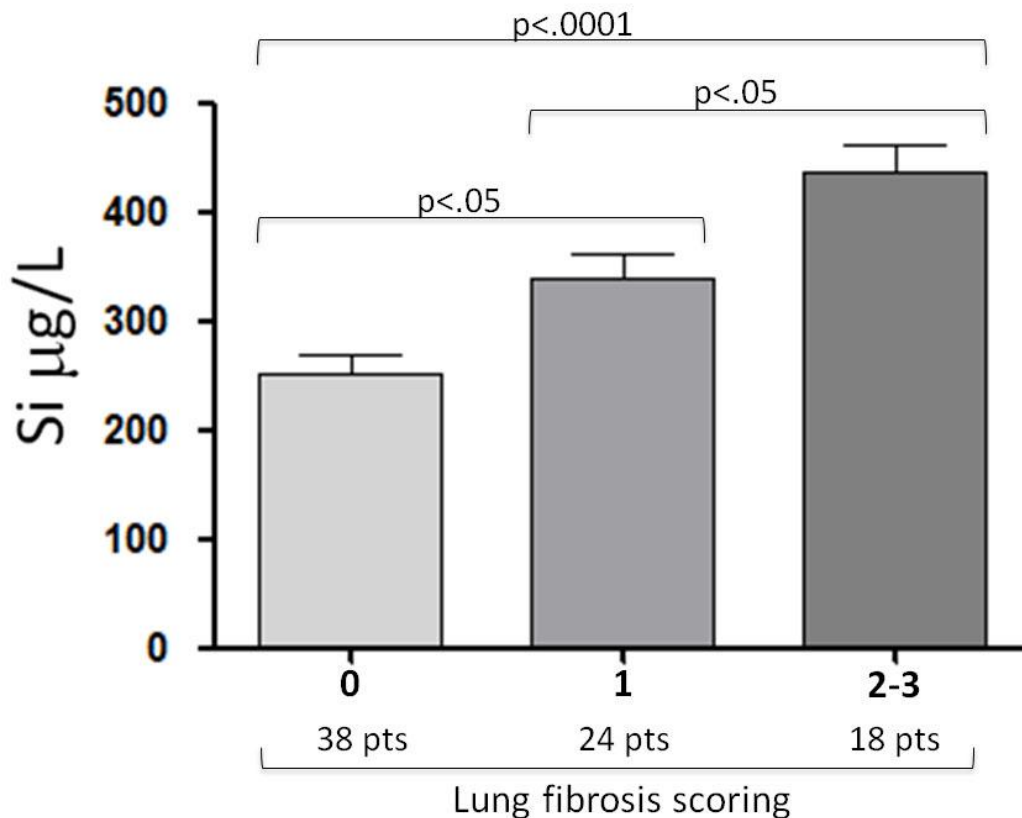


Fig. 3. Systemic sclerosis (SSc) patients with lung fibrosis, detected by high resolution computed tomography (HRCT) in 42/80 (53%) individuals, showed significantly higher levels of serum silicon (s-Si) compared to 38/80 (47%) without ($p < .0001$; Tab. 2). Moreover, the lung fibrosis scoring significantly correlated with serum silica levels; the highest mean levels of serum silica were found in patients with 2-3 degree of lung fibrosis. The s-Si levels are expressed as mean \pm SEM.

Moreover, abnormally increased ESR and CRP significantly correlated with high s-Si levels ($p = .0254$ and $p = .0072$, respectively). Similarly, the presence of serum anti-Scl70 antibodies was significantly associated with high s-Si levels ($p = .0068$), the opposite of that found for ACA-positive patients ($p = .0006$; Tab. 2).

Careful evaluation of radiological findings at HRCT invariably excluded the presence of typical silicotic alterations in all SSc patients. Finally, one male patient with long-term silica exposure and very high s-Si level died because of severe lung fibrosis complicated by adenocarcinoma during the time interval of the present study.

Discussion

The present study provided new insights on the possible role of silica as pathogenetic cofactor of SSc. In a series of scleroderma patients resident in the same geographical area with high density of industries individuals with anamnestic exposure to silica dust showed a statistically higher prevalence of some disease manifestations compared to those without. These epidemiological features were strengthened by the results of laboratory investigations showing significantly higher s-Si levels in exposed compared to unexposed scleroderma patients and healthy controls. This finding was significantly correlated with some important disease features; namely, diffuse cutaneous SSc variant, myositis, and/or interstitial lung involvement. In particular, the presence and severity of the lung fibrosis, evaluated by both HRCT and respiratory function tests, positively correlated with s-Si. Such clinical associations were in keeping with some laboratory parameter alterations; namely, patients with abnormally high values of inflammation reactants, i.e. ESR and CRP, and/or anti-Scl70 seropositivity showed significantly higher s-Si levels compared to those without; conversely, statistically lower s-Si were detected in ACA-positive compared to ACA-negative individuals. Overall, the above findings first suggest a significant association of high s-Si with some important SSc clinical manifestations and worse prognostic biomarkers (anti-Scl70, ESR and CRP) in patients with professional exposure to silica dust.

A possible link between silica exposure and systemic autoimmune disorders has been suggested by numerous epidemiological studies reporting an increased prevalence of some conditions such as systemic lupus erythematosus, rheumatoid arthritis, and systemic vasculitides, regardless the concomitancy of silicosis (11, 24, 25, 26, 27). In particular, the association between silica and SSc, the so-called Erasmus syndrome, was suggested by the observation of a significantly increased incidence of SSc in gold miners exposed to silica dust,

compared to general population; i.e. 2/1000 vs. 0.35/1000, respectively (9). During the last six decades several anecdotal observations and case-control series of patients with occupational exposure underlined a possible SSc development in individuals with silica exposure (28-35). On the other side, studies focusing on unselected SSc patients series (36-46), including a recent large meta-analysis (46), evidenced a significantly high prevalence of silica exposure that can be regarded as potential pathogenetic co-factor of the disease. Of note, a case-control study on occupational risk factors in SSc evidenced a significant higher risk (OR 5.57, 95% CI 1.60 to 18.37) in individuals exposed to crystalline silica compared to other compounds such as solvents (39). Overall, the above epidemiological observations (28-46) are consistent with the results of the present study, including the significant correlation with diffuse cutaneous subset, interstitial lung involvement, and serum anti-Scl70 antibodies (43-44).

The natural history of SSc is characterized by a variety of clinico-serological phenotypes observable since the disease onset, often with unpredictable clinical course, suggesting a multifactorial (host genetic and exogenous factors) through a multistep pathogenetic process (1-3; Fig. 4).

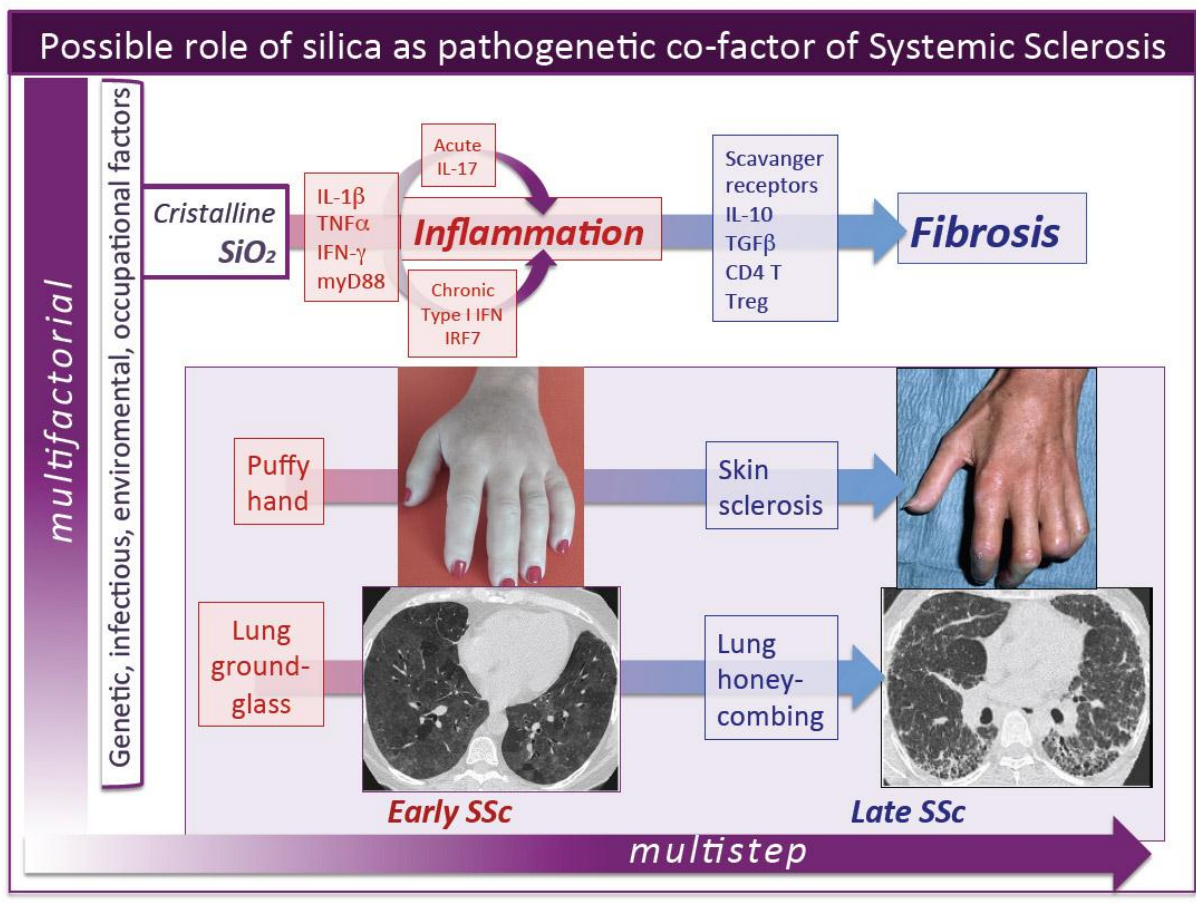


Fig. 4. In patients with occupational/environmental exposure to silica dust the presence of high serum levels of silica micro- and nanoparticles and s-Si can be regarded as additional co-factor potentially involved in the multifactorial and multistep etiopathogenesis of systemic sclerosis (SSc).

IL: interleukin; TNF: tumor necrosis factor; IFN: interferon; MyD88: myeloid differentiation primary response gene 88; IRF: interferon regulatory factor; TGF: transforming growth factor; Treg: regulatory T cells; HRCT: high resolution computed tomography.

In this scenario, the inhalation of silica dust might contribute to the development of specific scleroderma variants. The hypothesis of pathogenetic link between SSc and exogenous triggers, i.e. infectious and/or environmental/occupational toxic agents, in genetically predisposed individuals, has been suggested by different clinico-epidemiological and laboratory investigations (46, 48). SSc is an immune-mediated inflammatory disease characterized by concomitant histopathologic patterns, namely diffuse vascular alterations, tissue infiltration of inflammatory T- and B-lymphocytes, and increased synthesis/deposition of collagen by altered fibroblasts (1-3). Besides silica dust, numerous environmental/occupational agents, namely solvents (chlorinated, trichlorethylene, toluene, xylene, aromatic, ketones, any type of solvent), welding fumes, epoxy resins and pesticides may be potential triggering factors of SSc (46, 48). The development of autoreactive lymphocytes, with different autoantibody, cytokine, and chemokine production, may lead to immune-mediated inflammatory process and organ damage (1-3). In this context, a

pathogenetic role of silica as potential co-factor of SSc is also suggested by some molecular biology studies (11). In particular, the presence of lymphocyte activation has been demonstrated in silica-exposed workers (47); while, silica perturbation toward a profibrotic gene expression on scleroderma fibroblasts may represent a decisive contribute to the resulting fibrotic organ damage (48).

In addition, the hypothesis of a pathogenetic role of silica in the human diseases is strongly supported by laboratory investigations on the pathophysiology of silicosis (11); silica particles may yield multiple, profound alterations of both immune-system compartment and fibroblasts. Different molecular and cellular requirements may be involved in two distinct pathological processes leading to inflammasome activation and fibrosis production responsible for tissue damage (11; Fig. 3). The same pathogenetic mechanisms might be operative in the setting of SSc with silica exposure and specific genetic susceptibility; in this respect, the natural course of the disease may reproduce both pathological processes above-mentioned (1-3). In particular, typical inflammatory manifestations, i.e. puffy fingers and/or lung alveolitis, often characterize the early stages of the disease that very frequently may progress to overt fibrosis of the skin and visceral organs of advanced scleroderma (Fig. 4). The silica dust inhalation might be particularly relevant for the possible contribution in the lung involvement that may affect the overall SSc patient's outcome (1-3). In this light, lung fibrosis might represent a predisposing condition to the lung cancer development mainly observed in the late stages of SSc (49); the carcinogenic role of silica is suggested by several studies showing an elevated risk of lung cancer in both silicotic and non-silicotic individuals with occupational exposure to silica dust (50).

In conclusion, previous clinical observations suggesting a role of silica in a subset of genetically predisposed scleroderma patients seems to be reinforced by the results of the present study; it firstly evidenced abnormally high s-Si levels in exposed SSc patients along with a significant association with specific clinico-serological features. Further clinico-epidemiological and laboratory investigations should be directed at deeper comprehension of the actual role of this element in the pathogenesis of the whole SSc, and in particular of some prognostically harmful organ involvement, mainly lung fibrosis.

References

1. Ferri C, Valentini G, Cozzi F, Sebastiani M, Michelassi C, La Montagna G et al. Systemic Sclerosis: Demographic, Clinical, and Serologic Features and Survival in 1,012 Italian Patients. *Medicine (Baltimore)*. 2002; 81:139-153.
2. Steen VD. The many faces of scleroderma. *Rheum Dis Clin North Am*. 2008; 34:1-15.
3. Denton CP, Khanna D. Systemic sclerosis. *Lancet*. 2017; S0140-6736:30933-9.
4. Marie I, Gehanno JF, Bubenheim M, Duval-Modeste AB, Joly P, Dominique S et al. Systemic sclerosis and exposure to heavy metals: A case control study of 100 patients and 300 controls. *Autoimmun Rev*. 2017;16:223-230.
5. McCormic ZD, Khuder SS, Aryal BK, Ames AL, Khuder SA. Occupational silica exposure as a risk factor for scleroderma: a meta-analysis. *Int Arch Occup Environ Health*. 2010;83:763-9.
6. Rocha LF, Luppino Assad AP, Marangoni RG, Del Rio AP, Marques-Neto JF, Sampaio-Barros PD. Systemic sclerosis and silica exposure: a rare association in a large Brazilian cohort. *Rheumatol Int*. 2016;36:697-702.
7. Lee S, Hayashi H, Mastuzaki H, Kumagai-Takei N, Otsuki T. Silicosis and autoimmunity. *Curr Opin Allergy Clin Immunol*. 2017;17:78-84.
8. Parks CG, Conrad K, Cooper GS. Occupational exposure to crystalline silica and autoimmune disease. *Environ Health Perspect*. 1999;107:793-802.
9. Erasmus LD. Scleroderma in goldminers on the Witwatersrand with particular reference to pulmonary manifestations. *S Afr J Lab Clin Med*. 1957;3:209-31.
10. Bramwell B. Diffuse scleroderma: its frequency, its occurrence in stonemasons, its treatment by fibrinolysis, elevations of temperature due to fibrinolysin injections. *Edinbg Med J*. 1914;12:387-401.
11. Pollard KM. Silica, Silicosis, and Autoimmunity. *Front Immunol*. 2016;7:97.
12. Brown JM, Pfau JC, Holian A. Immunoglobulin and lymphocyte responses following silica exposure in New Zealand mixed mice. *Inhal Toxicol*. 2004;16:133.
13. Bates MA, Brandenberger C, Langohr I, Kumagai K, Harkema JR, Holian A et al. Silica triggers inflammation and ectopic lymphoid neogenesis in the lungs in parallel with accelerated onset of systemic autoimmunity and glomerulonephritis in the lupus-prone NZBWF1 mouse. *PLoS One*. 2015.
14. Van Den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A et al. Classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Arthritis*

15. Lane SE, Watts RA, Bentham G, Innes NJ, Scott DG. Are environmental factors important in primary systemic vasculitis? A case-control study. *Arthritis Rheum.* 2003;48:814-23.
16. Final Report for the Occupational Safety and Health Administration OSHA. 2011 https://www.osha.gov/silica/Employment_Analysis.pdf
17. Sebecić B, Japjec M, Dojcinović B, Zgaljardić I, Staresinić M. Aggressive granulomatosis after cementless total hip arthroplasty as a result of inflammatory reaction to metal debris: case report. *Acta Clin Croat.* 2013;52:492-6.
18. Fassina A, Corradin M, Murer B, Furlan C, Guolo A, Ventura L et al. Detection of silica particles in lung tissue by environmental scanning electron microscopy. *Inhal Toxicol.* 2009;21:133–40.
19. Alimonti A, Bocca B, Mannella E, Petrucci F, Zennaro F et al. Assessment of reference values for selected elements in a healthy urban population. *Ann Ist Super Sanità.* 2005;41:181-7.
20. Bocca B, Forte G, Petrucci F, Senofonte O, Violante N, Alimonti A. Development of methods for the quantification of essential and toxic elements in human biomonitoring. *Ann Ist Super Sanità.* 2005;41:165-70.
21. Giuggioli D, Lumetti F, Colaci M, Fallahi P, Antonelli A, Ferri C. Rituximab in the treatment of patients with systemic sclerosis. Our experience and review of the literature *Autoimmun Rev.* 2015;14:1072-8.
22. Sverzellati N, Calabrò E, Chetta A, Concari G, Larici AR, Mereu M et al. Visual score and quantitative CT indices in pulmonary fibrosis: relationship with physiologic impairment. *Radiol Med.* 2007;112:1160-72.
23. Hering KG, Hofmann-Preiß K, Kraus T. Update: standardized CT/HRCT classification of occupational and environmental thoracic diseases in Germany. *Radiologe.* 2014; 54: 363-84.
24. Barbhaiya M, Costenbader KH. Environmental exposures and the development of systemic lupus erythematosus. *Curr Opin Rheumatol.* 2016;28:497-505.
25. Schreiber J, Koschel D, Kekow J, Waldburg N, Goette A, Merget R. Rheumatoid pneumoconiosis (Caplan's syndrome). *Eur J Intern Med.* 2010;21:168-72.
26. Gómez-Puerta JA, Gedmintas L, Costenbader KH. The association between silica exposure and development of ANCA-associated vasculitis: systematic review and

27. Wichmann I, Sanchez-Roman J, Morales J, Castillo MJ, Ocaña C, Nuñez-Roldan A. Antimyeloperoxidase antibodies in individuals with occupational exposure to silica. *Ann Rheum Dis.* 1996;55:205-7.
28. Sluis-Cremer GK, Hessel PA, Nizdo EH, et al. Silica, silicosis, and progressive systemic sclerosis. *Br J Ind Med.* 1985;42:838–843.
29. Cowie RL. Silica-dust-exposed mine workers with scleroderma (systemic sclerosis). *Chest.* 1987;92:260-2.
30. Sanchez-Roman J, Wichmann I, Salaberri J, Varela JM, Nuñez-Roldan A. Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. *Ann Rheum Dis.* 1993;52:534-8.
31. Brown LM, Gridley G, Olsen JH, Mellekjaer L, Linet MS, Fraumeni JF Jr. Cancer risk and mortality patterns among silicotic men in Sweden and Denmark. *J Occup Environ Med.* 1997;39:633-8.
32. Conrad K, Stahnke G, Liedvogel B, Mehlhorn J, Barth J, Blasum C et al. Anti-CENP-B response in sera of uranium miners exposed to quartz dust and patients with possible development of systemic sclerosis (scleroderma). *J Rheumatol.* 1995;22:1286-94.
33. Rosenman KD, Moore-Fuller M, Reilly MJ. Connective tissue disease and silicosis. *Am J Ind Med.* 1999;35:375-81.
34. Walsh SJ. Effects of non-mining occupational silica exposure on proportional mortality from silicosis and systemic sclerosis. *J Rheumatol.* 1999;26:2179-85.
35. Makol A, Reilly MJ, Rosenman KD, Walsh SJ. Prevalence of connective tissue disease in silicosis (1985-2006)-a report from the state of Michigan surveillance system for silicosis. *Am J Ind Med.* 2011;54:255-62.
36. Burns CJ, Laing TJ, Gillespie BW, Heeringa SG, Alcsér KH et al. The epidemiology of scleroderma among women: assessment of risk from exposure to silicone and silica. *J Rheumatol.* 1996;23:1904-11.
37. Haustein UF, Ziegler V, Hermann K, Mehlhorn J, Schmidt C. Silica-induced scleroderma. *J Am Acad Dermatol.* 1990;22:444–448.
38. Englert H, Small-McMahon J, Davis K, et al. Male systemic sclerosis and occupational silica exposure-a population-based study. *Aust N Z J Med.* 2000;30:215–220.

39. Diot E, Lesire V, Guilmot JL, et al. Systemic sclerosis and occupational risk factors: a case-control study. *Occup Environ Med*. 2002;59:545–549.
40. Calvert GM, Rice FL, Boiano JM, Sheehy JW, Sanderson WT. Occupational silica exposure and risk of various diseases: an analysis using death certificates from 27 states of the United States. *Occup Environ Med*. 2003;60:122-9.
41. Gaultier J-B, Hot A, Cathébras P, Grange C, Ninet J, Rousset H. Systemic sclerosis in men. *Rev Med Interne*. 2008;29:181–6.
42. Freire M, Alonso M, Rivera A, Sousa A, Soto A et al. Clinical peculiarities of patients with scleroderma exposed to silica: A systematic review of the literature. *Semin Arthritis Rheum*. 2015;45:294-300.
43. Marie I, Menard JF, Duval-Modeste AB, Joly P, Dominique S et al. Association of occupational exposure with features of systemic sclerosis. *J Am Acad Dermatol*. 2015 Mar;72:456-64.
44. Marie I, Gehanno JF. Environmental risk factors of systemic sclerosis. *Semin Immunopathol*. 2015;37:463-73.
45. Rocha LF, Luppino Assad AP, Marangoni RG, Del Rio AP, Marques-Neto JF, et al. Systemic sclerosis and silica exposure: a rare association in a large Brazilian cohort. *Rheumatol Int*. 2016;36:697-702.
46. Rubio-Rivas M, Moreno R, Corbella X. Occupational and environmental scleroderma. Systematic review and meta-analysis. *Clin Rheumatol*. 2017;36:569-582.
47. Rocha-Parise M, Santos LM, Damoiseaux JG, Bagatin E, Lido AV et al. Lymphocyte activation in silica-exposed workers. *Int J Hyg Environ Health*. 2014;217:586-91.
48. Yang Y, Wei P, Guo XJ, Zhou D, Zhang WZ et al. Impact of age and autoantibody status on the gene expression of scleroderma fibroblasts in response to silica stimulation. *Eur J Inflamm*. 2013;11:631-639.
49. Colaci M, Giuggioli D, Sebastiani M, Manfredi A, Vacchi C et al. Lung cancer in scleroderma: results from an Italian rheumatologic center and review of the literature. *Autoimmun Rev*. 2013;3:374-9.
50. Poinen-Rughooputh S, Rughooputh MS, Guo Y, Rong Y, Chen W. Occupational exposure to silica dust and risk of lung cancer: an updated meta-analysis of epidemiological studies. *BMC Public Health*. 2016;16:1137.

Tab. 1. Occupational exposure categories in SSc patients and controls.

	Exposed individuals	
	SSc patients	Controls
Industries/Occupation	43/80 (54%)	6/50 (12%)
Ceramics	15	1
Chemicals	4	0
Textiles	5	0
Construction	5	1
Metalworking	6	2
Paint and Coatings	2	0
Dental Laboratories	2	1
Industrial supplies	4	1
Glass manufacturing	2	0
Kitchen utensil manufacturing	1	0

Tab. 2. Serum silica levels and clinico-serological features of 80 SSc patient
 HRCT: high resolution computed tomography; VC: vital capacity; FVC: forced vital capacity;

	Serum Si	
	$\mu\text{g/L}$ (mean \pm SD)	p
Males/Females (13/67)	378.9 \pm 185.6 / 330.6 \pm 111.6	0.4727
Smoke exposure +/- (39/41)	348.3 \pm 139.6 / 327.4 \pm 116.7	0.6255
Diffuse/Limited SSc (19/61)	409.9 \pm 106.7 / 326.1 \pm 127.5	0.0406
Telangectasias +/- (33/47)	308.3 \pm 128.1 / 351.6 \pm 130.1	0.1948
Calcinosis +/- (18/62)	283.1 \pm 132.3 / 349.2 \pm 124.4	0.0988
Skin ulcers +/- (53/27)	349.9 \pm 117.3 / 317.8 \pm 141.35	0.1403
Arthritis +/- (9/71)	328.0 \pm 176.3 / 338.3 \pm 122.0	0.7977
Myositis +/- (7/73)	419.4 \pm 53.0 / 317.0 \pm 130.0	0.0447
Sicca syndrome +/- (33/47)	348.4 \pm 129.5 / 320.3 \pm 124.6	0.3997
Thyroid inv. +/- (28/52)	318.0 \pm 129.2 / 347.0 \pm 126.8	0.305
Lung fibrosis HRCT +/- (42/38)	380.4 \pm 122.1 / 271.6 \pm 115.3	<0.0001
Ground glass opacities +/- (24/56)	408.8 \pm 134.6 / 308.1 \pm 113.3	0,003
Honeycombing +/- (8/72)	450.5 \pm 174.7 / 327.6 \pm 119.3	0.0496
Pulmonary function tests		
VC \leq/$>$80% (19/61)	390.0 \pm 113.1 / 304.3 \pm 125.3	0.0157
FVC \leq/$>$80% (19/61)	431.8 \pm 119.7 / 301.9 \pm 118.9	0.0004
DLCO \leq/$>$60% (48/32)	380.6 \pm 119.1 / 302.9 \pm 130.6	0.0127
Heart inv. +/- (16/64)	338.5 \pm 147.9 / 337.0 \pm 123.5	0.8129
PAPs $>$ / \leq 25mmHg (17/63)	347.1 \pm 151.2 / 332.4 \pm 119.6	0.8651
Esophageal inv. +/- (27/53)	364.4 \pm 141.1 / 324.1 \pm 119.7	0.2616
Kidney inv. +/- (2/78)	321.5 \pm 128.0 / 332.7 \pm 125.0	0.0785
Malignancies +/- (8/72)	294.5 \pm 93.3 / 340.0 \pm 129.4	0.3944
ESR $>$/\leq 34mm (20/60)	389.1 \pm 104.3 / 316.1 \pm 130.9	0.0254
CRP $>$/\leq 0.5mg/dl (22/58)	400.7 \pm 99.6 / 312.1 \pm 132.4	0.0072
ANA +/- (62/18)	336.7 \pm 111.5 / 338.0 \pm 147.6	0.865
ACA +/- (27/53)	265.6 \pm 108.6 / 369.9 \pm 122.9	0.0006
anti-Scl70 +/- (35/45)	383.4 \pm 122.0 / 301.9 \pm 121.5	0.0068

DLCO: lung diffusing capacity for carbon monoxide;

PAPs: pulmonary systolic arterial pressure; ESR: erythrocyte sedimentation rate;

CRP: C reactive protein; ANA: anti-nuclear antibodies; ACA: anti-centromere antibodies;

anti-Scl70: anti-Scl70 antibodies;

The p values were calculated by t-test.