Circulating levels of active transforming growth factor β1 are reduced in diffuse cutaneous systemic sclerosis and correlate inversely with the modified Rodnan skin score

M. Dziadzio, R. E. Smith, D. J. Abraham, C. M. Black and C. P. Denton

Objectives. To determine the relationship between clinical features and circulating levels of active transforming growth factor (TGF) β1 in the major subsets of systemic sclerosis (SSc).

Methods. In a cross-sectional study cases of diffuse cutaneous SSc (dose) (n = 27) or limited cutaneous SSc (dose) (n = 20) were compared with healthy controls (n = 22). Active and total TGFβ1 was measured in serum and plasma by a high-sensitivity enzyme-linked immunosorbent assay.

Results. There were no significant differences between levels of total serum TGFβ1. However, cases of dSSc had lower levels of active TGFβ1 than cases of lcSSc or controls. In addition, more cases of dSSc (18/27; 66%, P < 0.025) had no detectable active TGFβ1 than controls (7/22, 32%) or lcSSc (7/20, 35%). In dSSc, serum active TGFβ1 levels correlated negatively with skin score and positively with disease duration.

Conclusions. Contrary to expectation, levels of active TGFβ1 are reduced in dSSc and this correlates with two variables known to associate with disease activity, shorter duration and more extensive skin sclerosis. This suggests that active TGFβ1 may be sequestered in active involved SSc skin and that serum levels are reduced despite strong evidence implicating TGFβ isoforms in the pathogenesis of fibrosis. Our findings may have implications for systemic TGFβ-trapping therapies in this disease.

Key words: Scleroderma, Systemic sclerosis, Biological markers, Severity of illness index, Transforming growth factor beta.
Patients fulfilled the American College of Rheumatology (formerly the American Rheumatism Association) preliminary criteria for the classification of SSc [22]. Twenty-seven patients (20 female, 7 male) had diffuse cutaneous systemic sclerosis (dcSSc) and 20 patients (16 female, 4 male) had limited cutaneous systemic sclerosis (lcSSc) [23]. Disease duration was defined as the time from onset of the first non-Raynaud’s manifestation of SSc. Clinical assessment of skin sclerosis was performed concurrently with blood sampling: skin score was measured by the modified Rodnan skin scoring technique [24]. The presence of antinuclear antibodies, anticytomegolomer antibodies, and double-stranded DNA (dsDNA) antibodies, antitTG-1 antibodies, antitTG-1 antibodies, and antitTG-1 antibodies, also as rheumatoid factor was determined by validated assays. Patients were screened for major visceral complications of SSc according to current standard practice including pulmonary function tests, ECG, Doppler echocardiography and calculated creatinine clearance. When these basic tests were abnormal, further investigation by high-resolution CT scan (HRCT) or right heart catheterization was performed. Pulmonary hypertension (PHT) was defined by mean pulmonary artery pressure (PAP) above 25 mmHg at rest or 30 mmHg on exercise. Lung fibrosis was determined by HRCT, renal involvement by creatinine clearance of less than 60 ml/min or history of scleroderma renal crisis, and gastrointestinal tract involvement was determined by typical history or abnormal investigations prompted by symptoms. Skeletal muscle involvement was determined by creatinine kinase (CKP) greater than four times the upper limit of normal. Disease activity and severity were determined according to the guidelines formulated recently [21]. Briefly, in the assessment of disease activity, the following parameters were evaluated and scored: modified Rodnan skin score, presence of scleredema, any recent worsening of the cutaneous/vascular/cardipulmonary involvement, active digital ulcers, presence of arthritis, diffusing lung capacity for carbon monoxide (DLCO) <80%, serum complement: C3 and C4, erythrocyte sedimentation rate (ESR) >30 and low levels of serum complement. Scoring was different for each of those parameters with the total score ranging from 0 to 10. The disease was considered active if the score was ≥3. The assessment of disease severity was based on the evaluation of nine organs/systems: general, peripheral vascular, skin, joints and tendons, muscles, gastrointestinal tract, lung, heart and kidneys. The severity of the involvement of each organ was evaluated following guidelines and scored from 0–4, where 0 was normal or no involvement, 1 mild, 2 moderate, 3 severe and 4 end-stage. The single scores were summarized and the final value denoted disease severity.

Control samples were obtained from 22 healthy volunteers (Table 1).

### Blood sampling

Blood samples were taken from each subject in the morning. Serum and plasma samples were obtained by centrifugation of whole blood at 3000 g for 10 min and aliquots were stored at −20 °C until assayed. Levels of TGFβ1 were measured using a high-sensitivity sandwich immunosassay specific for the active ligand (Promega, WI, USA), according to the manufacturer’s protocol. Each sample was assayed in triplicate.

In brief, the 96-well microtitre plates were coated with 100 µl per well of anti-TGFβ1 monoclonal antibody diluted in carbonate coating buffer (concentration 1 µl/ml), which binds soluble TGFβ1. After incubation overnight at 4°C, TGFβ1 standard (Promega) or test samples (dilution 1:50) were added to the wells and reacted for 90 min at room temperature. The plates were then incubated with 1 µl/ml of biotinylated anti-TGFβ1 polyclonal antibody (Promega) for 2 h at room temperature and subsequently with 10 µl/ml of horseradish peroxidase (HRP) conjugated antibody for 2 h. The colour reaction was induced by the addition of substrate solution (tetramethylbenzidine/hydrogen peroxide) and was stopped with 100 µl 1 N HCl. An automated microplate reader was used to measure the optical density (OD) at a wavelength of 450 nm. Between each of these steps the plates were washed five times with TBST wash buffer. Baseline and acid-treated samples for each subject were analysed. For the measurements of naturally occurring active TGFβ1, serum or plasma samples were diluted 1:50 in the sample buffer provided. For total TGFβ1 levels, an aliquot of the sample was diluted 1:5 in Dulbecco’s phosphate-buffere saline and treated with 1 N HCl for 15 min (obtaining a sample pH of approximately 2.6); subsequently samples were neutralized with 1 N NaOH to a pH of approximately 7.6. Acid-treated standard TGFβ1 supplied at a concentration of 1 µg/ml was used as a calibration standard at concentrations ranging from 1.56 to 100 ng/ml.

### Statistical analysis

Medians and interquartile ranges were used to express summary statistics. The Kruskal–Wallis test was used to compare outcome between several groups, and the Mann–Whitney test for differences between two groups. Active TGFβ1 data are skewed and a logarithmic transformation was carried out in order to normalize them. Linear association between variables was explored using the method of least squares. The square of the correlation coefficient, R, is reported and confidence intervals on the slope of the lines presented. Correlations between active TGFβ1 and disease duration and skin score were sought using log10 transformed active TGFβ1 values. Group differences between the detectable and

### Table 1. Characteristics of healthy controls and scleroderma patients

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>lcSSc</th>
<th>dcSSc</th>
<th>SSc (lcSSc + dcSSc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>22</td>
<td>20</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>Sex (female:male)</td>
<td>15:7</td>
<td>16:4</td>
<td>20:7</td>
<td>36:11</td>
</tr>
<tr>
<td>Age (yr) [median (range)]</td>
<td>43 (22–73)</td>
<td>55 (40–80)</td>
<td>57.5 (30–67)</td>
<td>57 (30–80)</td>
</tr>
<tr>
<td>Age (yr) [mean ± s.d.]</td>
<td>47.3 ± 16.3</td>
<td>56.1 ± 9.9</td>
<td>54.8 ± 9.3</td>
<td>55.4 ± 9.5</td>
</tr>
<tr>
<td>RP duration (yr) [median (range)]</td>
<td>NA</td>
<td>17.5 (2–50)</td>
<td>3.3 (1–35)</td>
<td>9 (1–50)</td>
</tr>
<tr>
<td>RP duration (yr) [mean ± s.d.]</td>
<td>20.8 ± 14.3</td>
<td>7.9 ± 9.5</td>
<td>14.3 ± 13.6</td>
<td></td>
</tr>
<tr>
<td>SSC duration (yr) [median (range)]</td>
<td>NA</td>
<td>8 (3–20)</td>
<td>3.5 (0.5–15)</td>
<td>6 (0.5–20)</td>
</tr>
<tr>
<td>SSC duration (yr) [mean ± s.d.]</td>
<td>NA</td>
<td>10.3 ± 5.7</td>
<td>4.9 ± 4.5</td>
<td>7.2 ± 5.6</td>
</tr>
<tr>
<td>ANA positive</td>
<td>0</td>
<td>19</td>
<td>26</td>
<td>45</td>
</tr>
<tr>
<td>ACA positive</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Anti-Scl-70 positive</td>
<td>0</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
</tbody>
</table>

RP, Raynaud’s phenomenon; ANA, antinuclear antibodies; ACA, anticytomegolomer antibodies; NA, not applicable.
undetectable concentrations of TGFβ1 were tested using χ² test. Differences were considered significant if \( P \leq 0.05 \).

Results

Demographic and clinical features of study cohort

These are summarized in Tables 1 and 2. The healthy control group was younger than both patient groups \( (P = 0.001) \), but lcSSc and dcSSc groups were age-matched. Gender differences were ignored in all analyses as there was a 36:11 F:M ratio in the study cohort, reflecting the expected female preponderance for SSC. Five of 27 patients with dcSSc and seven of 20 patients with lcSSc had isolated pulmonary hypertension. Thirteen of 27 dcSSc patients and eight of 20 lcSSc patients had lung fibrosis. There was a non-linear relationship between skin score and time from diagnosis for the dcSSc group; \( (R^2 = 0.4995, P < 0.00006) \) reflecting the fact that in the first few years of the disease skin scores are at their highest.

Circulating TGFβ1 levels

The median, range and interquartile range of the serum TGFβ1 concentrations (µg/l) are shown in Table 3. Single patient measurements are shown in Fig. 1a and b. Total levels of TGFβ1 were detected in all HC and SSC samples. We did not detect circulating levels of active TGFβ1 in 7/22 of HC, 7/20 of lcSSc and 18/27 of dcSSc \( (\chi^2 = 7.39, P < 0.025) \). The samples with undetectable levels of TGFβ1 were analysed for the second time at lower dilution (1:10) and the levels remained undetectable in all samples. Whilst total TGFβ1 levels were marginally higher in dcSSc than lcSSc or HC, these differences did not reach significance \( (P < 0.08 \text{ HC vs dcSSc}) \). The values for active TGFβ1 were skewed and therefore a logarithmic transformation (log10) was carried out. Zero values, evenly spread across the groups \( (n = 5 \text{ in HC, n = 6 in lcSSc and n = 4 in dcSSc}) \) were tested using a log10 circulating serum active TGFβ1 levels and disease duration \( (R^2 = 0.198, P < 0.04) \). There was a positive correlation between TGFβ1 and disease duration in lcSSc \( (R^2 = 0.229, P < 0.05) \) but not in dcSSc (Fig. 3). Confidence intervals were calculated for the slopes of those plots in Figs 2 and 3, where a significant relationship was found. For active TGFβ1 in in dcSSc vs skin score, the slope was \(-0.038 \) [confidence interval \( (CI) = -0.072 \) to \(-0.004) \]. The slopes for active TGFβ1 in dcSSc vs disease duration was \( 0.093/\text{yr} (CI 0.111 \) to \( 0.174) \) and total TGFβ1 in lcSSc vs disease duration was \( 3.10 \text{µg/ml/yr} (CI 0.40 \) to \( 5.80) \). No correlation was found between total TGFβ1 and skin score (Fig. 3). Also, no correlation was found between the circulating levels of either active TGFβ1 or total TGFβ1 and disease activity or severity scores in both dcSSc and lcSSc. No difference in concentration of either active TGFβ1 or total TGFβ1 was observed when patients were categorized as those with PHT or those without PHT as well as those with or without lung fibrosis. Finally, there was no correlation between active or total TGFβ1 and age in any of the groups (HC, lcSSc and dcSSc).

Discussion

In this study we have used a high-sensitivity enzyme-linked immunosorbent assay (ELISA) to measure circulating levels of TGFβ1 in patients with SSC and in healthy controls (HC). We have determined and compared active and total TGFβ1 levels; the latter obtained after serum acidification, and compared then from the final analysis and from the graphical representation of the data (Fig. 1a). The Kruskal–Wallis test was significant \( (P < 0.01) \) and dcSSc was significantly different from HC \( (P < 0.01) \) and lcSSc \( (P < 0.02) \). For comparison, plasma TGFβ1 levels reflected those of contemporary serum samples but were, on average, 30% lower than in serum in all groups, which is in keeping with published reports [16].

In dcSSc (but not in lcSSc), there was a negative correlation between skin score and log10 circulating serum active TGFβ1 \( (R^2 = 0.194, P < 0.04) \) and also a positive correlation between log10 circulating serum active TGFβ1 levels and disease duration \( (R^2 = 0.198, P < 0.04) \) (Fig. 2). There was a positive correlation between total serum TGFβ1 and disease duration in lcSSc \( (R^2 = 0.229, P < 0.05) \) but not in dcSSc (Fig. 3). Confidence intervals were calculated for the slopes of those plots in Figs 2 and 3, where a significant relationship was found. For active TGFβ1 in in dcSSc vs skin score, the slope was \(-0.038 \) [confidence interval \( (CI) = -0.072 \) to \(-0.004) \]. The slopes for active TGFβ1 in dcSSc vs disease duration was \( 0.093/\text{yr} (CI 0.111 \) to \( 0.174) \) and total TGFβ1 in lcSSc vs disease duration was \( 3.10 \text{µg/ml/yr} (CI 0.40 \) to \( 5.80) \). No correlation was found between total TGFβ1 and skin score (Fig. 3). Also, no correlation was found between the circulating levels of either active TGFβ1 or total TGFβ1 and disease activity or severity scores in both dcSSc and lcSSc. No difference in concentration of either active TGFβ1 or total TGFβ1 was observed when patients were categorized as those with PHT or those without PHT as well as those with or without lung fibrosis. Finally, there was no correlation between active or total TGFβ1 and age in any of the groups (HC, lcSSc and dcSSc).

Table 2. Scleroderma activity and severity scores [20, 21]

<table>
<thead>
<tr>
<th>Organ involvement</th>
<th>lcSSc</th>
<th>dcSSc</th>
<th>SSc (lcSSc + dcSSc)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y/N</td>
<td>Y/N</td>
<td>Y/N</td>
</tr>
<tr>
<td>General</td>
<td>8/11</td>
<td>12/15</td>
<td>20/27</td>
</tr>
<tr>
<td>Peripheral vascular</td>
<td>19/0</td>
<td>19/0</td>
<td>20/27</td>
</tr>
<tr>
<td>Skin</td>
<td>16/13</td>
<td>16/13</td>
<td>16/13</td>
</tr>
<tr>
<td>Joint/tendon</td>
<td>26/0</td>
<td>26/0</td>
<td>26/0</td>
</tr>
<tr>
<td>Muscle</td>
<td>13/6</td>
<td>13/6</td>
<td>13/6</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>11/10</td>
<td>11/10</td>
<td>11/10</td>
</tr>
<tr>
<td>Lung</td>
<td>11/10</td>
<td>11/10</td>
<td>11/10</td>
</tr>
<tr>
<td>Heart</td>
<td>3/16</td>
<td>3/16</td>
<td>3/16</td>
</tr>
<tr>
<td>Kidney</td>
<td>1/18</td>
<td>1/18</td>
<td>1/18</td>
</tr>
<tr>
<td>Severity score</td>
<td>7/22</td>
<td>7/22</td>
<td>7/22</td>
</tr>
</tbody>
</table>

Scleroderma activity is scored between 0–10; active disease is denoted by a score ≥ 3. The severity of organ involvement is scored between 0 and 4, with 0 denoting no involvement. The median and range shown is for all patients in a group. Scleroderma severity score ranges from 0 to 36.

Table 3. Serum TGFβ1 concentrations

<table>
<thead>
<tr>
<th>TGFβ1 (µg/l)</th>
<th>Healthy controls (n = 22)</th>
<th>lcSSc (n = 20)</th>
<th>dcSSc (n = 27)</th>
<th>SSc (lcSSc + dcSSc) (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active</td>
<td>Total</td>
<td>Active</td>
<td>Total</td>
</tr>
<tr>
<td>Median</td>
<td>1.23</td>
<td>71.1</td>
<td>1.59</td>
<td>67.6</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–20.75</td>
<td>14.0–238.4</td>
<td>0.00–8.01</td>
<td>13.6–152.8</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.11–3.87</td>
<td>45.6–121.7</td>
<td>0.00–4.30</td>
<td>58.8–98.8</td>
</tr>
</tbody>
</table>
with clinical features. We found low or undetectable levels of active TGFβ1 in early dcSSc, which correlated inversely with skin score. In contrast, levels of active TGFβ1 in lcSSc did not differ from HC; there were no significant differences in total TGFβ1 between the groups. The presence of substantial levels of active TGFβ1 in HC in this study and in other reports [15–17] suggests that it is not detrimental and raises the possibility that TGFβ1 may be important, for example, as a modulator of immune cell function. Low serum levels of active TGFβ1 in early dcSSc and the inverse correlation with modified Rodnan skin score have clinical relevance in the context of the evaluation of the extent of skin sclerosis. Skin score constitutes a key clinical parameter for assessing disease severity and predicting prognosis: severe skin involvement has been associated with poor prognosis, decreased survival and a greater risk of developing scleroderma renal crisis, pulmonary fibrosis and myocardial disease [25].

The biological significance of our results should be interpreted in the context of the physiology of TGFβ1. Important potential sources of circulating TGFβ1 are activated macrophages, T lymphocytes, platelets and endothelial cells. Active TGFβ1 is generated from latent TGFβ1 complex at sites of injury by a combination of proteolysis and low pH. In human serum TGFβ1 is bound covalently by alpha-2 macroglobulin (α2M) in an inactive form; the interaction of TGFβ1 with α2M accounts for the latency of serum TGFβ1 [26]. It has been suggested that the majority of serum TGFβ in the form of a covalent complex with α2M may represent TGFβ targeted for clearance via the α2M receptor whereas the non-covalent complex might reflect a protected serum pool of potentially active TGFβ to be carried and released at target sites [26]. Active TGFβ1 is rapidly cleared by mechanisms which include binding to α2M, decorin and soluble TGFβ receptors, followed by its clearance via the liver [2, 27, 28]. In the context of systemic sclerosis, in vitro protein and mRNA expression studies have revealed that SSc fibroblasts do not
secrete more TGFβ than normal cells [29]; it has been shown that overexpression of either type I or type II TGFβ receptors significantly increases alpha2(I) collagen promoter activity in transient transfection assays in dermal fibroblasts [30]. Addition of anti-TGFβ/α antibody abolished the stimulatory effect of receptor overexpression on collagen promoter activity. Comparison of TGFβ receptor type I and type II mRNA expression levels in SSc and normal fibroblasts have shown elevated (2-fold) expression of both receptor types in SSc cells, which correlated with increased binding of TGFβ and with elevated alpha2(I) collagen mRNA levels [30, 31].

A plausible explanation for our findings is that increased binding sites or altered turnover of active TGFβ1 may reduce the level of active cytokine in dCSSc. There are several reports suggesting increased expression of TGFβ receptors in SSc [30–32]. In addition, a number of potential TGFβ-interacting proteins such as fibrillin or decorin are present at elevated levels in SSc, and extracellular matrix binding sites are likely to be increased [33–35]. Whether this is a primary pathogenetic process or a bystander phenomenon is unclear. However, it is interesting to speculate that in the active phase of early dcSSc and in cases with extensive skin involvement, an increase in cell surface and extracellular matrix TGFβ binding sites may sequester active TGFβ1 as it becomes released from the latent complex. This may augment the matrix stimulating activity of TGFβ1 and explain why relatively low levels are detectable in SSc since bound TGFβ1 may not be readily accessible for immunolocalization; low circulating level of TGFβ1 may reduce endogenous immunosuppressive activity and contribute to the increased immunological activity in early dcSSc that is a hallmark of this disease subset.

Previous studies of circulating serum or plasma TGFβ levels in SSc [12–18] have generated discordant results. For example, Falanga and Julien [12] did not detect any difference in the levels of circulating plasma TGFβ between SSc patients and healthy controls; they reported, however, increased TGFβ binding to SSc fibroblasts in culture and immunocytochemically detectable TGFβ in inflammatory infiltrates from SSc skin biopsy samples, and it has recently been reported that mononuclear cells in dcSSc produce increased amounts of TGFβ1 in vitro [36]. Keystone et al [13] found elevated serum TGFβ in patients with dcSSc and lcSSc; correlations with clinical variables have not been analysed in this abstract. Higley et al [14] found no significant difference in plasma concentrations of active TGFβ1 between SSc patients and HC, using a low sensitivity ELISA assay (1 µg/l).

In the same study, significantly higher plasma levels were observed in patients with morphea when compared with HC and SSc; a similar trend of high values was also observed in patients with primary Raynaud’s. The authors reported technical difficulties in TGFβ activation so total levels of TGFβ1 were not estimated. Snowden et al [15] measured active TGFβ levels in plasma from 39 SSc, nine primary Raynaud’s phenomenon patients and 60 HC: active TGFβ1 was detected in six out of 39 SSc patients only and in none of the other samples. No significant correlation was found between the concentrations of TGFβ1 and the pattern of cutaneous or visceral involvement, or the levels of N-terminal peptide of type III procollagen (PIIINP). Giacomelli et al [16] looked for any correlation between the in vitro spontaneous and phytohemagglutinin (PHA)-induced production of TGFβ1 by peripheral blood mononuclear cells (PBMC) of SSc patients and total serum and plasma levels of TGFβ1 in the same SSc subject and in HC. No significant difference in total TGFβ1 levels in plasma, serum or supernatants from unstimulated and stimulated cultures of PBMC was found. Sato et al [17] measured serum levels of TGFβ1 in 32 SSc patients and 20 controls using a commercial ELISA kit (R&D); it is unclear whether the authors measured active or total levels of TGFβ1. TGFβ1 was found in all
samples, with no difference between SSC and HC; no correlation between serum TGF/1 and modified Rodman skin score was found in that study. Finally, Choi et al. [18], who found increased serum levels of vascular endothelial growth factor (VEGF) in SSC, reported positive correlation of VEGF with serum TGF/1 levels. No numerical TGF/1 concentrations or clinical correlations are described in that paper. In summary, marked variability between the studies is likely to reflect disease heterogeneity, different methodologies employed to study TGF/1 levels and bioactivity and the complex nature of TGF/1 secretion and activation. Differences in study designs, samples, techniques, antibodies and lack of consensus in describing clinical details make any attempt at systematic review or meta-analysis of these data complicated. Finally, although a contemporaneous evaluation of the active TGF/1 levels in the affected SSC skin and its serological measurements appears attractive, this approach has some limitations, in particular substantial technical issues concerning the reliable detection of TGF/1 ligand in lesional skin and the discrimination between active and latent forms of this growth factor. In fact a number of experiments have been performed with various results [12, 14, 30].

There are no conclusive data in the literature on any correlation between TGF/1 serum concentrations and age in both healthy subjects and various disease groups. Grainger et al. [37] showed no correlation between active TGF/1 and age in either healthy controls or subjects with atherosclerosis, whereas Stefoni et al. [38] reported a weak negative correlation between total TGF/1 and age in healthy subjects and in patients on haemodialysis. The latter study did not, however, evaluate active TGF/1. In our study we found no correlation between either active or total TGF/1 levels and age; our healthy controls were slightly younger than scleroderma patients and yet their serum TGF/1 levels were lower than those found in the dcSSc group, which would be in contrast with previous findings [38]. Also, any potential age effect would not negate our findings of lower active TGF1 in dcSSc compared with lcSSc as these groups were very well matched for age.

Difficulties in accurate measurements of active TGF/1 are related to its complex regulation and short half-life in biological fluids (2–3 min). Latent TGF/1 is present in large amounts in platelets, and therefore inaccurate sample handling may lead to the release and activation of latent TGF/1 into plasma from platelets during separation and storage or, in case of serum, during clotting, leading to falsely high concentrations of active TGF/1. In fact, significantly higher levels of TGF/1 are found in the serum when compared with plasma, which may reflect the release of TGF/1 from activated platelets during clot formation [16, and our data]. Therefore, careful and quick sample preparation is mandatory for reliable serological assays of TGF/1. Finally, antibodies not specific for active 25kDa TGF/1 peptide have been used in older assays, detecting also latent TGF/1; at present, highly sensitive and specific immunoassays for active 25kDa TGF/1 are being developed.

In summary, detailed assessment and comparison with clinical features may further elucidate the role of circulating pro-fibrotic factors as markers or mediators of disease in SSC. The potential significance of low levels of TGF/1 and the relevance of our findings to systemic TGF/1-trapping strategies in early stage dcSSc justify further study, including prospective longitudinal assessment of circulating levels of active TGF/1 in SSC.

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