Circulating Gremlin-1 is elevated in systemic sclerosis patients

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Abstract
Introduction: Systemic sclerosis is an autoimmune connective tissue disease in which there is activation of the immune system, vascular disease and fibrosis. Activation of quiescent fibroblasts to myofibroblasts is key to disease pathogenesis. Gremlin-1 is a bone morphogenetic protein antagonist which is important in development and we recently reported in skin fibrosis. The aim of this study was to determine the serum circulating levels of Gremlin-1 in early diffuse systemic sclerosis.

Methods: Twenty-one early diffuse systemic sclerosis patients (less than 2 years from first non-Raynaud’s symptom) were included and age and sex-matched healthy controls. Serum was isolated from blood and measured with a specific enzyme-linked immunoassay for Gremlin-1. Clinical variables were also measured.

Results: Significantly elevated Gremlin-1 was found in sera of early diffuse systemic sclerosis patients (p < 0.001). In patients with interstitial lung disease, this compared to systemic sclerosis without evidence of interstitial lung disease, Gremlin-1 was significantly elevated (p < 0.0007). A correlation was found between circulating Gremlin-1 and modified Rodnan Skin Score, albeit weak.

Discussion: In early diffuse systemic sclerosis patients, elevated Gremlin-1 is found in serum. This is particularly prominent in systemic sclerosis–associated interstitial lung disease. This suggests that Gremlin-1 may be a biomarker for systemic sclerosis interstitial lung disease.

Keywords
Gremlin-1, bone morphogenetic protein, lung fibrosis, scleroderma, transforming growth factor-beta

Introduction
Systemic sclerosis (SSc) is an idiopathic autoimmune connective tissue disease which is characterised by vascular abnormalities, inflammation and fibrosis.1,2 The disease is likely initiated by a vascular injury, leading to inflammation and finally fibrosis. The fibrosis primarily affects the skin and can affect the lungs.3 The extent of skin thickening and organ involvement can delineate this disease into two subtypes: limited cutaneous SSc in which only limited to discrete areas of the skin and diffuse SSc in which there is larger involvement of the skin and internal organs. Currently, no treatment is licenced for the skin fibrotic element of the disease, although recent treatments have been licenced for lung disease associated with SSc, such as nintedanib. Activation of quiescent fibroblasts to activated myofibroblasts that secrete copious amounts of extracellular matrix is at the core of the disease, and understanding of how these cells are activated remains elusive.

Gremlin-1 is a bone morphogenetic protein (BMP) antagonist that is required for embryogenesis.4 Gremlin-1 is part of the transforming growth factor (TGF)-β family and acts to block by sequestering soluble BMPs. BMPs have to be tightly regulated, and thus, Gremlin-1 is one such protein that regulates this.4,5 Recently, Gremlin-1 has been found to be associated with kidney,6,7 lung,8,9 and skin fibrosis.10 We described very recently that overexpression of Gremlin-1 led to increased myofibroblast transition that was partially dependent on TGF-β1 signalling, as blockade of TGF-β1 mitigated fibrosis.11 Analysis of SSc fibroblasts compared to controls did not find elevated
levels of Gremlin-1. The aim of this study is to determine the levels of Gremlin-1 in the sera of SSc patients with early diffuse SSc disease.

**Methods**

Twenty-one patients with early diffuse SSc were involved in the study; this is a retrospective study in a single-centre study. Patients were defined as early diffuse SSc defined as <2 years since the first non-Raynaud’s symptom. All patients fulfilled the American College of Rheumatology (ACR) criteria for a diagnosis of diffuse SSc and full informed consent was provided. The study has full ethical approval with the local research ethics committee (REC) with approval no. REC/13/NE/0089 and followed the declaration of Helsinki guidelines. Healthy controls (HCs) were age and gender matched (n = 20). 15 mL blood was drawn from each donor arm, and serum was isolated by centrifugation at 2000g for 15 min.

**ELISA**

A commercially available enzyme-linked immunosorbent assay (ELISA) was used purchased from Reagent Genie (Dublin, Republic of Ireland). This recognises human Gremlin-1. A standard curve was constructed from known amounts of Gremlin-1, and data were calculated from this curve.

Data were tested for normality using the Kolmogorov–Smirnov test of normality. Normal distribution as determined by the Kolmogorov–Smirnov test, Student’s t-test was undertaken. Student’s t-test was used to test differences between HC and SSc patients with a p-value < 0.05 considered statistically significant. The Mann–Whitney U test was used to compare differences between SSc without lung disease and those with. For correlation analysis, Pearson’s correlation r value was calculated using Prism™ software package.

**Results**

Twenty-one early diffuse SSc patients were included in this study with 20 HCs. Table 1 gives the patients’ demographics. Of the SSc patients, 5 had interstitial lung disease (ILD) and 16 did not have any evidence of ILD. Diffusing capacity of the lung for carbon monoxide (DLCO)% predicted of 60% or less was used to define ILD. No patient was receiving any treatment medications.

Gremlin-1 levels in SSc were significantly higher compared to HCs (Figure 1): mean = 1.14 ng/mL (standard deviation (SD) = 0.7) versus mean = 14.32 ng/mL (SD = 5.1), n = 21, p < 0.0001; Student’s t-test.

Patients with SSc-associated ILD had significantly elevated serum Gremlin-1 levels (p = 0.0007; Mann–Whitney U test; Figure 2).

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**Table 1.** SSc diffuse patient demographics.

| Patient number | Age (years) | Sex | Autoantibodies | mRSS (1) | Treatment | ILD | DLCO% |
|----------------|-------------|-----|----------------|----------|-----------|-----|-------|
| Patient_1      | 48          | F   | Scl-70         | 9        | None      | N   | 85    |
| Patient_2      | 54          | F   | Scl-70         | 16       | None      | N   | 82    |
| Patient_3      | 51          | F   | RNA-polIII     | 10       | None      | N   | 89    |
| Patient_4      | 66          | F   | Scl-70         | 11       | None      | N   | 73    |
| Patient_5      | 39          | F   | Scl-70         | 12       | None      | N   | 79    |
| Patient_6      | 52          | F   | Scl-70         | 10       | None      | N   | 87    |
| Patient_7      | 41          | M   | Scl-70         | 14       | None      | Y   | 57    |
| Patient_8      | 49          | F   | Scl-70         | 17       | None      | Y   | 52    |
| Patient_9      | 55          | F   | RNA-polIII     | 21       | None      | N   | 91    |
| Patient_10     | 42          | F   | Scl-70         | 14       | None      | N   | 75    |
| Patient_11     | 57          | M   | Scl-70         | 19       | None      | N   | 82    |
| Patient_12     | 35          | F   | Scl-70         | 11       | None      | N   | 76    |
| Patient_13     | 47          | F   | Scl-70         | 15       | None      | N   | 74    |
| Patient_14     | 61          | F   | Scl-70         | 21       | None      | Y   | 50    |
| Patient_15     | 55          | F   | Scl-70         | 18       | None      | N   | 78    |
| Patient_16     | 48          | F   | Scl-70         | 22       | None      | Y   | 52    |
| Patient_17     | 51          | F   | Scl-70         | 16       | None      | N   | 77    |
| Patient_18     | 39          | F   | RNA-polIII     | 12       | None      | N   | 93    |
| Patient_19     | 42          | F   | Scl-70         | 26       | None      | N   | 70    |
| Patient_20     | 37          | M   | Scl-70         | 19       | None      | N   | 74    |

ILD: interstitial lung disease; DLCO: carbon monoxide lung diffusion capacity % predicted.

1 mRSS is the modified Rodnan Skin Score based on 1–51.

![Figure 1](image1.png)

![Figure 2](image2.png)
We next sought to ascertain if there is a correlation between sera Gremlin-1 levels and skin thickness using the modified Rodnan Skin Score (mRSS); a weak negative correlation was found between Gremlin-1 amounts and mRSS ($r = -0.466; p = 0.033, n = 21$) (see Figure 3). This suggests a weak inverse correlation.

Discussion

We have recently described a pro-fibrotic role of Gremlin-1 in dermal fibrosis in SSc. The aim of this study was to assess the circulating levels of Gremlin-1 in early diffuse SSc. We identified significantly elevated levels of Gremlin-1 compared to HCs. Furthermore, we also demonstrated significantly elevated levels of Gremlin-1 in SSc-associated ILD compared to patients without ILD.

Gremlin-1 is a BMP antagonist that regulates BMP signalling, but it is associated with certain tumours including breast cancer, and fibrosis of the kidney, lungs and we recently described the skin as a target cell type. Overexpression of Gremlin-1 led to a significant increase in Extra cellular matrix (ECM) and cell migration; however, we did not see elevated levels of Gremlin-1 in the fibroblasts. This study sought to identify if SSc early diffuse patients had elevated Gremlin-1 levels. We found among the 21 SSc patients elevated Gremlin-1, and in the patients subdivided into those with ILD and those without, we could see significantly elevated levels of Gremlin-1. This is suggestive of a biomarker for ILD disease associated with SSc.

Given that SSc-associated ILD is responsible for the high mortality associated with the disease, this could be of possible clinical utility to differentiate patients. Tissue Gremlin-1 has been associated with IPF, and in IPF cell, Gremlin-1 has been found to be fibrotic and regulated by microRNA27b. Furthermore, overexpression of Gremlin-1 in rat lungs by adenoviral vector results in lung fibrosis. Interestingly, in sarcoidosis, the associated development of fibrosis in these patients was associated with a specific polymorphism in Gremlin-1 gene. Most recently, although serum levels of Gremlin-1 have been found to be significantly elevated compared to controls and also patients with idiopathic interstitial pneumonia also had higher serum Gremlin-1 levels. This all suggests that...
Gremlin-1 could be used a clinical biomarker. We particularly focused on early diffuse SSc patients as we had previously found an induction with IL-6 trans signalling and with IL-6 being such an important molecule in early disease we focussed on this. It is now accepted that interventions in early disease are the best possible way to modify disease course. Interestingly, Gremlin-1 has also been found to be critically involved in hereditary pulmonary hypertension in mice and patients with hereditary pulmonary hypertension. It is possible that there is an interrelationship between IL-6 and Gremlin-1 levels. Given that IL-6 appears to regulate Gremlin-1 in vitro, it maybe that high IL-6 serum correlates with Gremlin-1 levels, although in this study we did not measure IL-6. The patients were early diffuse and thus likely to be much more ‘inflammatory’ subsets so one would predict high IL-6 levels.

We also analysed a possible association with skin thickness (by mRSS) and serum Gremlin-1 levels and found a weak negative correlation ($r = -0.466$). This is a relatively weak correlation, and the modestly likely reflects the number of patients. The biological relevance of this, if any, remains unclear to the authors. In summary, we demonstrate elevated levels of the pro-fibrotic morphogen Gremlin-1 and that this is particularly marked in SSc patients with ILD. Whether Gremlin-1 can predict disease progression is currently unknown and we did not perform a prospective study. This should be examined in prospective follow-up studies to determine if it can predict disease. We recently demonstrated that inhibition of Gremlin-1 with small interfering RNA in fibroblasts retarded collagen deposition, suggesting that Gremlin-1 is a therapeutic target in this disease.

Declaration of conflicting interests
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References
1. Varga J and Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007; 117(3): 557–567.
2. Dowson C, Simpson N, Duffy L, et al. Innate immunity in systemic sclerosis. *Curr Rheumatol Rep* 2017; 19(1): 2.
3. Denton CP and Khanna D. Systemic sclerosis. *Lancet* 2017; 390(10103): 1685–1699.
4. Stafford DA, Brunet LJ, Khokha MK, et al. Cooperative activity of noggin and gremlin 1 in axial skeleton development. *Development* 2011; 138(5): 1005–1014.
5. Brazil DP, Church RH, Surrue S, et al. BMP signalling: agony and antagony in the family. *Trends Cell Biol* 2015; 25(5): 249–264.
6. McMahon R, Murphy M, Clarkson M, et al. IHG-2, a mesangial cell gene induced by high glucose, is human gremlin. Regulation by extracellular glucose concentration, cyclic mechanical strain, and transforming growth factor-$\beta$. *J Biol Chem* 2000; 275(14): 9901–9904.
7. Li G, Li Y, Liu S, et al. Gremlin aggravates hyperglycemia-induced podocyte injury by a TGF/$\beta$/smad dependent signaling pathway. *J Cell Biochem* 2013; 114(9): 2101–2113.
8. Koli K, Mylläriemi M, Vuorinen K, et al. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. *Am J Pathol* 2006; 169(1): 61–71.
9. Worrell JC, Walsh SM, Fabre A, et al. CXCR3A promotes the secretion of the antifibrotic decoy receptor sIL-13R$\alpha$2 by pulmonary fibroblasts. *Am J Physiol Cell Physiol* 2020; 319(6): C1059–C1069.
10. O’Reilly S, Ciechomska M, Cant R, et al. Interleukin-6 (IL-6) trans signaling drives a STAT3-dependent pathway that leads to hyperactive transforming growth factor-$\beta$ (TGF-$\beta$) signaling promoting SMAD3 activation and fibrosis via Gremlin protein. *J Biol Chem* 2014; 289(14): 9952–9960.
11. Duffy L, Henderson J, Brown M, et al. Bone morphogenetic protein antagonist Gremlin-1 Increases Myofibroblast transition in dermal fibroblasts: implications for systemic sclerosis. *Front Cell Dev Biol* 2021; 9: 681061.
12. Farkas L, Farkas D, Gauldie J, et al.Transient overexpression of Gremlin results in epithelial activation and reversible fibrosis in rat lungs. *Am J Respir Cell Mol Biol* 2011; 44(6): 870–878.
13. Yin M, Tissari M, Tamminen J, et al. Gremlin-1 is a key regulator of the invasive cell phenotype in mesothelioma. *Oncotarget* 2017; 8(58): 98280–98297.
14. Ren J, Smid M, Iaria J, et al. Cancer-associated fibroblast-derived Gremlin 1 promotes breast cancer progression. *Breast Cancer Res* 2019; 21(1): 109.
15. Mayes MD, Lacey JV Jr, Beebe-Dimmer J, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003; 48(8): 2246–2255.
16. Graham JR, Williams CM and Yang Z. MicroRNA-27b targets gremlin 1 to modulate fibrotic responses in pulmonary cells. *J Cell Biochem* 2014; 115(9): 1539–1548.
17. Heron M, van Moorsel CH, Grutters JC, et al. Genetic variation in GREM1 is a risk factor for fibrosis in pulmonary sarcoidosis. *Tissue Antigens* 2011; 77(2): 112–117.
18. Aoshima Y, Enomoto Y, Muto S, et al. Gremlin-1 for the treatment of subcutaneous fibrosis in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* 2016; 387(10038): 2630–2640.
19. Cahill E, Costello CM, Rowan SC, et al. Gremlin plays a key role in the pathogenesis of pulmonary hypertension. *Circulation* 2012; 125(7): 920–930.