Serum IGFBP-2 in Systemic Sclerosis as a Prognostic Factor of Lung Dysfunction

Julien Guiot
CHU de Liege - Hopital du Sart Tilman Pneumologie-Allergologie
Makon-Sébastien Njock (ms.njock@chuliege.be)
CHU de Liege - Hopital du Sart Tilman https://orcid.org/0000-0001-8137-1978
Béatrice André
CHU de Liege - Hopital du Sart Tilman
Fanny Gester
CHU de Liege - Hopital du Sart Tilman Pneumologie-Allergologie
Monique Henket
CHU de Liege - Hopital du Sart Tilman Pneumologie-Allergologie
Dominique de Seny
CHU de Liège: Centre hospitalier universitaire de Liege
Catherine Moermans
CHU de Liege - Hopital du Sart Tilman Pneumologie-Allergologie
Michel G Malaise
CHU de Liège: Centre hospitalier universitaire de Liege
Renaud Louis
CHU de Liege - Hopital du Sart Tilman Pneumologie-Allergologie

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Abstract

Background: Systemic sclerosis (SSc) is a rare connective tissue disease associated with rapid evolving interstitial lung disease (ILD), driving its mortality. Specific biomarkers associated with the progression of this lung disease are highly needed. We aimed to identify specific biomarkers of SSc-ILD to predict the evolution of the disease.

Methods: We compared prospectively serum levels of several biomarkers associated with lung fibrosis in SSc patients (n=102), among which SSc-no ILD (n=63) and SSc-ILD (n=39), compared to healthy subjects (HS) (n=39). We also performed a longitudinal study in a subgroup of 28 patients analyzing biomarkers variations and pulmonary function tests over a period of 2 years.

Results: Serum level of IGFBP-2 was significantly increased in SSc patients compared to HS, and negatively correlated with pulmonary function (assessed by carbon monoxide transfer coefficient (KCO)) ($r=-0.29$, $p<0.01$). Two-year longitudinal analysis in a subgroup of 28 SSc patients determined that IGFBP-2 variation was positively correlated with KCO at 2-year follow-up ($r=0.6$, $p<0.001$). SSc patients with a lower variation of IGFBP-2 (less than 22%) presented significant degradation of pulmonary function at 2-year follow-up ($p<0.01$). ROC curve analysis enabled us to identify that baseline IGFBP-2 > 105 ng/ml was associated with a poor outcome (KCO <70% predicted) at 2-year follow-up (AUC=0.75, $p<0.05$).

Conclusions: We showed for the first time that serum levels of IGFBP-2 might be a prognostic factor of the development of SSc-ILD. Indeed, initial level of IGFBP-2 above 105 ng/ml was associated with a poor patient's outcome (KCO <70% predicted) two years later.

Introduction

Systemic sclerosis (SSc) is a complex systemic disease of unknown origin associated with a multi-organic affection involving a complex interplay of microvasculopathy, disturbances in fibroblastic function and abnormalities of the immune system [1–3]. While any organ may be involved in the disease process, pulmonary complications of SSc, including interstitial lung disease (ILD) and pulmonary hypertension (PH), remain one of the major causes of morbidity and mortality in the disease [4–7]. Indeed, ILD and PH represent together 60% of SSc-related deaths [8]. SSc-ILD have many common clinical and pathological characteristics with some other major ILDs, mainly idiopathic pulmonary fibrosis (IPF) [9–12]. Lung fibrosis is present in approximately 25% of SSc patients [13]. Contrary to what is seen in IPF [14], treatment is mainly based on an aggressive immunosuppressive therapy specifically proposed in the progressive forms of SSc-ILD [15, 16]. One of the major problem clinicians have to deal with is to identify patients with increased risk of ILD progression for early intervention [6, 17–20]. In this context, prognostic biomarkers are highly needed in order to help clinicians to predict ILD development and provide adequate treatment.

To date, the most frequently used diagnostic biomarkers for SSc are serum autoantibodies. Indeed, more than 90% of SSc patients harbor antinuclear antibodies (ANA) in their serum [21–23]. Some of these are

highly specific for SSc, including anti-Scl-70 (also called anti-topoisomerase I) and anti-centromere (anti–CENP-B) antibodies [24, 25]. Although ANA are historical biomarkers available for Ssc, they are not able to predict the occurrence of ILD. Several serum biomarkers, including surfactant protein-D (SP-D) [26, 27], Krebs Von Den Lungen 6 (KL-6) [28, 29] and chemokine ligand-18 (CCL18), have been associated with SSc-ILD. Furthermore, transforming growth factor beta (TGF-β) is known to be involved in the pathophysiology of many lung fibrotic diseases by stimulating the deposition of collagen and increasing lung remodeling [30, 31]. Besides TGF-β, previous studies identified that insulin-like growth factor-binding proteins (IGFBPs) were also clearly associated with IPF and of interest as new potential biomarkers for SSc-ILD [32, 33].

The aim of our study was to quantify serum level of several SSc- and IPF-associated growth factors in SSc patients in order to identify novel biomarkers to predict the occurrence of ILD.

Methods

Subject characteristics

In this study, we prospectively recruited patients with SSc (SSc-ILD and SSc-no ILD) and healthy subjects (HS) from our ambulatory care policlinic at CHU Liege. The blood of the patients was collected at time of diagnosis of SSc in our center. The diagnosis of SSc was made according to the international recommendations of ACR/Eular [3]. SSc-ILD was defined by a combination of specific HRCT images of at least 10% of all parenchyma (reticulations, honey combing and/or ground glass opacities) with clinical signs (velcros or crackels) or symptoms (cough, shortness of breath) and alteration of pulmonary function tests. We excluded all other causes of ILD (such asbestosis, IPF, idiopathic non specific interstitial pneumonia, hypersensitivity pneumonitis or toxic pneumonitis). All cases were validated after a multidisciplinary discussion in order to confirm the presence or absence of SSc-ILD. Then, we performed a longitudinal study, resampling blood 2 years after the first analysis (n = 28). HS were recruited by advertisement in our policlinic waiting room. They all denied any respiratory disease and had normal spirometric values with FEV1 > 80% predicted and FEV1/FVC ratio > 70%. The impact of maintenance of immunosuppressive drugs on cell count and biomarker levels was not relevant in our study. The protocol was approved by the ethics committee of CHU of Liège, and all subjects gave written consent before their enrollment (Belgian number: B707201422832 ; ref : 2014/302).

Pulmonary function tests

All tests were performed according to the recommendations of the European Respiratory Society (ERS). The results were expressed in percent predicted. The total lung capacity (TLC) was measured by body plethysmography and expressed in percent predicted. The diffusion capacity of CO (DLCO) and the report DLCO/AV (alveolar volume) were measured by the single-breath carbon monoxide gas transfer method and expressed in percent predicted (SensorMedics2400He /CO Analyzer System, Bilthoven, Netherlands).

Biomarkers measurements in serum

Levels of Interleukin (IL)-8, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-7, Chitinase-3-like protein 1 (YKL-40), IGFBP-1 and IGFBP-3 were assessed by ELISA multiplex using Fluorokine-1. Multianalyte Profiling Kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The detection limit for this assays were 3-3-200-230-170-705 pg/ml respectively. The concentration of the other proteins were measured separately by ELISA: TGF-β, MMP-9, IGF-1, IGFBP-2 (DuoSet kit, R&D systems); The detection limits for these kits were 7-25-25-32 pg/ml respectively.

Statistical analysis

Demographic and functional data were expressed as mean ± standard deviation (SD). The biomarkers levels were expressed as median (IQR). Comparisons between groups were performed by Dunn's test of multiple comparisons following a significant Kruskal-Wallis test, or by Mann-Witney or unpaired "t" test (according to the distribution of the variable) for pairwise comparison. Correlations between variables were performed using Spearman's rank correlation test. A $p < 0.05$ was considered as significant. Statistical analysis and graph were performed with Prism Graph Pad software® v6. San Diego.

Results

Study population, patient characteristics, and clinical data

We prospectively recruited patients with SSc (SSc-no ILD, n = 63; SSc-ILD, n = 39) from our ambulatory care policlinic at CHU Liege and compared them to healthy subjects (HS) (n = 39) (Fig. 1). Demographic, functional and treatment characteristics of the subjects are given in Table 1. The average age of patients compared to HS was similar. FEV1 was moderately lowered in the SSc-no ILD and SSc-ILD groups compared to HS ($p < 0.05$ and $p < 0.05$, respectively). SSc-ILDs present lower levels of FEV1, FVC, TLC and DLCO compared to SSc-no ILD patients ($p < 0.05$; $p < 0.001$; $p < 0.001$ and $p < 0.001$, respectively). Of note, 30% of patients were receiving maintenance treatment with immunosuppressive drugs and 27% were receiving oral corticosteroids.

Table 1
Demographic and clinical characteristics of HS and SSc patients.

	HS (n = 39)	SSc-no ILD (n = 63)	SSc-ILD (n = 39)
Age, yrs	59 ± 10	55 ± 12	61 ± 12
Gender (M/F)	13/26	17/46	8/31
BMI, Kg/m²	26 ± 4	25 ± 4	25 ± 5
Smokers (NS/FS/S)	17/16/6	31/17/15	23/11/05
Paq-year	12 ± 18	10 ± 14	6 ± 12
Haemoglobin	-	13.93 ± 1.98	12.98 ± 2.94
FEV1 post-BD, %pred.	106 ± 18	99 ± 21°	88 ± 22°*
FVC post-BD, %pred.	112 ± 18	105 ± 19	90 ± 22***
FEV1/FVC post-BD, %pred.	78 ± 6	78 ± 10	81 ± 8
TLC, %pred.	-	100 ± 15	85 ± 18***
DLCO, %pred.	-	72 ± 20	57 ± 17***
KCO, %pred.	-	79 ± 19	76 ± 16
Immunosupressor (yes/no)	-	16/47	15/24
OCS (yes/no)	-	19/44	9/30
PAH/asthma (%)		4.8/0	13/2.5
UIP/NSIP/mixed pattern			2.5/10/7.5
Disease duration (y)	-	7.2 ± 8.5	6.3 ± 7.0
Rodnan skin score	-	3.4 ± 5.1	4,8 ± 6.9
ACR/Eular score	-	10.5 ± 5.6	13.7 ± 9.45
Limited SSc/lcSSc/dSSc/ SS	-	20/34/4/2	11/17/6/1

Data are expressed as mean ± SD. dSSc: diffuse cutaneous SSc; DLCO: Diffusion lung capacity for CO; FEV1: Forced expired volume in one second; FS: Former smoker; FVC: Forced Vital Capacity; HS: healthy subjects; ILD: Interstitial lung disease; IT: Immunosuppressive therapy (Mycophenolate Mofetil, methotrexate, cyclophosphamide); KCO: The carbon monoxide transfer coefficient; lcSSc: limited cutaneous SSc; NS: Non smoker; NSIP: Nonspecific interstitial pneumonia; OCS: Oral corticosteroid; PAH: Pulmonary arterial hypertension; S: smoker; SS: Sine scleroderma; SSc: Systemic sclerosis; TLC: Total lung capacity; UIP: usual interstitial pneumonia.

°$p < 0.05$ compared to HS

*$p < 0.05$ and ***$p < 0.001$ compared to SSc-no ILD

	HS (n = 39)	SSc-no ILD (n = 63)	SSc-ILD (n = 39)
Musculoskeletal involvement (%)	-	9	16
Renal crisis (%)	-	4	0
Cardiac involvement (%)	-	2	4

Data are expressed as mean ± SD. dSSc: diffuse cutaneous SSc; DLCO: Diffusion lung capacity for CO; FEV1: Forced expired volume in one second; FS: Former smoker; FVC: Forced Vital Capacity; HS: healthy subjects; ILD: Interstitial lung disease; IT: Immunosuppressive therapy (Mycophenolate Mofetil, methotrexate, cyclophosphamide); KCO: The carbon monoxide transfer coefficient; lcSSc: limited cutaneous SSc; NS: Non smoker; NSIP: Nonspecific interstitial pneumonia; OCS: Oral corticosteroid; PAH: Pulmonary arterial hypertension; S: smoker; SS: Sine scleroderma; SSc: Systemic sclerosis; TLC: Total lung capacity; UIP: usual interstitial pneumonia.

°$p < 0.05$ compared to HS

*$p < 0.05$ and ***$p < 0.001$ compared to SSc-no ILD

Serum biomarkers at baseline

First, we compared the levels of different serum biomarkers associated with lung fibrosis (IGF-1, IGFBP-1, IGFBP-2, IGFBP-3, TGF-β, IL-8, TNF-α, YKL-40, MMP-7, MMP-9 and CRP) between HS and SSc groups (Fig. 2). There is a significant increase in IGFBP-1 (8 to 12.9 ng/ml, $p < 0.05$), IGFBP-2 (83 to 117 ng/ml, $p < 0.001$), IL-8 (3.6 to 9.3 pg/ml, $p < 0.001$), MMP-9 (412 to 967 ng/ml, $p < 0.001$) and CRP (0.7 to 2.1 mg/l, $p < 0.001$) levels in SSc patients compared to HS (Fig. 2b, c, e, f and see additional file 1: Table S1). Of note, IGF-1 and IGFBP-3 were significantly reduced SSc patients compared to HS (13 to 8.9 ng/ml, $p < 0.05$; and 806 to 694 ng/ml, $p < 0.05$, respectively) (Fig. 2a, d).

Then, we compared the levels of serum biomarkers between the two subgroup of SSc patients (SSc-ILD *vs* SSc-no ILD) and HS (Table 2). There is a significant increase of the levels of IGFBP-2, IL-8 and MMP-9, as well as a decrease of IGFBP-3 in SSc-no ILD and SSc-ILD patients compared to HS. Of note, the level of IGFBP-1 was only increased in SSc-no ILD patients compared to HS ($p < 0.05$). On the other side, the level of IGF-1 was reduced and CRP increased only in SSc-ILD patients compared to HS ($p < 0.05$ and $p < 0.001$, respectively). Then, we focused our analysis on the difference between SSc-no ILD and SSc-ILD. Interestingly, we observed a significant reduction of the levels of IGFBP-1 and IGFBP-3 in SSc-ILD compared to SSc-no ILD ($p < 0.01$ and $p < 0.05$, respectively*)*. We did not find any significant relation between biomarkers and therapies at baseline (immunosuppressive agent or systemic corticosteroids).

Table 2
Concentrations of serum biomarkers in subgroups of SSc patients (SSc-no ILD and SSc-ILD) and HS.

	HS (n = 39)	SSc-no ILD (n = 63)	SSc-ILD (n = 39)
IGF-1 (ng/ml)	13 (8–17)	8.9 (5.3–15.3)	9 (5.2–15.8)°
IGFBP-1 (ng/ml)	8 (3–16)	15 (7–25)°	5.8 (2.2–15.8)**
IGFBP-2 (ng/ml)	83 (51–109)	113 (69–145)°	132 (85–213)°°°
IGFBP-3 (ng/ml)	806 (675–926)	740 (598–877)°°°	656 (479–758)°*
ratio IGF-1/IGFBP-1	5 (3–15)	2.2 (0.7–6.2)°°°	5.3 (1.4–20.6)**
ratio IGF-1/IGFBP-2	0.7 (0.4–1.4)	0.4 (0.2–0.8)°	0.3 (0.2–0.9)°°°
ratio IGF-1/IGFBP-3	0.1 (0 -0.1)	0.05 (0.03–0.09)	0.07 (0.04–0.1)
TGF-B (ng/ml)	26 (24–31)	29 (24–34)	29 (22–35)
IL-8 (pg/ml)	3.6 (1.5 -7)	9.3 (3.8–17.4)°°°	11 (6–19)°°°
TNF (pg/ml)	1.5 (1.5–1.5)	1.5 (1.5–1.5)	1.5 (1.5–1.5)
YKL40 (ng/ml)	33 (24–49)	37 (19–60)	46 (22–64)
MMP-7 (ng/ml)	1.7 (1.4 -2)	1.7 (1–2.9)	2.4 (1.4–3.9)
MMP9 (ng/ml)	412 (221–818)	796 (413–1292)°	1183 (482–1575)°°°
CRP (mg/l)	1.2 ± 1.4	3.5 ± 4	6.4 ± 9.1°°°

Data are expressed as median (interquartile range). CRP: C-Reactive Protein; HS: healthy subjects; IGF-1: Insuline like growth factor-1; IGFBP-1,-2,-3: Insuline-like growth factor − 1,-2,-3; IL-8: Interleukin-8; MMP-7,-9: Metalloproteinase-7 and − 9; SSc: Systemic sclerosis; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosing factor-α; YKL-40: Chitinase-3-like protein 1.

°$p < 0.05$ and °°°$p < 0.001$ compared to HS

*$p < 0.05$ and **$p < 0.01$ compared to SSc-no ILD

Correlation between serum biomarkers and pulmonary function tests at baseline

We performed correlation analysis to assess whether biomarkers were associated with pulmonary function tests at baseline. IGFBP-2 was negatively correlated with alveolo-capillar function assessed by carbon monoxide transfer coefficient (KCO) (r=-0.29, p < 0.01) (Fig. 3). In addition, YKL-40 was also negatively correlated with Forced Vital Capacity (FVC) as well as the Diffusion Lung capacity for CO (DLCO) (r=-0.31, p < 0.01 and r=-0.24, p < 0.05 respectively) (see additional file 2: Table S2).

Longitudinal analysis on serum biomarker variations and pulmonary function tests

To assess whether the variation over the time of the levels of serum biomarkers was associated with pulmonary function declines, we performed a longitudinal study in a subgroup of 28 SSc patients analyzing biomarkers variations and pulmonary function tests over a period of 2 years (Fig. 1). Demographic and biological characteristics of SSc patients at baseline and after 2 years are given in Table 3.

Table 3
Demographic and biological characteristics of SSc patients at baseline and 2-year follow-up.

	Baseline SSc (n = 28)	**2 years follow-up SSc (n = 28)**
Age, yrs	57 ± 12	59 ± 12
Gender (M/F)	22/6	22/6
BMI, Kg/m²	24 ± 4	24 ± 4
Smokers (NS/ES/S)	14/10/5	14/10/5
Paq-year	0 (0–26)	1 (0–26)
FEV1 post-BD, %pred.	100 ± 20	98 ± 19
FVC post-BD, %pred.	103 ± 20	101 ± 18
TLC %pred.	95 ± 16	94 ± 13
DLCO %pred.	66 ± 17	65 ± 13
KCO %pred.	81 ± 14	73 ± 12***
ILD yes/no	8/20	11/17
IT yes/no	10/18	10/18
OCS yes/no	5/23	5/23
Data are expressed as mean (SD). DLCO: Diffusion lung capacity for CO; FEV1 = Forced expired volume in one second; FS: Former smoker; FVC: Forced Vital Capacity; ILD: Interstitial lung disease; IT: Immunosuppressive therapy (Mycophenolate Mofetil, methotrexate, cyclophosphamide); KCO: The carbon monoxide transfer coefficient; NS: Non smoker; OCS: Oral corticosteroid; S: smoker; SSc: Systemic sclerosis; TLC: Total lung capacity.		
****p* < 0.001 compared to Baseline		

The 2-year longitudinal analysis of pulmonary function revealed that KCO was significantly reduced (Baseline: 81 (± 14) % and 2-year 73 (± 12) %, $p < 0.001$) (Fig. 4a). Next, we performed analysis to determine if pulmonary function decline was associated to the variation of serum biomarkers (see additional file 3: Table S3). Interestingly, we found a positive correlation between the variation of IGFBP-2 and KCO at 2-year follow-up ($r = 0.6$, $p < 0.001$) (Fig. 4b).

Then, we investigated if IGFBP-2 could predict the progression of SSc disease. First, SSc patients were divided into two groups: patients with higher or lower variation of IGFBP-2 (ΔIGFBP-2 $\geq$ or $\leq$ 22%). Interestingly, SSc patients with a lower variation of IGFBP-2 (less than 22%) presented significant degradation of pulmonary function at 2-year follow-up (Baseline: 77 (± 12) % and 2-year follow-up: 66 (± 9) %, $p < 0.01$), whereas the ones with higher variation of IGFBP-2 (more than 22%) conserved their pulmonary function (Fig. 4c). Furthermore, baseline level of IGFBP-2 was elevated in the subgroup of SSc patients with lower variation of IGFBP-2 (less than 22%) compared the ones with higher variation of IGFBP-2 (more than 22%) (Fig. 4d). ROC curve analysis enabled us to identify that baseline IGFBP-2 of 105ng/ml discriminate the two subgroup of SSc patients (AUC = 0.75 at 80% sensibility and 75% specificity, $p = 0.028$) (Fig. 3E). Indeed, baseline IGFBP-2 $\geq$ 105 ng/ml was associated with a poor patient's outcome at 2-year follow-up (KCO < 70% predicted) (Fig. 3F). These results suggest that serum level of IGFBP-2 (105 ng/ml) might predict the evolution of SSc disease.

Discussion

SSc is a complexe multi-organ disorder with heterogeneous clinical features. As the diagnosis of SSc-ILD is complex, there is a need to develop novel biomarkers to identify early patients in order to deliver more appropriate treatment. Here, we quantified serum level of several SSc- or IPF-associated growth factors in SSc patients compared to HS. SSc patients featured a marked increase in serum levels of IGFBP-1, IGFBP-2, IL-8, MMP-9 and CRP whereas IGF-1 and IGFBP-3 were significantly reduced compared to HS. Of interest, IGFBP-2 was negatively correlated to KCO at baseline. Two-year longitudinal analysis determined that IGFBP-2 variation was positively correlated with the KCO measurement. Of great interest, initial levels of IGFBP-2 above 105 ng/ml were associated with a poor patient's outcome 2 years later (KCO < 70% predicted), suggesting that serum levels of IGFBP-2 might predict the evolution of SSc-ILD.

In previous studies, we have identified that IGFBP-2 was positively associated with lung fibrosis in serum and induced sputum of IPF patients [32, 34]. Moreover, IGFBP-2 was reduced in IPF patients receiving anti-fibrotic therapy, although serum levels remained higher in IPF patients than in HS [32]. Other studies on lung fibrosis identified a significant increase of IGFBP-2 in bronchoalveolar lavage (BAL) fluid and in lung tissue of ILDs without focusing on systemic sclerosis [35]. In this study, we showed that patients suffering from SSc exhibited higher levels of IGFBP-2 than HS, but to a lesser extent than patients suffering from IPF (as previously shown in one of our study [32]). Of interest, we demonstrated that level variation of IGFBP-2 was associated with the severity of lung dysfunction. Indeed, baseline serum level of IGFBP-2 above 105 ng/ml allows identifying patients with a poor prognosis at 2-year follow-up (KCO < 70% predicted). This interesting observation suggests the potential prognostic value of baseline IGFBP-2

to identify SSc patients with risk of rapid evolution. Integrating new biomarkers in the follow up of SSc-ILD is challenging taking into account the variability of other clinical markers like symptoms, CRP, DLCO or FVC. Moreover, it is suitable to avoid repeated chest imaging in the follow-up of the patients to limit as much as possible irradiation. The use of serum biomarker IGFBP-2 could be a good candidate to predict the progression of SSc-ILD and need to be explored.

In our study, serum levels of TGF-β were similar for all groups even though TGF-β is widely known to be associated with the pathophysiology of fibrosing lung disease [36]. Similarly, our previous study focusing on IPF did not find any difference in TGF-β levels between HS and patients suffering from IPF leading to the conclusion that serum TGF-β is not a good biomarker of lung fibrosis [19].

YKL-40 was negatively correlated with pulmonary function tests in our study (FEV1, FVC, DLCO). Confirming previous studies, we identified that YKL-40 is associated with the lung function impairment of patients suffering from SSc [37–39]. Therefore, these observations need further explorations to see whether YKL-40 could act as a predictor of lung degradation for patients with SSc.

IL-8 was also increased in our study in SSc patients. IL-8 is known to be a strong chemotactic agent for neutrophils and can impact the pathophysiological process of SSc by recruiting neutrophils in lungs [40, 41]. Of interest, it should be noted that blood neutrophils were increased in SSc patients compared to HS. Furthermore, several studies have shown that patients with SSc-ILD patients have elevated levels of pro-inflammatory cytokines such as interleukin IL-8, IL-6, TNF-α in BAL fluid and serum [6, 42, 43]. In the same line, MMP-9 was also increased in SSc context. MMP-9 is known to be actively secreted by neutrophils [44, 45], which are increased in SSc patients.

Conclusions

Among all the molecules that we studied, only serum level of IGFBP-2 was able to predict the occurrence of ILD in SSc patients. Indeed, serum IGFBP-2 above 105 ng/ml might be a prognostic factor of alveolo-capillary dysfunction. We need to validate those results in a larger longitudinal trial to confirm the clinical value of these observations.

Abbreviations

ANA: Antinuclear Antibodies

BRDU: Bromodeoxyuridine

CRP: C-Reactive Protein

DLCO: Diffusion lung capacity for CO

FEV1: Forced expired volume in one second

FVC: Forced vital capacity

IGF-1: Insuline like growth factor-1

IGFBP-1,-2,-3: Insulin like Growth Factor Binding Protein -1,-2,-3

IL-8: Interleukin-8

ILD: Interstitial lung disease

IPF: Idiopathic pulmonary fibrosis

IQR: Interquartile range

KCO: The carbon monoxide transfer coefficient

MMP-7,-9: Matrix metalloproteinase -7 and -9

Pro-Col I: Pro-collagen type I

SD: Standard deviation

SSc: Systemic sclerosis

SSc-ILD: Systemic sclerosis associated interstitial lung disease

TGF-β: Transforming growth factor β

TLC: Total lung capacity

TNF-α: Tumor necrosing factor α

YKL-40: Chitinase-3-like protein 1

Declarations

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Availability of data and materials

The data underlying this article are available in the article and in its online Additional Files. Further inquiries will be shared on reasonable request to the corresponding author.

Ethics approval and consent to participate

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Competing interests

The authors have declared no conflicts of interest. MH is employee of Belgian Volition SPRL.

Consent for publication

Not applicable.

Authors' contributions

JG, M-SN, RL, MGM designed the study and coordinated the research. JG, FG and MH contributed to collect samples, and carried out the clinical evaluation of patients. M-SN, MH, BA, CM and DDS performed experiments and analysed the datas. JG and M-SN drew figures and wrote the manuscript. All authors reviewed the final version of the manuscript. All authors read and approved the final version of the manuscript. JG, M-SN, RL, MGM guaranty the integrity of the work as a whole, from inception to published article.

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Figures

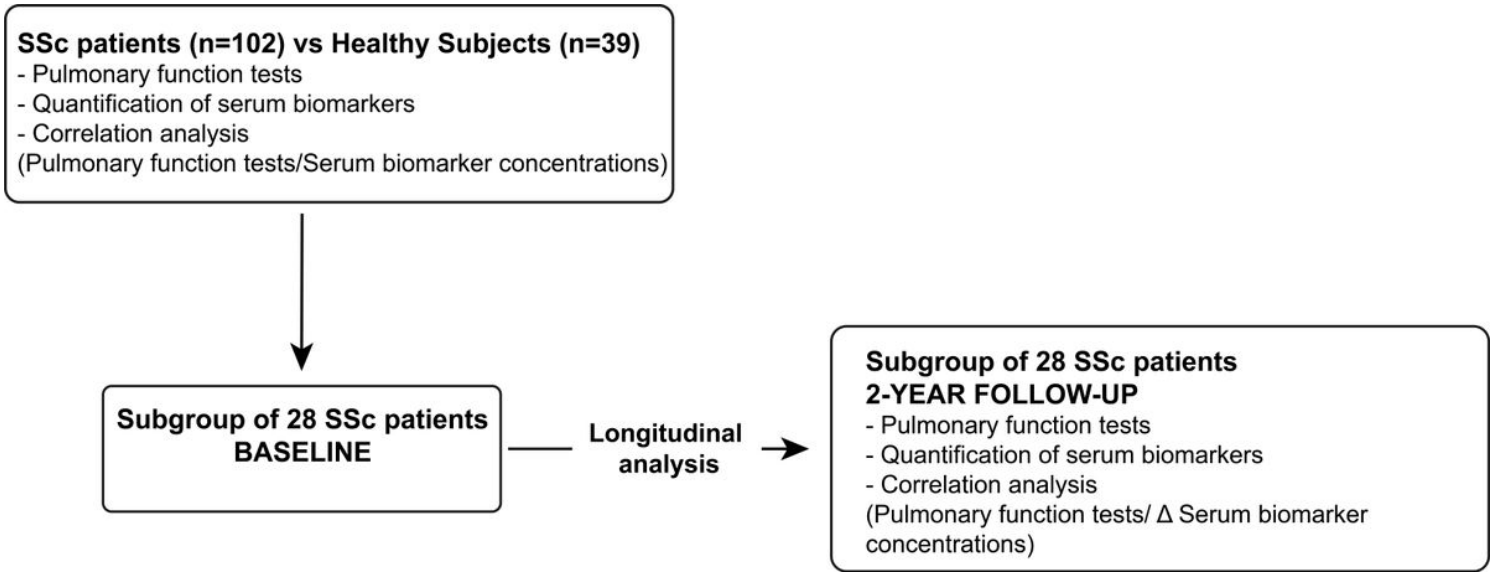

Figure 1

Study design.

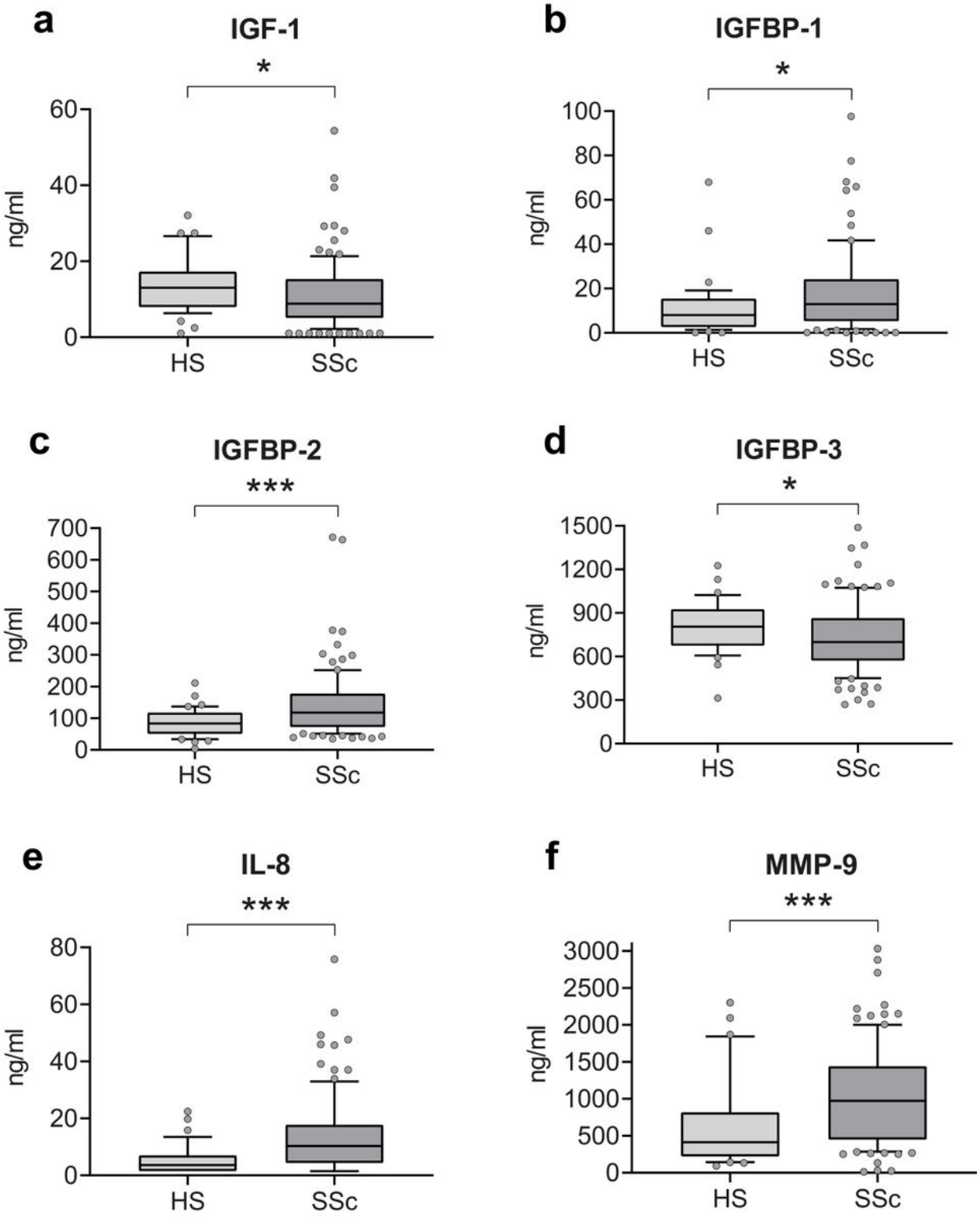

Figure 2

Serum biomarkers in SSc patients compared to HS. Comparison of the concentration of a IGF-1, b IGFBP-1, c IGFBP-2, d IGFBP-3, e IL-8 and f MMP-9 in SSc patients and HS. Data are expressed as median (IQR – CI 90%). * $p<0.05$ ** $p<0.01$ *** $p<0.001$ compared to HS. HS: healthy subjects; IGF-1: Insuline like growth factor-1; IGFBP-1 , -2, -3 : Insulin like Growth Factor Binding Protein -1,-2,-3; IL-8: Interleukin-8; MMP-9: Matrix metalloproteinase-9; SSc: Systemic sclerosis.

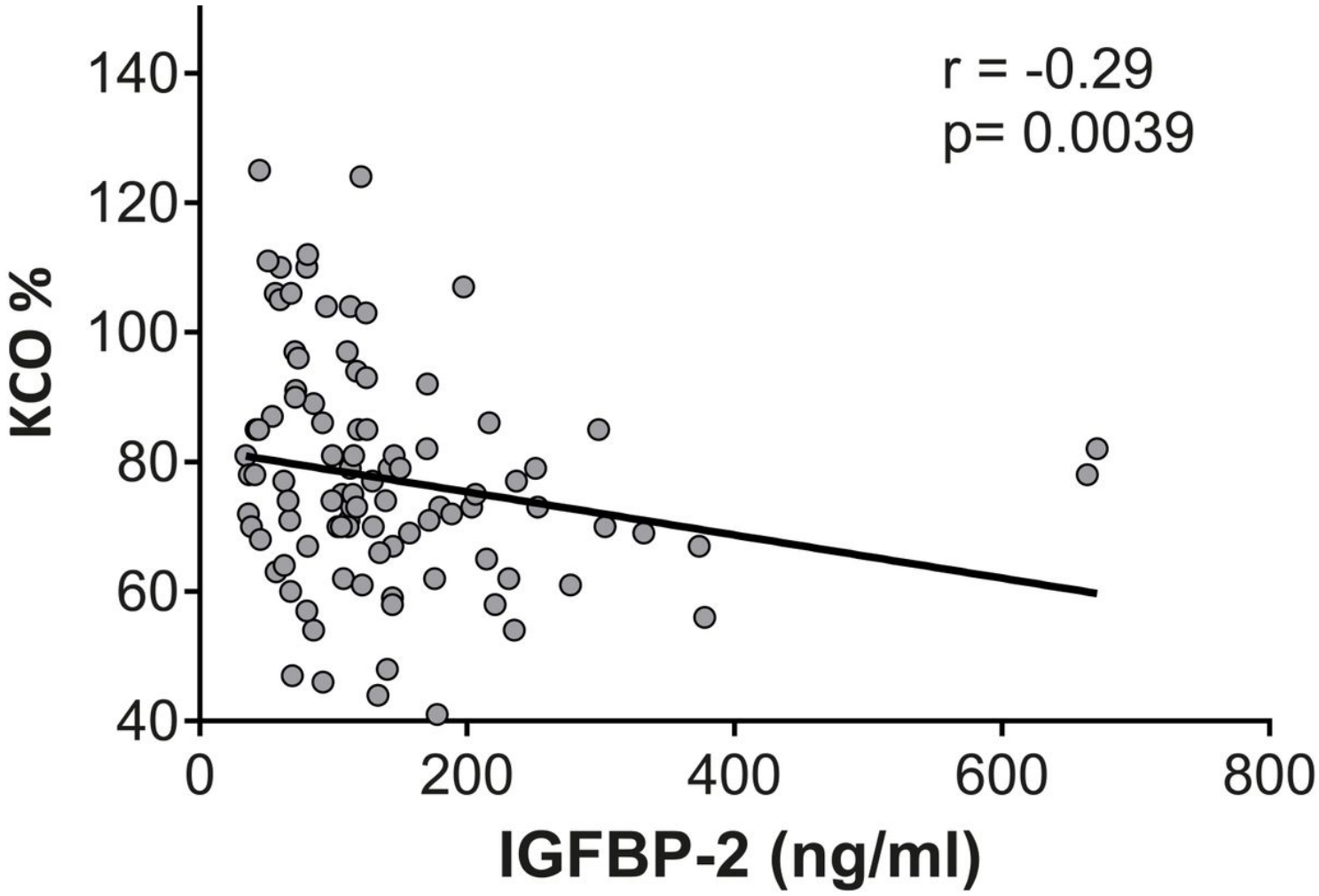

Figure 3

Correlation at baseline between KCO (% pred) and IGFBP-2 levels in SSc cohort. IGFBP-2: Insuline-like growth factor-2; KCO: The carbon monoxide transfer coefficient.

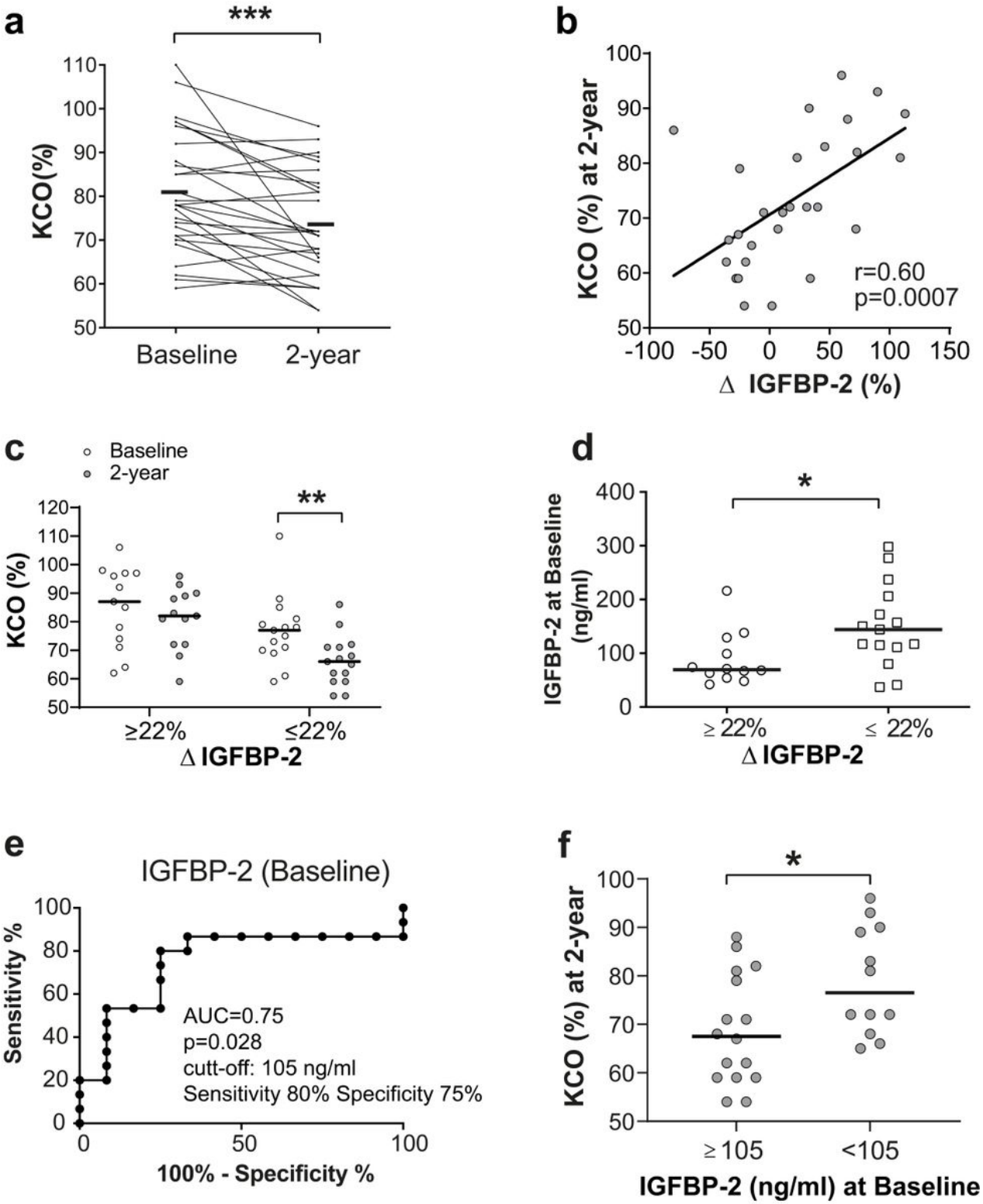

Figure 4

Identification of prognostic value of IGFBP-2 for the development of SSc-ILD. a KCO (%) variation in the 2-year longitudinal analysis. b Correlation study between the variation of IGFBP-2 (between baseline and 2-year follow-up) and KCO (at 2-year follow-up). c Longitudinal analysis of KCO (%) in SSc subgroups with higher or lower variation of IGFBP-2 (ΔIGFBP-2 ≥ or ≤ 22%). d Levels of baseline IGFBP-2 for SSc subgroups with higher or lower variation of IGFBP-2 (≥ or ≤ 22%). e ROC curve analysis to determine the

level of baseline IGFBP-2 which will enable to discriminates the two group of SSc patients (ΔIGFBP-2 ≥ and ≤ 22%). f KCO (%) at 2-year follow-up of SSc patients with presenting baseline IGFBP-2 higher or lower than 105 ng/ml. * p<0.05 **p<0.01 ***p<0.001. AUC: area under the curve; IGFBP-2: Insuline-like growth factor-2; KCO: The carbon monoxide transfer coefficient.

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