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Original Research

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

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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±11.9SD years, mean disease duration 10.1±7.8SD) 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].

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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±11.9SD years, mean disease duration 10.1±7.8SD) 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±10.8SD 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 μm) (Fig. 1).

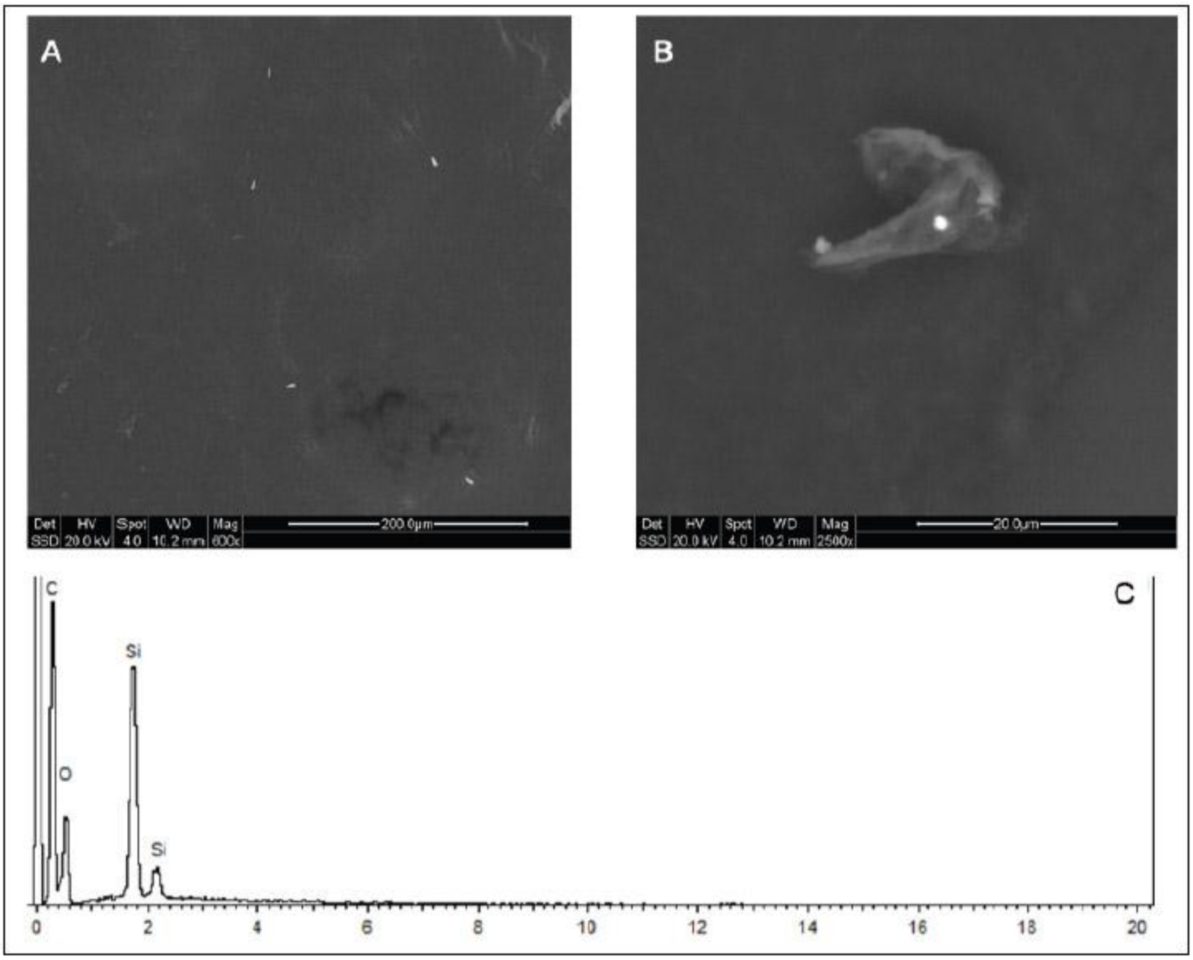

Fig. 1. ESEM analysis of serum sample from an SSc patient: image (A) with magnification of 600x and (B) with magnification 2500x showing a cluster of nanoparticles. The chemical analysis of this cluster (C) by means of EDS shows the presence of Si element.

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).

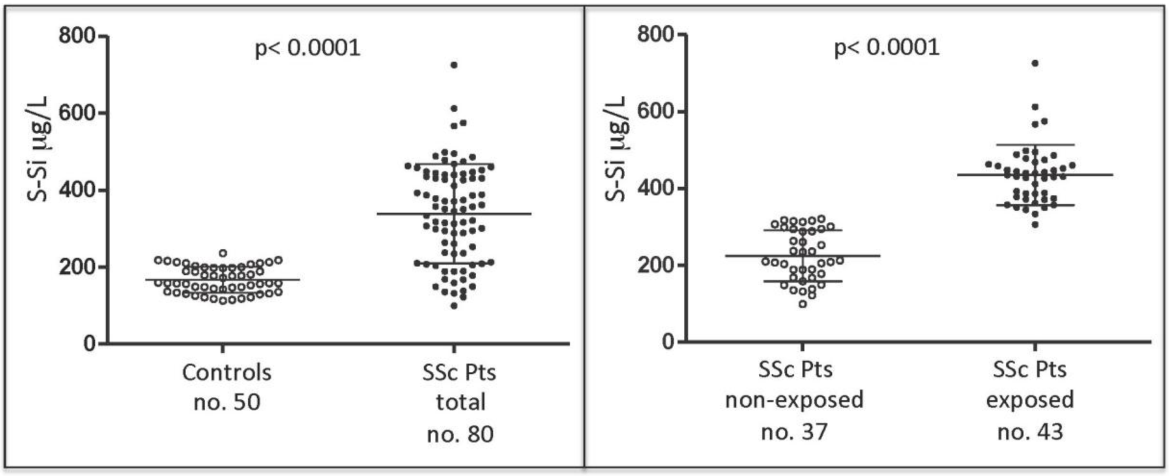

Fig. 2. Comparison between silica levels in SSc patients and control subjects (left), and between silica-exposed and non-exposed SSc patients (right). The results are reported as mean±SD.

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=.0406), myositis (p=.0447), lung fibrosis (p<.0001), ground glass opacities (p=.003), and honeycombing (p=.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<.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).

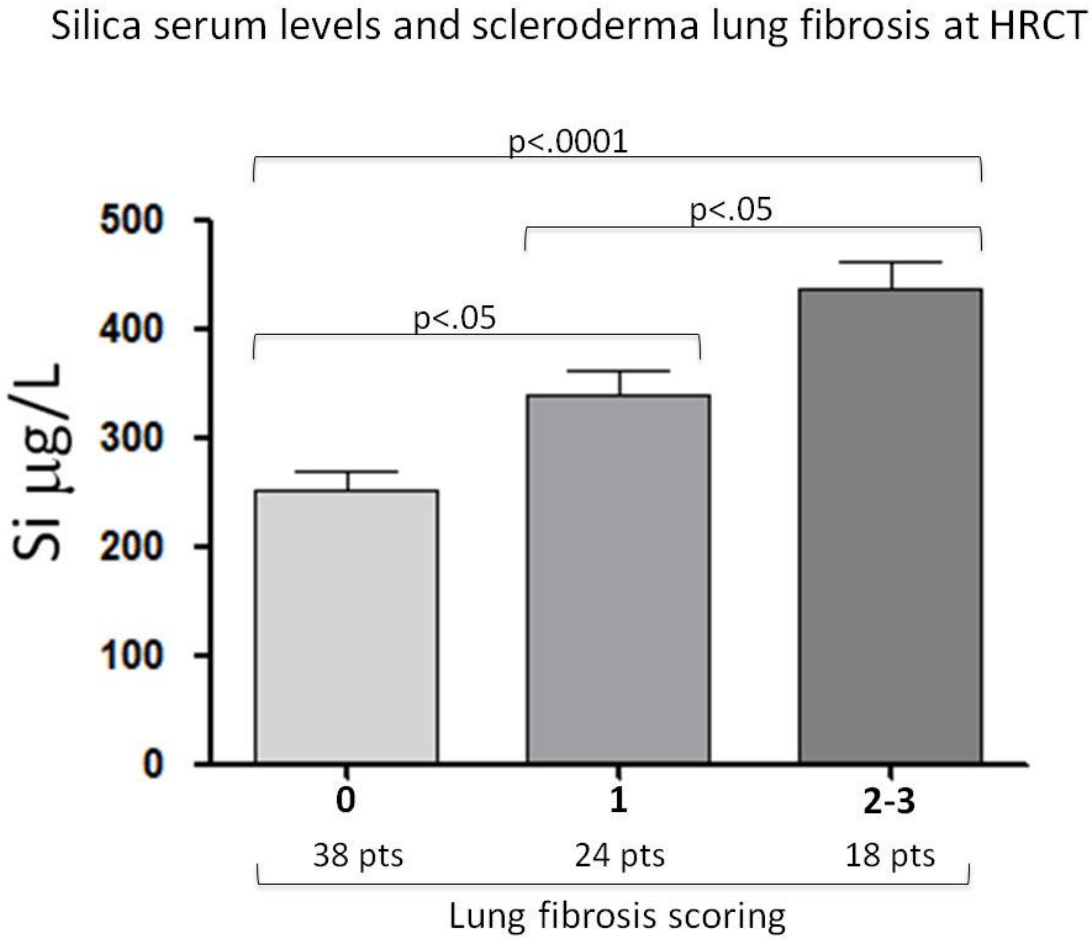

Fig. 3. Systemic sclerosis (SSc) patients with lung fibrosis, detected by high resolution computed tomography (HRCT) in 42/80 (53%) individuals, showed significantly higher levels of serum silicon (s-Si) compared to 38/80 (47%) without (p<.0001; Tab. 2). Moreover, the lung fibrosis scoring significantly 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±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 co-factor 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).

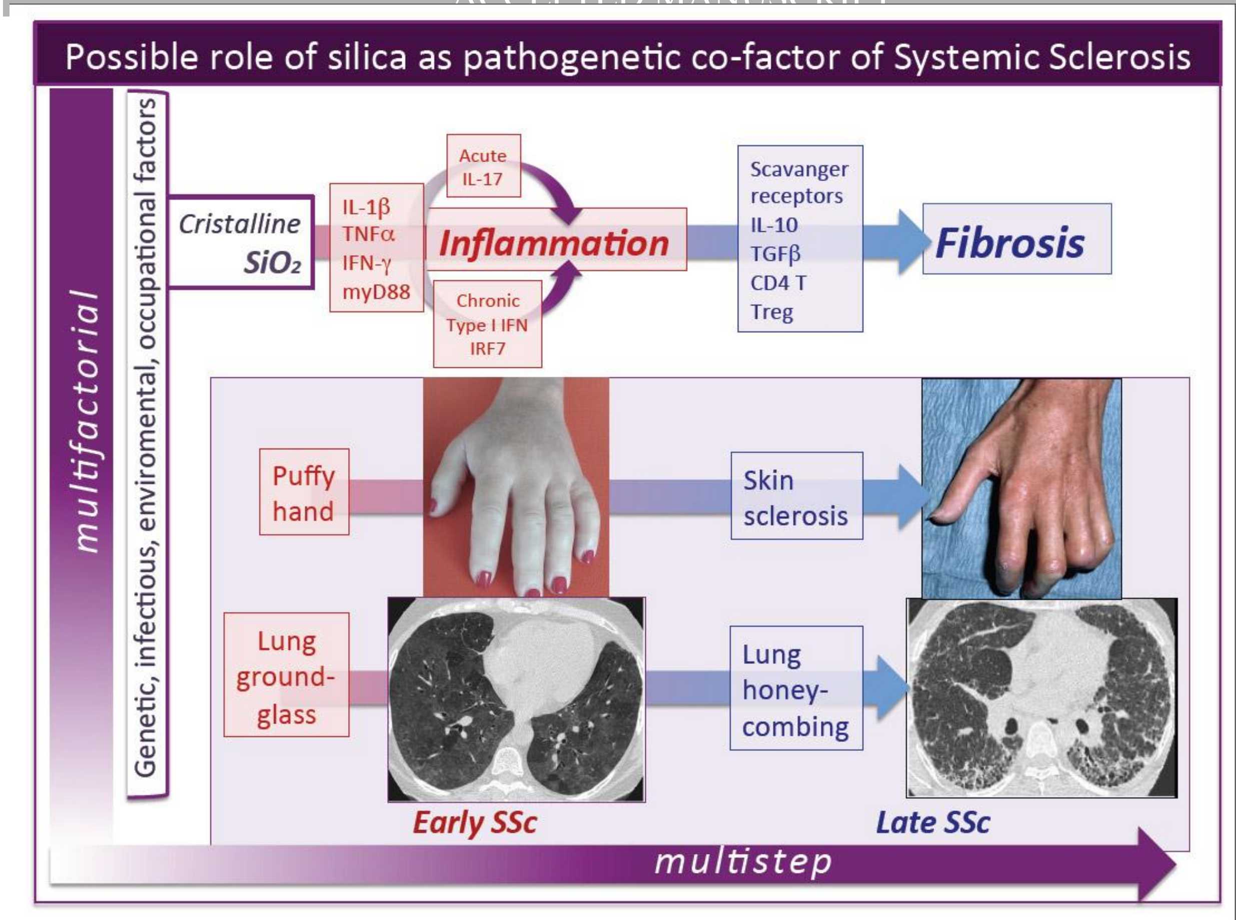

Fig. 4. In patients with occupational/environmental exposure to silica dust the presence of high serum levels of silica micro- and nanoparticles and s-Si can be regarded as additional co-factor potentially involved in the multifactorial and multistep etiopathogenesis of systemic sclerosis (SSc).

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.

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

DLCO: lung diffusing capacity for carbon monoxide;
PAPs: pulmonary systolic arterial pressure; ESR: erytrocyte 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.