Levels of F2-isoprostanes in systemic sclerosis: correlation with clinical features


Objective. Oxidative stress may be one of the important complex pathogenetic mechanisms that lead to damage in scleroderma; free radicals may provoke endothelial injury, fibroblast proliferation and fragmentation of autoantigens favouring induction of autoantibodies. The present study investigates the oxidant status in scleroderma patients by measuring the urinary concentration of 8-isoprostaglandin-F2α, an F2-isoprostane, and a product of free radical-mediated peroxidation of arachidonic acid.

Methods. Forty-three scleroderma patients (42 women and 1 man, mean age 54.1 yr, mean disease duration 9.0 yr) underwent clinical evaluation and instrumental investigations in order to assess skin, vascular, lung and heart involvement. Von Willebrand factor was evaluated as marker of vascular dysfunction in 36 out of the 43 cases. The urinary level of 8-isoprostaglandin-F2α was measured in all scleroderma patients and in the 43 age- and sex-matched healthy controls.

Results. Urinary levels of 8-isoprostaglandin-F2α were higher in scleroderma patients than in the healthy control group (341.7 vs 147.6 pg/mg creatinine; P < 0.001). Values of 8-isoprostaglandin-F2α were strongly correlated with the nailfold videocapillaroscopy pattern and lung involvement (P = 0.002 and 0.003, respectively), showing increasing levels with the progression of pulmonary severity. Correlation between 8-isoprostaglandin-F2α level and von Willebrand factor narrowly failed to reach statistical significance (P = 0.05). There was no correlation between 8-isoprostaglandin-F2α concentration and disease activity, vascular, skin and heart involvement, disease pattern or autoantibody profile.

Conclusions. Our study further supports the role of oxidant stress in the pathogenesis of scleroderma, showing a strong correlation between a marker of free radical damage with both the severity of lung involvement and the videocapillaroscopic patterns.

Key words: Systemic sclerosis, Oxidative stress, F2-isoprostanes.

Systemic sclerosis (SSc) is a connective tissue disease characterized by tissue fibrosis, vascular endothelial dysfunction and specific immunological abnormalities. Its pathogenesis is very complex and still largely unknown [1], but vascular perturbation is supposed to be a primary event which may trigger the fibrotic process. Endothelial damage can be found early in the progress of SSc, as indicated by increased plasma concentration of von Willebrand factor antigen (vWF) in the precocious phase of the disease [2]. Of the factors suspected to induce endothelial cell dysfunction in SSc, excessive oxidative stress has received considerable attention since it was first suggested by Murrell [3]. Oxidative stress is mediated by free radicals, the generation of which is physiologically counteracted by antioxidant mechanisms; this balance may be impaired by either an increased production of free radicals or a deficient antioxidant defence. Free radicals may be generated by ischaemia-reperfusion [4], which represents a characteristic chronic widespread phenomenon in SSc. In the past 10 yr in vitro and in vivo studies have shown the overproduction of free radicals in the pathophysiology of SSc. Lau et al. noticed at first that plasma thiol concentration, which is connected with the degree of plasma oxidation, was reduced in patients with SSc, indicating an increased production of free radicals [5] and then that plasma levels of malondialdehyde, an indicator of free radical activity, were high [6]. They suggested increased free radical production by neutrophil activation. Moreover an elevated release of superoxide by monocytes of SSc patients was demonstrated [7] as well as an enhanced activity of plasma superoxide dismutase [8]. In forearm biopsies increasing severity of skin fibrosis was correlated with a switch in dermal endothelial cells from endothelial nitric oxide synthase to the inducible form of the enzyme, which is a potent inducer of nitric oxide production; therefore these observations furnish a direct link between the severity of skin fibrosis, the hallmark of the disease, and nitric oxide-related free radical damage [9].

Increased free radical status directly damages the endothelium and it may induce chromosomal breakage [10] and promote fibroblast proliferation [3] and alterations in the fluidity of erythrocyte membranes [11] and provoke fragmentation of self-antigens revealing novel epitopes to the immune system favouring the induction of the typical SSc autoantibodies [12]. The mechanism that leads to generation of autoantibodies in an oxidative environment seems to be specific to SSc.

All these studies have aroused increasing interest in the concept that free radicals may play a significant role in SSc.
F₂-isoprostane levels in systemic sclerosis

The concentration of F₂-IsoPs [16] neither does medication with more indicative of an integrated evaluation over time compared to their measurement in 12- or 24-h urine collection is considered more indicative of an integrated evaluation over time compared with plasma determination [15]. Lipid intake does not influence the concentration of F₂-IsoPs [16] neither does medication with non-steroidal anti-inflammatory drugs (NSAIDs) [17]. Moreover, some drugs, including calcium channel blockers and angiotensin-converting enzyme inhibitors, do not seem to interfere with their urinary concentration [18].

Few studies have focused on the measure of these compounds in patients affected by SSc. Stein et al. [15] found that the urinary concentration of the tetranordicarboxylic acid metabolite of F₂-Isop (F₂-IP-M) was significantly higher in eight patients with SSC than in healthy controls. Cracowski et al. [19] confirmed this evidence in a larger group of patients affected by either a limited pattern of SSC (16 cases) or a diffuse form of SSC (21 cases) or undifferentiated connective tissue disease (15 cases), the latter defined on the basis of Raynaud’s phenomenon (RP) in association with either the typical SSC videocapillaroscopic pattern or positivity for anti-Scl70 or anticientromere antibodies. This study showed that the urinary concentration of 8-isoprostaglandin-F₂α (8-iso-PGF₂α), a biochemically stable F₂-Isop, was about twice that in normal subjects, without any significant difference among the three above-mentioned SSC spectrum entities [19]. In a later study Cracowski et al. [20] found that urinary levels of 15-F₂-IsopS, another member of the F₂-Isop family, were increased in 11 patients with SSC but not in 11 patients with primary RP.

The aim of our study was to measure in a large group of SSc patients the urinary concentrations of 8-iso-PGF₂α, considered a reliable marker of oxidant injury in vivo [17, 21], and to evaluate whether there was a correlation with clinical features.

Patients and methods

Study population

Forty-three Italian patients referred to our department (42 women and 1 man, mean age 54.1 ± 14.8 yr, range 23–75 yr, mean disease duration 9.0 yr with 95% CI 7.8–10.3, range 2–46 yr) were consecutively enrolled for the study between January and May 2004. All patients fulfilled the American College of Rheumatology criteria for the diagnosis of SSC [22]. The distinction between limited cutaneous SSC (lcSSC) and diffuse cutaneous SSC (dcSSC) was made according to the criteria of LeRoy et al. [23]. Exclusion criteria comprised cigarette smoking, diabetes and moderate to severe hypercholesterolaemia (cholesterol level >240 mg/dl). The application of these criteria increased the sex disproportion (i.e. four men and only one woman were excluded for active smokers). Hypertension was diagnosed before SSC onset in 11 patients.

Patients were receiving a wide range of drugs, including vasodilators, cyclophosphamide, low-dose prednisolone (<10 mg/day), aspirin and H₂-receptor antagonists.

Patients underwent examination and comprehensive laboratory evaluation of full blood count, erythrocyte sedimentation rate (ESR), renal and liver function indices, C3 and C4 level and antinuclear and anti-extractable nuclear antigen (ENA) antibody determination. Anticentromere antibodies (ACA) were tested by indirect immunofluorescence on HEP-2 cells; anti-Scl70 antibodies were determined by enzyme-linked immunosorbent assay (ELISA). vWF was measured in 36 patients by enzyme-linked fluorescent assay (Vidas vWF; normal <120%); the intra-assay and inter-assay variabilities of the method with different levels of antigen were 3.7–4.5% and 3.2–4.5%, respectively.

In all the patients skin involvement was assessed by the modified Rodnan total skin score (TSS). Briefly, the TSS uses physical examination to measure dermal thickening. Seventeen anatomical sites were evaluated by the same operator using a score from 0 to 3 (0 indicates normal), thus resulting in a total score from 0 to 51 [24].

All the patients underwent the following instrumental investigations: electrocardiogram (ECG), chest radiograph, pulmonary function test with diffusing capacity for carbon monoxide adjusted to haemoglobin (DLCO) and Doppler echocardiogram to evaluate left ventricular ejection fraction (LVEF) and to estimate pulmonary artery systolic pressure (sPAP). If no tricuspid regurgitation could be detected, sPAP was assessed as normal, estimated sPAP was considered abnormal if it was >35 mmHg. Pulmonary high-resolution computed tomography (PhrCT) was performed in 20 cases on the grounds of clinical evaluation; fibrosing alveolitis was considered active if ‘ground glass’ lesions were found. The coefficient of variation of the pulmonary function test with DLCO was <5%.

Skin, vascular, pulmonary and cardiac involvement was then evaluated based on a severity scale classification proposed by Medsger et al. [25]. Briefly, the skin involvement was judged absent (stage 0) if TSS was 0, mild (stage 1) if TSS was between 1 and 14, moderate (stage 2) if TSS was between 15 and 25, and severe (stage 3) if TSS was between 26 and 39 and endstage (stage 4) if TSS was >40. Peripheral vascular involvement was judged absent (stage 0) in the case of absence of RP or RP not requiring vasodilators, mild (stage 1) in the case of RP requiring vasodilators, moderate (stage 2) in the case of digital pitting scars, severe (stage 3) in the case of digital tip ulcerations or endstage (stage 4) in the case of digital gangrene. Pulmonary involvement was judged absent (stage 0) in the case of normal values of DLCO and forced vital capacity (FVC), absence of pulmonary fibrosis on chest radiograph and sPAP <35 mmHg, mild (stage 1) in the case of DLCO or FVC between 70 and 80% of the predicted value or basilar rales or fibrosis on radiograph or sPAP between 35 and 49 mmHg, moderate (stage 2) in the case of DLCO or FVC between 50 and 69% of the predicted value or sPAP between 50 and 64 mmHg, severe (stage 3) in the case of DLCO or FVC <50% of predicted value or sPAP >65 mmHg or endstage (stage 4) in the case of oxygen required. Heart involvement was judged absent (stage 0) in the case of normal ECG and LVEF >50%, mild (stage 1) in the case of ECG conduction defect or LVEF between 45 and 49%, moderate (stage 2) in the case of ECG arrhythmia or LVEF between 40 and 44%, severe (stage 3) in the case of ECG arrhythmia requiring therapy or LVEF between 30 and 40% or endstage (stage 4) in the case of congestive heart failure or LVEF <30%.

The disease activity was assessed according to Valentini et al. [26].

The same operator, blind to clinical features, performed nailfold videocapillaroscopy (NVC) in all patients. NVC was performed using an optical probe videocapillaroscope equipped with 100× and 200× contact lenses and connected to image analysis software (VideoCap; DS MediGroup, Milan, Italy). The nailfolds of all 10 fingers were examined in each patient after a drop of immersion oil had been placed on the nailfold bed to improve the image resolution. The microvascular alterations were classified into three different patterns according to Cutolo et al. [27]. These include the ‘early’ pattern of few enlarged/giant capillaries, few capillary haemorrhages, relatively well-preserved capillary distribution, and no evident loss of capillaries; the ‘active’ pattern of frequent giant capillaries, frequent capillary haemorrhages, moderate loss of capillaries, mild disorganization of the capillary architecture, absent or mild ramified capillaries and the presence of oedema; the ‘late’ pattern of irregular enlargement.
of the capillaries, few or no giant capillaries and haemorrhages, severe loss of capillaries with extensive avascular areas, disorganization of the normal capillary array and the presence of ramified/bushy capillaries. Images from each examination were revised by a second rheumatologist, always obtaining the same result.

All the investigations were performed in our hospital. All the patients underwent physical examination and laboratory investigations at the same time as urinary collection. ECG, chest radiograph, pulmonary function test and NVC were performed no more than 7 days apart. Doppler echocardiogram and, as indicated, PhrCT were performed in a 3-month interval from 8-iso-PGF2α measurement.

The study conformed to standards currently applied in Italy. All the investigations were performed as part of the normal clinical evaluation of our scleroderma patients. All the patients gave written informed consent.

The urinary level of 8-iso-PGF2α was quantified in the SSc patients and in an age- and sex-matched group of 43 healthy volunteers (mean age 52.9 ± 14.1 yr, range 24–73 yr) selected from the general population as a control group. All the control subjects were non-smokers and did not receive any drugs.

Measurement of urinary 8-iso-PGF2α

Urinary excretion of 8-iso-PGF2α was evaluated, as previously described [28], from overnight urine collection (from 8 p.m. to 8 a.m.). The timing and total volume were recorded and two 50-ml aliquots were stored at −70°C until extraction. To prevent the formation of 8-iso-PGF2α in vitro, 1 mmol/l of the antioxidant 4-hydroxy-Tempo (Sigma) was added to one aliquot of each urine sample. A small amount of authentic tritium-labelled thromboxane (TX) B2 (6000 dpm, Amersham) was added to each urine sample. A small amount of authentic tritium-labelled thromboxane (TX) B2 (6000 dpm, Amersham) was added to each urine sample. A small amount of authentic tritium-labelled thromboxane (TX) B2 (6000 dpm, Amersham) was added to each urine sample. A small amount of authentic tritium-labelled thromboxane (TX) B2 (6000 dpm, Amersham) was added to each urine sample.

In the first step pre-packed octadecylsilane columns washed with successive chromatographic steps after acidification to pH 3. The peak corresponding to the studied isoeicosanoid was identified from chemically related compounds was obtained by high-performance liquid chromatography (HPLC). Reverse-phase gradient chromatography was performed using 5S ODS2 columns (Supelco, Sigma-Aldrich) with a mobile phase consisting of water-acetonitrile acidified with trifluoroacetic acid. The peak corresponding to the studied isoeicosanoid was identified on the basis of the retention coefficient K, calculated using aliquots of pure compounds (8-iso-PGF2α, TXB2, 2,3-dinor-TXB2, 6keto-PGF1α and 2,3-dinor-6keto-PGF1α) detected at 214 nm by means of an on-line UV system. One-millilitre samples were collected using an automated sample collector. Eluates containing 8-iso-PGF2α were dried under vacuum, resuspended in 1 ml assay buffer and assayed by EIA using commercial antisera (Cayman Chem Co.). The percentage of recovery at the end of the purification procedure was determined in each individual sample measuring the amount of eluted [3H-TXB2, its value was 53.7 ± 7.7. The urinary excretion of 8-iso-PGF2α was expressed as pg/mg urinary creatinine. The intra-assay variability of 8-iso-PGF2α measurement was calculated and found to be 8.6% (n = 9); the inter-assay variability was 12.9% (n = 9).

Statistical analysis

Age was expressed as mean with standard deviation; highly skewed continuous variables (disease duration, haemoglobin and creatinine levels, TSS, FVC, DLCO and 8-iso-PGF2α levels) were expressed as geometric means with 95% confidence interval (95% CI) after logarithmic transformation. Quantitative data were assessed using Student’s t-test or by analysis of covariance with Tukey’s post hoc comparison of the means. Qualitative data were compared by the χ² test with the Yates corrections and the Fischer exact test when necessary. A value of P < 0.05 was considered significant.

The correlation between 8-iso-PGF2α levels and continuous variables (age, disease duration, haemoglobin level, creatinine value, TSS, FVC, DLCO) was evaluated using Pearson’s bivariate correlation.

The SSc population was divided into two subgroups on the basis of the 50th percentile of 8-iso-PGF2α urinary concentration: one with high oxidative status (8-iso-PGF2α levels <352.8 pg/mg of creatinine) and the second with very high oxidative status (8-iso-PGF2α levels ≥352.8 pg/mg of creatinine); the different distribution of the clinical parameters and of the visceral involvement was then evaluated. All P values are two-sided. A value of less than 0.05 indicates a significant difference.

All calculations were performed with the SPSS (version 11.5) statistical package (SPSS, Chicago, IL, USA).

Results

Basal demographic and clinical characteristics of SSc patients

The patients’ demographic, clinical, biochemical and instrumental data, and the severity of degree of organ involvement are summarized in Table 1. All the patients suffered from RP; none of them had clinical evidence of renal involvement.

Levels of 8-iso-PGF2α in patients with SSc vs healthy controls

Urinary excretion of 8-iso-PGF2α was significantly higher (2.3 times) in patients with SSc (341.7 pg/mg creatinine with 95% CI 340.6–342.8) than in healthy controls (147.6 pg/mg creatinine with 95% CI 146.4–148.8) (P<0.001) (Table 2).

Correlation between 8-iso-PGF2α levels and clinical features

The correlation between 8-iso-PGF2α levels and the clinical parameters are reported in Table 3 (Part A).

Correlation between 8-iso-PGF2α levels and NVC pattern

In SSc patients there was a significant correlation between excretion of 8-iso-PGF2α and NVC pattern (P = 0.002) (Table 3, part A). In patients with the early SSc pattern the concentration of 8-iso-PGF2α was 277.2 pg/mg creatinine (95% CI 276.0–278.5); in patients with the active and late pattern the level increased to 319.5 pg/mg creatinine (95% CI 318.4–320.7) and to 485.9 pg/mg creatinine (95% CI 484.6–487.1), respectively. The NVC pattern did not correlate with disease duration.

Correlation between 8-iso-PGF2α levels and pulmonary involvement

The severity of pulmonary involvement was related to 8-iso-PGF2α levels (P = 0.003), which progressively increased from the patients without lung disease (264.1 pg/mg creatinine, CI 262.7–265.4) to the patients with mild (299.5 pg/mg creatinine, CI 298.3–300.7), moderate (360.4 pg/mg creatinine, CI 359.2–361.6) and severe plus endstage pulmonary involvement (515.1 pg/mg creatinine, CI 513.8–516.4) (Table 3, Part A). In particular the worst stages presented 8-iso-PGF2α levels that were 2-fold higher than the cases without lung involvement. This strong association mainly...
TABLE 1. Demographic and clinical characteristics of the 43 patients affected by SSc (expressed as absolute number (%) unless otherwise indicated)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>54.1 ± 14.8</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>9.0 (7.8–10.3)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.4 (12.3–14.4)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 (0.2–1.8)</td>
</tr>
<tr>
<td>TSSS</td>
<td>10.3 (9.1–11.5)</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>93.4 (92.3–94.5)</td>
</tr>
<tr>
<td>DLCO (%)</td>
<td>76.7 (65.9–68.1)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>14/22 (63.7)</td>
</tr>
</tbody>
</table>

Subsets
- lcSSc: 27 (62.8)
- dcSSc: 16 (37.2)

Autoantibody pattern
- ACA positivity: 24 (55.8)
- Anti-Scl70 positivity: 15 (34.9)
- ANA positivity (absent ACA and anti-Scl70): 4 (9.3)

Disease activity
- Inactive: 32 (74.4)
- Active: 11 (25.6)

ESR: Normal 31 (72.1)
High: 12 (27.9)

vWf: Normal 28 (65.1)
Moderate: 11 (25.6)
Severe: 0 (0)
Endstage: 0 (0)

Hypertension
- Absent: 32 (74.4)
- Present: 11 (25.6)

Skin involvement
- Absent: 0 (0)
- Mild: 32 (74.4)
- Moderate: 11 (25.6)
- Severe: 0 (0)
- Endstage: 0 (0)

Peripheral vascular involvement
- Absent: 0 (0)
- Mild: 28 (65.1)
- Moderate: 8 (18.6)
- Severe: 7 (16.3)
- Endstage: 0 (0)

NVC pattern
- Early: 11 (25.6)
- Active: 21 (48.8)
- Late: 11 (25.6)

Lung involvement
- Absent: 0 (0)
- Mild: 10 (23.3)
- Moderate: 10 (23.3)
- Severe: 15 (34.8)
- Endstage: 6 (14.0)

PhrCT (n = 20)
- Normal: 15 (75)
- Active fibrosing alveolitis: 5 (25)

Pulmonary hypertension
- Absent: 36 (83.7)
- Present: 7 (16.3)

Heart involvement
- Absent: 31 (72.1)
- Mild: 8 (18.6)
- Moderate: 4 (9.3)
- Severe: 0 (0)
- Endstage: 0 (0)

TABLE 2. The levels of 8-iso-PGF<sub>2α</sub> in patients with SSc and in healthy controls

<table>
<thead>
<tr>
<th>8-iso-PGF&lt;sub&gt;2α&lt;/sub&gt; (pg/mg creatinine)</th>
<th>Mean</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC patients (n = 43)</td>
<td>341.7</td>
<td>340.6–342.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Healthy controls (n = 43)</td>
<td>147.6</td>
<td>146.4–148.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

318 F<sub>2</sub>-isoprostane levels in systemic sclerosis

Other clinical characteristics

The concentration of 8-iso-PGF<sub>2α</sub> was higher in patients with an elevated vWf level compared with patients with a normal vWf level (378.4 vs 292.8 pg/mg creatinine); the correlation narrowly failed to reach statistical significance (P = 0.05) (Table 3, Part A).

The urinary concentration of 8-iso-PGF<sub>2α</sub> also progressively increased with the worsening of skin, vascular and heart involvement without reaching statistical significance.

No significant correlation was found between 8-iso-PGF<sub>2α</sub> levels and patient age, disease duration, disease activity, disease subsets, autoantibody pattern, haemoglobin level, creatinine level or ESR.

Different distribution of disease severity
in relation to oxidative status

We divided the patients in a subgroup with high oxidative status (8-iso-PGF<sub>2α</sub> level < 352.8 pg/mg of creatinine, that represents the 50th percentile of 8-iso-PGF<sub>2α</sub> concentration in our SSc population) and in a subgroup with very high oxidative status (≥352.8 pg/mg of creatinine). An advanced grade of pulmonary involvement and late NVC pattern were more frequently represented in those with very high oxidative status (Table 3, Part B). Moreover, SSc patients who were also affected by systemic high pressure were more numerous in the subgroup with very high oxidative status.

Evaluation of 8-iso-PGF<sub>2α</sub> levels in relation to the severity of pulmonary involvement and NVC abnormalities

Dividing the patients on the grounds of the most severe grades of lung involvement (i.e. grades 3 and 4) and the most advanced microangiopathy as evaluated by NVC (i.e. late pattern) we obtained three subgroups: subgroup 1 (18 cases) with no severe lung involvement or advanced microangiopathy; subgroup 2 (17 cases) with either severe lung involvement or advanced microangiopathy; subgroup 3 (8 cases) with both severe lung involvement and advanced microangiopathy. By this composite index a progressively higher increase of 8-iso-PGF<sub>2α</sub> concentration in relation to the severity of involvement in one or in both areas was found (P < 0.001) (Table 4).
Clinical feature | Part A: Correlation of 8-iso-PGF$_{2\alpha}$ levels with the clinical parameters | Part B: Different distribution of clinical parameters in relation to high (8-iso-PGF$_{2\alpha}$ level < 352.8 pg/mg creatinine) or very high (8-iso-PGF$_{2\alpha}$ level ≥ 352.8 pg/mg creatinine) oxidative status
--- | --- | ---
DLCO %* | High antioxidant status: absolute number (%) | Very high antioxidant status: absolute number (%)
**vWf** ($n = 36$) | Correlation coefficient | 74.2 (73.0–75.3) | 60.8 (59.6–61.9) | 0.05
Normal | 12 (60) | 5 (31.2) | n.s. (0.1)
High | 8 (40) | 11 (68.8) | n.s.
Hypertension | Absent | 19 (90.5) | 13 (59.1) | 0.03
Present | 2 (9.5) | 9 (40.9) | n.s.
Skin involvement | | | |
Mild | 16 (76.2) | 16 (72.7) | n.s.
Moderate | 5 (23.8) | 6 (27.3) | n.s.
Peripheral vascular involvement | | | |
Mild | 16 (76.2) | 12 (54.5) | n.s.
Moderate | 2 (9.5) | 6 (27.3) | n.s.
Severe | 3 (14.3) | 4 (18.2) | n.s.
NVC pattern | | | |
Early | 7 (33.3) | 4 (18.2) | 0.01
Active | 13 (61.9) | 8 (36.4) | n.s.
Late | 1 (4.8) | 10 (45.5) | n.s.
Lung involvement | | | |
Absent | 6 (28.6) | 4 (18.2) | 0.03
Mild | 7 (33.3) | 3 (13.6) | n.s.
Moderate | 7 (33.3) | 8 (36.4) | n.s.
Severe | 1 (4.8) | 7 (31.8) | n.s.
Heart involvement | | | |
Absent | 17 (81.0) | 14 (63.6) | n.s.
Mild | 2 (9.5) | 6 (27.3) | n.s.
Moderate | 2 (9.5) | 2 (9.1) | n.s.

*Values for DLCO expressed as geometric mean with 95% CI. 8-iso-PGF$_{2\alpha}$, 8-isoprostaglandin-F$_{2\alpha}$; DLCO, diffusing capacity for carbon monoxide, % predicted; vWf, von Willebrand factor antigen; NVC, nailfold videocapillaroscopy.

Table 3. Correlation between 8-iso-PGF$_{2\alpha}$ levels and clinical features (Part A) and different distribution of clinical parameters in relation to oxidative status (Part B)

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>8-iso-PGF$_{2\alpha}$ levels (pg/mg creatinine)</th>
<th>Part A: Correlation of 8-iso-PGF$_{2\alpha}$ levels with the clinical parameters</th>
<th>Part B: Different distribution of clinical parameters in relation to high (8-iso-PGF$<em>{2\alpha}$ level &lt; 352.8 pg/mg creatinine) or very high (8-iso-PGF$</em>{2\alpha}$ level ≥ 352.8 pg/mg creatinine) oxidative status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Group 1 ($n = 18$)</td>
<td>277.4</td>
<td>276.2–278.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 2 ($n = 17$)</td>
<td>360.4</td>
<td>359.2–361.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 3 ($n = 8$)</td>
<td>527.4</td>
<td>526.2–528.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

8-iso-PGF$_{2\alpha}$, 8-isoprostaglandin-F$_{2\alpha}$; mean = geometric mean. Group 1: patients with no severe lung involvement nor late NVC pattern. Group 2: patients with either severe lung involvement or late NVC pattern. Group 3: patients with both severe lung involvement and late NVC pattern.

Discussion

The present study confirms marked oxidative stress in SSc; in fact the F$_2$-IsoPs urinary concentration in SSc patients was significantly higher than in the control group. More interestingly, levels of F$_2$-IsoPs showed a strong correlation with peripheral microangiopathy as directly observed by NVC and with lung involvement.

Recently, NVC abnormalities were associated with different serum levels of both IL-13 [29], a profibrotic and pro-inflammatory cytokine, and E-selectin [30]. However, to our knowledge the observation that the concentration of F$_2$-IsoPs increases with the severity of SSc microvascular bed involvement represents the first evidence of a clear association between the three aforementioned NVC patterns and a measurable biochemical parameter. In our study the disease duration did not correlate with either F$_2$-IsoP levels or with the NVC pattern; therefore higher values of F$_2$-IsoPs seem to be predictive of a more severe microangiopathy.

A previous study did not find any correlation between F$_2$-IsoP levels in SSc patients and the presence of lung involvement as defined on the basis of clinical data and chest radiograph and then confirmed by CT scan [19]. The present study confirms that pulmonary fibrosis as shown by the chest radiograph was not correlated with F$_2$-IsoP concentration; even in patients with active fibrosing alveolitis the F$_2$-IsoP concentration was not significantly higher, but this subgroup of patients was too small to draw any meaningful information. Nevertheless when the SSc patients were considered on the basis of the lung severity assessment score [25] we found a significant correlation with F$_2$-IsoP concentration, the level of which progressively increased as the score worsened; in fact patients with severe and endstage lung involvement showed F$_2$-IsoP levels two times higher than patients without pulmonary disease. It is known that lung involvement, which occurs in more than 75% of the SSc patients, significantly impacts on morbidity and mortality [31]. The DLCO measure has a great influence on lung assessment severity scoring; this parameter is strongly correlated with F$_2$-IsoP concentration. Reduction of the DLCO is the first and most sensitive alteration in case of lung involvement in SSc. Either pulmonary fibrosis or pulmonary hypertension can impair DLCO, and it has been suggested that isolated reduction of DLCO might also be indicative of lung microangiopathy [32].
The correlation of F2-Isop levels with both NVC pattern and pulmonary involvement may be due to the common pathogenetic mechanisms of SSc involving microvasculature at different sites, as suggested by the evidence of correlation between a component of DLCO—the diffusing capacity of the alveolocapillary membrane—and NVC abnormalities [33]. Quite interestingly, a relation between the severity of microvascular disease and oxidative stress was observed in essential hypertension, where only patients with retinal microangiopathy showed increased excretion of 8-iso-PGF2α [34].

The correlation between F2-Isop concentration and vWF level, a marker of vascular injury, narrowly failed to reach statistical significance. The vWF level was only evaluated in 36 cases; a larger number of SSc patients would probably allow us to reach a significant correlation. vWF level is frequently elevated in SSc; high values are associated with the extent of visceral involvement [35] and with a poor prognosis [36]. SSc patients with more severe skin, heart and peripheral vascular involvement presented higher F2-Isop levels without reaching statistical significance. Patients with limited and diffuse pattern of SSC showed similar F2-Isop levels, as previously described [19]. Hypertension, which was found to be slightly related to 8-iso-PGF2α excretion in our SSC population, may be seen as another factor participating in the microangiopathy that increases the oxidative burden. A similar additional effect of several oxidative stimuli has already been demonstrated in patients with coronary heart disease [37].

The aetio-pathogenesis of SSC is still largely unknown, and it has still to be demonstrated if the oxidative stress causes or participates in the initial endothelial damage. However, the relation existing with severity of both lung and microangiopathic symptoms, as observed in our study, suggests that oxidative stress plays a crucial role in the progression of vascular damage. In SSC endothelial damage may initially induce all or drive the proliferation of smooth-muscle cells and fibroblasts, followed by the recruitment of platelets and inflammatory cells. It might be speculated that all these cell types are involved in the generation of a pro-oxidant environment. This hypothesis could explain the relationship between microangiopathy as directly observed by NVC and oxidative status.

8-iso-PGF2α is a potent kidney [17] and lung [38] vasoconstrictor; therefore besides its utility as marker of lipid peroxidation it might directly participate in the complex pathogenetic mechanism of SSc.

At present we think that measurement of F2-isoprostanes, which is considered the ‘gold standard’ assessment of oxidative stress in vivo [39], is not a suitable test for routine evaluation of scleroderma patients, since the measurement is difficult to perform. Whether oxidative stress is a suitable target for therapeutic intervention needs to be assessed in properly designed intervention trials.

Our study shows that the oxidative status evaluated by measuring the urinary concentration of F2-Isop is markedly increased in SSC, and above all it shows a strong correlation with both lung involvement and videocapillaroscopic abnormalities, further suggesting that free radicals may play an pivotal role in SSc damage. F2-Isop levels may represent a marker of aggressive disease.

### Key messages

- The urinary concentration of F2-isoprostanes, a marker of oxidative stress, is very high in scleroderma patients.
- Levels of F2-isoprostanes show a strong correlation with the severity of both microangiopathy and lung involvement.

The authors have declared no conflicts of interest.


