High serum levels of YKL-40 in patients with systemic sclerosis are associated with pulmonary involvement

C Nordenbæk[1], JS Johansen[1,2], P Halberg[1], A Wiik[3], C Garbarsch[4], S Ullman[5], PA Price[6], S Jacobsen[1,7]

[1]Department of Rheumatology, Hvidovre Hospital, [2]Department of Rheumatology, Herlev Hospital, [3]Department of Autoimmunology, Statens Serum Institute, Copenhagen, [4]Institute of Medical Anatomy, The Panum Institute, Copenhagen, [5]Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark, [6]Department of Biology, University of California San Diego, La Jolla, CA, USA, and [7]Department of Rheumatology, Rigshospitalet, Copenhagen, Denmark

Objectives: YKL-40, a growth factor of connective tissue cells, is elevated in sera from patients with diseases characterized by inflammation, tissue remodelling, or fibrosis. The aim of the study was to determine serum YKL-40 levels in patients with systemic sclerosis (SSc) and to explore any possible clinical and prognostic associations.

Methods: YKL-40 was measured in sera from 88 patients with SSc (26 with diffuse and 62 with limited skin involvement) and in sera from 88 matched healthy controls. Immunohistochemical staining for YKL-40 antigen was performed in a biopsy from a patient with pulmonary SSc.

Results: Serum YKL-40 levels of the SSc patients were significantly higher than those of the controls ($p < 0.00001$). Patients with pulmonary fibrosis by chest X-ray, obstructive ventilatory pattern, reduced diffusing capacity (DLco), and digital joint deformity due to skin retraction had significantly higher serum YKL-40 compared with patients without these findings. Patients with elevated serum YKL-40 had shorter survival times than patients with normal serum YKL-40 ($p = 0.0005$), although this was not independent of age and pulmonary function. YKL-40 protein expression was found in inflammatory cells in fibrosing pulmonary tissue from a patient with SSc.

Conclusions: Serum YKL-40 is elevated in patients with SSc with pulmonary involvement.

YKL-40, also known as human cartilage glycoprotein-39 (1), is a member of the 'mammalian chitinase-like proteins'. The gene (*CHI3L1*) for YKL-40 (2) and its crystallographic structure (3) are known, but the site and mode of binding to cell surface receptors have not yet been described. YKL-40 is a heparin and chitin-binding lectin (4) without chitinase activity (1, 4). It is produced by macrophages (4, 5), neutrophils (6), synovial cells, chondrocytes from arthritic joints (1), differentiating smooth muscle cells, hepatic stellate cells, and cancer cells. The precise biological function of YKL-40 is unknown, but it increases growth rates of fibroblastic cell lines derived from human osteoarthritic synovium, foetal lung and adult skin, and works synergistically with insulin-like growth factor (IGF)-1 in stimulating the growth of fibroblasts (7). YKL-40 also increases the number of chondrocytes and synovial cells and proteoglycan synthesis (8). Furthermore, YKL-40 is an adhesion and migration factor for vascular smooth muscle cells (9) and umbilical vein endothelial cells and modulates vascular endothelial cell morphology by promoting the formation of branching tubules. This indicates that YKL-40 may function in angiogenesis. Besides having biological effects, YKL-40 has been shown to be an autoantigen in rheumatoid arthritis.

Elevated serum YKL-40 levels are found in patients with a variety of diseases characterized by inflammation, remodelling of the extracellular matrix, and development of fibrosis, such as rheumatoid arthritis, osteoarthritis, giant cell arteritis, pneumonia, liver fibrosis, and different types of solid tumours (10, 11). The serum YKL-40 levels vary according to the disease activity of the patients and elevated serum YKL-40 is associated with a poor prognosis.

Systemic sclerosis (SSc) is an autoimmune disease characterized by initial inflammation followed by fibrotic changes in the skin, blood vessels, and several other organs. The pathogenesis of SSc is unclear. However, various growth factors seem to be involved in the fibrotic processes (12). Elevated serum YKL-40 is found in approximately 30% of patients with SSc and particularly in those with joint involvement (13). The aim of the present study was to assess

Søren Jacobsen, Department of Rheumatology, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.
E-mail: sj@dadlnet.dk

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whether serum YKL-40 in patients with SSc has clinical and prognostic associations.

Material and methods

Patients

Eighty-eight patients (median age 58 years, range 20–82) who fulfilled the American College of Rheumatology (ACR) preliminary criteria for the classification of SSc were included, and were part of a longitudinally followed population (14). The patients were selected according to the availability of serum samples collected during the follow-up. The median duration of cutaneous sclerosis was 7.2 years (range 0–39) and the median duration of follow-up after the time of blood sampling was 3.2 years (range 0–17). Skin involvement was diffuse in 26 (30%) patients and limited in 62 (70%). Fifty-five patients were (or had been) treated with penicillamine (300–1200 mg daily, median 750 mg). Skin involvement was classified according to the maximum cutaneous involvement by means of a two-subset model (limited and diffuse involvement; dividing lines: elbows and knees). Demographic data, signs, and symptoms (i.e. gender, age, duration of cutaneous involvement, years of follow-up after blood sampling, digital pitting scars, subcutaneous calcinosis, distal osteolysis, chronic polyarthritis, SSc-related deformities of the finger joints (due to retraction of skin and tendons), and the presence of anti-centromere and anti-Scl70 antibodies, pulmonary fibrosis) were registered at the time of blood sampling: Vital status was obtained from the Danish Central Person Register. Causes of death were based on information obtained from hospital charts, autopsy reports, and death certificates.

Pulmonary imaging and function tests

At inclusion, the patients had pulmonary function tests (PFTs) and routine chest X-ray examinations. The largest of three technically acceptable efforts was used to determine the forced expiratory volume during the first second (FEV1), inspiratory vital capacity (VC), and the ratio of FEV1/VC. The diffusing capacity of CO (DLco) was determined using a single breath helium, CO-dilution technique. The PFT results were transformed to standardized values calculated as percentages of the predicted values with respect to sex, age, and height. Three subsets of patients were delineated: (i) restrictive ventilatory pattern (VC $\leqslant$ 80% and FEV1/VC $\geqslant$ 70%), (ii) obstructive ventilatory pattern (FEV1/VC $<$ 70%), and (iii) DLco reduced to less than 70% of the expected values. Twenty-seven patients (31%) had radiographically demonstrated pulmonary fibrosis. The ventilatory pattern was restrictive in 17 (19%) patients, obstructive in 11 (13%), and low DLco was found in 39 (44%). In patients with pulmonary fibrosis the median values of VC and DLco were 76% and 56%, respectively, of the expected value.

Serological methods

Serum YKL-40 was determined by an in-house radioimmunoassay (RIA). An age- and sex-matched control population consisting of 70 women and 18 men (median age 58 years, range 21–79) was used to determine the cut-off limit of normal versus elevated serum YKL-40 (i.e. the 95th confidence limit of serum YKL-40 in healthy subjects = 275 μg/L).

Statistical analysis

Statistical analyses were performed by means of SPSS for Windows version 10 (SPSS Inc., Chicago, USA) and included the Mann–Whitney rank test, Fisher's exact test, stepwise conditional logistic regression analysis, and survival analyses by means of Kaplan–Meier plotting, log-rank statistics, and Cox regression analysis. Statistical significance was set at $p < 0.05$.

Results

Serum YKL-40 was higher in the patients (median 161 μg/L, quartiles 112–284 μg/L, 95th percentile 602, $p < 0.00001$) than in the controls (median 104 μg/L, quartiles 89–149 μg/L, 95th percentile 275). Twenty-seven per cent had elevated serum YKL-40 (i.e. $>$ the 95th percentile of the controls). Anticentromere antibodies (ACA) and antitopoisomerase-I antibodies (ATA) were found in 34 and 11 patients, respectively, and did not correlate with serum YKL-40.

Table 1 shows serum YKL-40 levels in relation to demographic and clinical features. Serum YKL-40 was significantly elevated in patients with pulmonary fibrosis, obstructive ventilatory pattern, SSc joint deformity, and old age ($>$ 60 years). Table 2 shows the demographic and clinical features of the 88 SSc patients stratified according to elevated or normal serum YKL-40. Elevated serum YKL-40 was significantly related to old age, obstructive ventilatory pattern, decreased vital capacity, and diffusing capacity. Elevated serum YKL-40 was not associated with restrictive ventilatory pattern. To evaluate independent contributions to elevated serum YKL-40, a logistic stepwise regression analysis of serum YKL-40 using the factors of Table 2 as independent variables was performed. The factors independently related to elevated serum YKL-40 included decreased values of DLco ($p = 0.005$) and decreased values of FEV_1/VC ($p = 0.05$).

Table 1. Serum concentrations of YKL-40 in 88 patients with systemic sclerosis according to the presence of selected demographic, clinical and serological features.

	Serum YKL-40 (μg/L)		
	Feature present	Feature absent	p*
Male sex (n=18)	224 (108–312)	156 (116–312)	0.38
Age >60 years (n=38)	220 (142–352)	136 (96–224)	0.004
Diffuse SSc (n=26)	155 (136–284)	170 (108–284)	0.95
Pitting scars (n=59)	188 (128–288)	136 (96–248)	0.19
Calcinosis (n=41)	188 (136–304)	146 (96–276)	0.07
Distal osteolysis (n=19)	154 (100–276)	162 (100–276)	0.75
Arthritis (n=7)	276 (156–312)	156 (108–272)	0.25
SSc joint deformity (n=61)	194 (136–288)	128 (80–224)	0.03
Pulmonary fibrosis (n=27)	222 (136–288)	152 (96–248)	0.03
Isolated reduced DLco (n=37)	152 (108–244)	183 (116–288)	0.62
Restrictive pattern (n=17)	222 (136–288)	156 (108–272)	0.36
Obstructive pattern (n=11)	284 (208–360)	154 (108–248)	0.02
Smoking (n=46)	162 (120–284)	161 (108–276)	0.65
Anti-centromere antibody (n=34)	158 (96–284)	167 (126–288)	0.81
Anti-topoisomerase antibody (n=11)	154 (80–288)	162 (116–284)	0.77

Values are medians (quartiles). *Mann–Whitney test.

Table 2. Demographic and clinical features stratified according to serum levels of YKL-40 in 88 patients with systemic sclerosis.

	High serum YKL-40 (>275 μg/L), n=24	Normal serum YKL-40 (⩽275 μg/L), n=64	
Binary variables	No. (%)	No. (%)	p*
Male sex	8 (33)	10 (16)	0.08
Smoking	11 (46)	31 (48)	0.98
Treated with penicillamine	13 (54)	42 (66)	0.46
Diffuse SSc	7 (29)	19 (39)	0.83
Arthritis	4 (17)	3 (5)	0.08
SSc joint deformity	19 (79)	42 (66)	0.33
Pitting scars	17 (71)	42 (66)	0.83
Calcinosis	12 (50)	29 (45)	0.88
Distal osteolysis	6 (25)	13 (20)	0.85
Pulmonary fibrosis	11 (46)	16 (25)	0.10
Restrictive ventilatory pattern	7 (29)	10 (16)	0.22
Obstructive ventilatory pattern	7 (29)	4 (6.3)	0.008
Isolated reduction of DLco	8 (33)	29 (45)	0.44
Continuous variables	Median (quartiles)	Median (quartiles)	p†
Age (years)	66 (54–71)	56 (44–67)	0.01
Disease duration (years)	7.8 (3.4–17)	6.9 (3.9–15)	0.86
Vital capacity (% of expected)	92 (70–105)	102 (86–117)	0.01
FEV_1/VC (%)	81 (69–85)	82 (77–87)	0.11
Diffusing capacity (% of expected)	58 (52–73)	74 (62–86)	0.003

*Fisher's exact test. †Mann–Whitney test.

Immunohistochemical analysis for YKL-40 protein expression in a lung biopsy with inflammation and severe fibrosis from a patient who died of SSc showed YKL-40 expression in macrophages and leucocytes in areas with inflammation (data not shown).

Twenty-two (25%) patients died during follow-up, and 11 of these had elevated serum YKL-40. Nine patients died of causes related to SSc [pulmonary fibrosis (six), renal crisis (one), and malabsorption (two)], and six of these had elevated serum YKL-40. The patients who died had higher serum YKL-40 (median 264 μg/L, quartiles: 188–352 μg/L) than the surviving patients (median 144 μg/L, quartiles: 96–244 μg/L, $p<0.001$). Patients with high serum YKL-40 had shorter survival from the time of the

blood sample than patients with normal serum YKL-40 (p=0.004) (Figure 1). When controlling for age, reduced vital capacity, and reduced diffusing capacity, patients with increased serum YKL-40 still had the poorest survival, although this was not statistically significant (p=0.1).

Discussion

We found elevated serum YKL-40 in 27% of patients with SSc, similar to other reports (13). SSc patients with various signs of pulmonary involvement had elevated serum YKL-40, and this was associated with features of obstructive lung disease and decreased vital and diffusing pulmonary capacity. The cellular abnormalities in SSc lungs often result in complex alterations of the lung function. Interstitial fibrosis, vascular lesions including fibrous thickening of the intima, media hypertrophy, and perivascular fibrosis may lead to a restrictive lung function pattern and/or impairment of the diffusing capacity. High serum YKL-40 was related to reduced DLco, possibly caused by interstitial and perivascular fibrosis. Interestingly, the present study shows that SSc patients with elevated serum YKL-40 had shorter survival than patients with normal serum YKL-40 and died more often due to extensive interstitial or vascular fibrosing processes (e.g. pulmonary fibrosis, SSc renal crisis). However, this association is partly explained by age, reduced vital capacity, and reduced diffusing capacity.

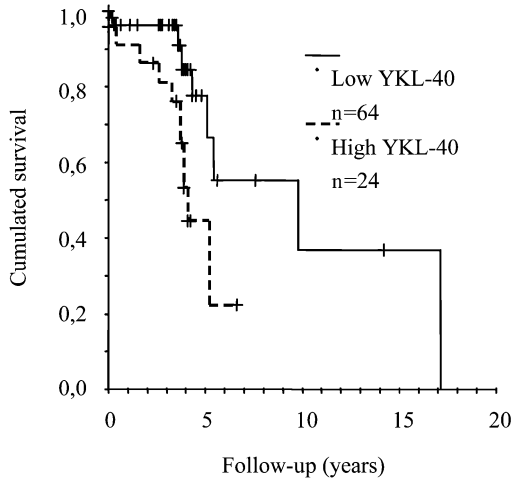

Figure 1. The impact of serum YKL-40 on overall survival of patients with systemic sclerosis. The patients were grouped by a high versus normal (age-adjusted) serum YKL-40. The strata are: 1 (—) patient having normal serum YKL-40 (i.e. ⩽275 μg/L); 2 (----) patients having elevated serum YKL-40 (i.e. >275 μg/L). The cut-off limit used for the determination of normal versus high serum YKL-40 was the 95th confidence limit of serum YKL-40 in healthy age-matched subjects. The p-value shown is for the log rank test for equality of strata.

Immunohistochemical analysis of a lung biopsy from an SSc patient with lung inflammation and fibrosis showed YKL-40 protein expression in macrophages and neutrophils in areas with inflammation. YKL-40 is a growth factor for fibroblasts (7), but it is not known whether YKL-40 plays a role in the pathogenesis of pulmonary fibrosis in patients with SSc. YKL-40 may actively take part in inflammation and tissue remodelling processes and has a role in pathological conditions leading to organ fibrosis. YKL-40 was one of the most differentially expressed genes in liver tissue with hepatitis C cirrhosis (15) and serum YKL-40 was elevated in most patients with moderate to severe liver fibrosis and cirrhosis, independently of disease aetiology (11) associated with shorter survival of patients with alcoholic liver disease compared to patients with normal serum YKL-40.

The precise role of YKL-40 in the pathogenesis of pulmonary fibrosis in SSc is still unknown and large prospective studies of patients with SSc are needed to determine any prognostic significance of high serum YKL-40.

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