Cross-Sectional Evaluation of YKL-40 Serum Concentrations in Patients with Systemic Sclerosis. Relationship with Clinical and Serological Aspects of Disease

GIOVANNI LA MONTAGNA, SALVATORE D'ANGELO, and GABRIELE VALENTINI

ABSTRACT. ***Objective.*** To investigate the behavior of serum YKL-40 in a cohort of patients with systemic sclerosis (SSc).

Methods. Forty SSc patients (35 women, 5 men) were investigated for serum YKL-40, soluble interleukin 2 receptor alpha (sIL-2Rα; by ELISA), von Willebrand factor (vWF; ELISA), and aminoterminal propeptide of type III procollagen (PIIINP; radioimmunoassay) concentrations. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were evaluated. Skin and organ system involvement were scored according to the Medsger organ/system severity scale.

Results. Serum YKL-40 in SSc patients (mean 132.9 ng/ml; median 75.5, 95% CI 87.8–175) was significantly higher than in controls (mean 66.6 ng/ml; median 52, 95% CI 54.6–78.6). Fourteen patients had levels > 135 ng/ml (cutoff value) with a mean of 264.7 ± 160 ng/ml. Serum YKL-40 values were found to be more frequently increased in patients with arthralgias/arthritis (10/18 cases) than in patients without such features (4/22) ($p = 0.021$). Significant differences were found comparing serum YKL-40 concentrations in the patients with or without joint involvement (median 138 vs 57.5 ng/ml, respectively; $p = 0.007$). Serum YKL-40 levels correlated with the joint involvement severity score ($p = 0.018$) and sIL-2Rα levels ($p < 0.001$). No differences were found with any therapeutic regimen.

Conclusion. This preliminary study shows that YKL-40 serum levels are increased in SSc and that they are correlated with sIL-2Rα and joint involvement, suggesting a relationship with cartilage and/or fibroblast activity. (J Rheumatol 2003;30:2147–51)

Key Indexing Terms:
YKL-40 SERUM LEVELS
JOINT INVOLVEMENT
SOLUBLE INTERLEUKIN 2 RECEPTOR
SYSTEMIC SCLEROSIS

Systemic sclerosis (SSc) is a connective tissue disorder characterized by microvascular damage and fibrosis involving the skin and target organs: lungs, heart, gut, and kidneys[1]. During the course of the disease, many patients develop rheumatic symptoms (arthralgias and myalgias). A few patients, with diffuse cutaneous SSc, develop symmetrical polyarthritis occurring early in the disease[2].

YKL-40 or human cartilage glycoprotein 39 (HC gp-39) is a major glycoprotein secreted by human chondrocytes and synovial fibroblasts[3,4]. Other human tissues such as liver, kidney, brain, and placenta have been found to express low concentrations of YKL-40 mRNA. In contrast, no YKL-40 mRNA has been found in preparations from either human embryonic lung fibroblast or human skin fibroblasts[5]. Nevertheless, YKL-40 has been reported to promote the growth of both skin and fetal lung fibroblasts[6] and to be a serological marker related to hepatic fibrosis[7,8].

Synovial and serum concentrations of YKL-40 are higher in patients with active rheumatoid arthritis (RA) and osteoarthritis (OA) than in controls[9-12], and it has been suggested as a surrogate marker for inflammatory and degenerative joint diseases.

We evaluated serum YKL-40 in a cohort of patients with SSc.

MATERIALS AND METHODS

Patients. Forty consecutive SSc patients, admitted 1999-2000 to our rheumatology unit, were studied, 35 women, 5 men aged 15 to 72 years (mean 48.4 ± 14.7; median 48). Disease duration ranged from 1 to 34 years (mean 12.3 ± 9.5; median 10), considering Raynaud's phenomenon as the onset manifestation of disease. All patients satisfied the American College of Rheumatology (previously American Rheumatism Association) preliminary criteria for the classification of SSc[13]. They were subgrouped into 3

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clinical subsets according to Giordano, *et al*[14], as limited cutaneous (lSSc) (including SSc sine scleroderma), intermediate cutaneous (iSSc), and diffuse cutaneous (dSSc). The patients were also divided in 3 serological subsets, i.e., anticentromere antibody (ACA) positive, anti-DNA topoisomerase I antibody (anti-Scl-70) positive; antinuclear antibody (ANA) positive, ACA negative; and anti-Scl-70 negative. These subsets was defined by measuring ANA on HEp-2 cells (cutoff level 1:40) and anti-Scl-70 by ELISA (cutoff level 20 EU/ml).

During the 6 months prior to study entry, all patients were treated with low doses of glucocorticoids (≤ 10 mg daily prednisone equivalent; 20 cases), d-penicillamine (5 cases), hydroxychloroquine (2 cases), and cyclophosphamide (12 cases). Other supportive drugs (prostacyclin agonists and other vasoactive drugs, angiotensin-converting inhibitors, prokinetics, anti H2-receptors, or omeprazole) were also used.

Thirty-four apparently healthy subjects (24 women, 10 men) aged 20 to 60 years (median 46 yrs) served as controls.

Testing. Fasting sera from SSc patients were collected, after drug withdrawal for more than 3 days, and stored at –20°C until tested. Serum YKL-40 levels were determined by a quantitative immunoassay[9] according to manufacturer's instructions (Quidel Corp., formerly Metra Biosystems, Santa Clara, CA, US). Briefly, the YKL-40 assay is a sandwich immunoassay in a microtiter stripwell format. The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin is bound to streptavidin on the strip and captures YKL-40 in a standard, control, or patient sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected with p-nitrophenyl phosphatase as substrate. All YKL-40 assays were performed in duplicate with minimum detection limit of 20 ng/ml.

Skin involvement was assessed by the modified Rodnan skin score grading from 1 to 3[15].

Joint complaints, including arthralgias (pain without other signs of inflammation) and true arthritis (i.e., tenderness and/or joint swelling), were also identified.

The involvement of target organ/systems was investigated by electrocardiogram, B mode echocardiography with Doppler examination, capillaroscopy, pulmonary function tests, diffusing capacity for carbon monoxide (DLCO), high resolution computed tomography of the chest, esophageal and/or gastrointestinal barium study, and oral xylose absorption test.

Disease severity was determined by the preliminary 9 organ/system severity scale according to Medsger, *et al*[16].

In addition to YKL-40, the patients were investigated for variables reflecting endothelial activity/damage (von Willebrand factor, vWF)[17], fibroblast function (aminoterminal propeptide of type III procollagen, PIIINP)[18], lymphocyte activation (soluble serum interleukin 2 receptor alpha, sIL-2Rα)[19], and for acute phase reactants (erythrocyte sedimentation rate, ESR; C-reactive protein, CRP).

The blood, plasma, or serum samples for ESR measured according to Westergren and CRP by immunonephelometry (using a Behring nephelometer) were taken together with YKL-40.

Serum sIL-2Rα was measured by a quantitative sandwich ELISA using a monoclonal antibody specific for sIL-2Rα in a precoated microplate according to manufacturer's instructions (Quantikine, R&D Systems, Minneapolis, MN, USA) with a detection limit < 6 pg/ml.

PIIINP was measured by commercially available radioimmunoassay (RIA; PIIINP RIA kit, Orion Diagnostica, Espoo, Finland) with a detection limit < 0.2 µg/l.

vWF was detected using microtiter strips coated with a preparation of purified murine anti-vWF IgG monoclonal antibody that recognizes a functional epitope of vWF according to the manufacturer's procedure (Shield Diagnostics ELISA kit, Dundee, UK) with a detection limit < 1.6% of the activity.

We relied on our previous studies specifically devoted to each variable (unpublished observations) to provide normal concentrations for comparison with our patients with SSc.

Statistical analysis. Results were analyzed using the Statistical Package for Social Sciences (SPSS) v. 6.1 for Windows. Data are expressed as mean ± standard deviation (SD) and confidence interval, median, and ranges when appropriate. Differences between groups were assessed by nonparametric tests. To analyze correlations between continuous variables, Spearman's rho test was performed, using log transformed data of serological variables not normally distributed. P values < 0.05 were considered significant.

RESULTS

The SSc patients were divided into 13 cases with lSSc, 18 with iSSc, and 9 with dSSc. ANA, assessed in all cases, showed a centromere pattern in 7, anti-Scl-70 antibodies in 29, and ANA positivity in absence of either ACA or anti-Scl-70 antibodies in 4 patients.

Table 1 shows the mean (95% CI), median, and range of serum YKL-40 in SSc patients and controls. Serum YKL-40 concentrations were significantly higher in patients than in controls, as indicated by the exclusion of the mean value of the SSc patients from the confidence interval of the control group.

No significant differences were seen between the sexes (data not shown). Fourteen patients (35%; 95% CI 19.5–50.4%) had levels > 135 ng/ml (upper limit of normal: mean ± 2 SD). No differences emerged when we compared the levels of YKL-40 in the patients subdivided into either the 3 clinical subgroups of SSc (Figure 1a), or into the 3 serological subgroups (data not shown).

Figure 2 shows the concentrations of serum YKL-40 from all patients with and without arthralgias/arthritis (18 vs 22 cases, respectively; median 138 vs 57.5 ng/ml). The difference between the 2 groups was statistically significant (Kruskal-Wallis test, p = 0.007). Moreover, cross-tabulation of the SSc patients with and without increased serum YKL-40 levels showed that the frequency of arthralgias/arthritis was significantly greater (10/18 cases) in those with increased levels than in those without (4/22 cases) (Fisher's exact test, p = 0.021).

The modified Rodnan skin score was 14.9 ± 10.6 (range 0–42, median 13). No differences emerged when the scores of patients were stratified according to the clinical subsets (data not shown).

Organ system involvement was as follows: general health in 22 patients (score 1, 42.5%; score –2, 12.5%); peripheral vessels in 34 (score 1, 15%; –2, 35%; –3, 35%); heart in 11 (score 1, 12.5%; 2, 30%; 4, 2.5%); lung in 38 (score 1, 35%; 2, 30%; 3, 30%); gastrointestinal tract in 18 (score 1, 85%; 2, 12.5%); kidney in 5 (score 1, 2.5%; 2, 2.5%; 3, 5%; 4, 2.5%); muscle in 1 (score 1); joint/tendon in 11 (score 1, 7.5%; 2, 17.5%; 4, 2.5%).

Table 1. Serum YKL-40 levels in SSc patients and controls.

	Serum YKL-490, ng/ml				
	n	Mean	95% CI	Median	Range
SSc patients	40	132.9	87.8–175	75.5	24.2–584
Controls	34	66.6	54.6–78.6	52.0	24.0–150

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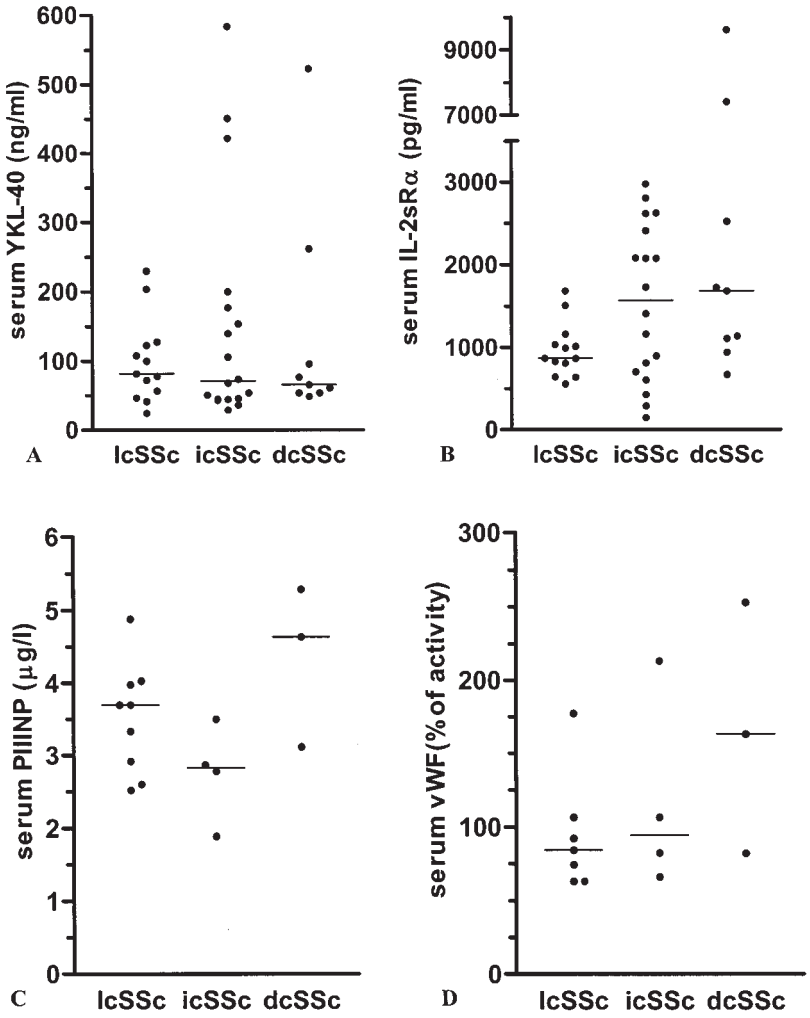

Figure 1. YKL-40, sIL-2Rα, PIIINP, and vWF serum concentrations in patients with SSc according to clinical subsets (bars are median values). lcSSc: limited cutaneous SSc; icSSc: intermediate; dcSSc: diffuse SSc.

Comparing the levels of serum YKL-40 in SSc patients with a greater severity score (2–3) of heart, lung, and gastrointestinal involvement with those in patients with no organ involvement, no significant differences emerged. In addition, no significant differences were found between serum YKL-40 values in patients with 1–2 renal severity score (2 cases; median 61 ng/ml, range 54–78) with respect to those without kidney involvement (score 0) (35 cases; 72.6 ng/ml, range 24.2–584). In this regard, 3 cases with a renal severity score 3–4 were excluded from the analysis; the median serum creatinine was 6.3 mg/dl (range 3.3–7.3), and the median serum YKL-40 level in these patients was 451 ng/ml (range 262–523), probably due to impaired kidney function.

Moreover, there were no differences when serum PIIINP levels were compared according to the presence or absence of internal organ involvement (data not shown).

Table 2 shows the median (range) and percentages of SSc patients with abnormal levels of serological variables analyzed in our series.

Figure 1 shows the median and individual serum sIL-2Rα, PIIINP, and vWF levels in the SSc clinical subgroups. No significant differences were found.

Univariate analyses relating YKL-40 and clinical (age, disease duration, skin, and internal organ/system involvement) and serological variables (ESR, CRP, rheumatoid factor, sIL-2Rα, vWF, PIIINP levels) were made. Significant Spearman's rho correlations were found with age (r_s = 0.394; p = 0.01), joint involvement according to Medsger, *et al* (r_s = 0.369; p = 0.019), and serum sIL-2Rα concentrations (r_s = 0.349; p =0.027). These last 2 correlations were confirmed after adjustment for confounding variables such as age (p = 0.015 and p = 0.001, respectively).

Dividing the patients into 2 groups according to therapeutic regimen, we were unable to observe any significant difference in YKL-40 serum levels between patients treated

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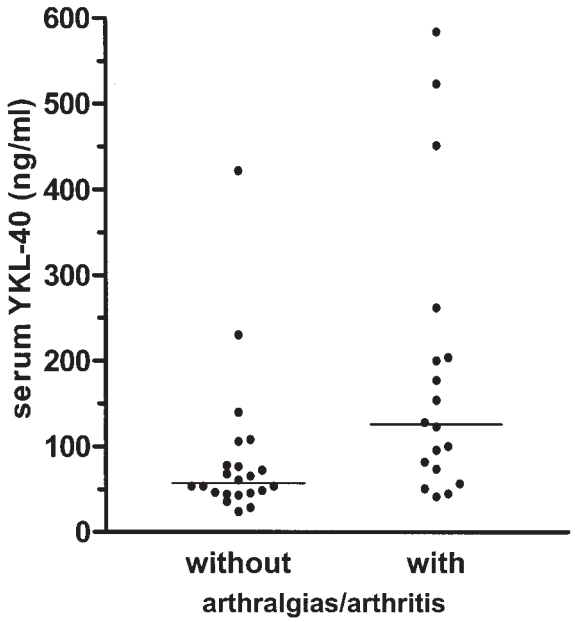

Figure 2. Concentrations of serum YKL-40 in patients with SSc with and without arthralgias/arthritis (bars are median values).

with cyclophosphamide or not (p = 0.49), or with or without low doses of glucocorticoids (p = 0.059). On the whole, no difference emerged between the patients who used anti-inflammatory and disease modifying antirheumatic drugs and those who did not (data not shown).

DISCUSSION

This is the first report of YKL-40 behavior in patients with SSc. Here, 35% of patients showed increased concentrations of YKL-40, suggesting a low grade of synovial and/or cartilage involvement despite the absence of a clinically detectable arthritis[2,20]. Histological evidence of synovitis was found in more than 66% of synovial biopsies from SSc patients in one study[21]. Therefore, it can be hypothesized that the YKL-40 increase in the serum of SSc patients follows the chondrocyte, synovial cell, and macrophage activation, as in active RA or in OA[22-24].

In agreement with findings by other investigators in OA[24,25] and in cancer[26], we found no differences between the sexes, but there was a significant although weak correlation with age. This suggests that the increase of YKL-40 is most probably due to the influence of other factors, such as disease changes.

Unlike other reports of a significant correlation between CRP and YKL-40 in OA and RA[11,24], we were unable to find a significant correlation in SSc. This suggests that CRP and YKL-40 reflect different aspects of joint disease. Indeed, CRP, an acute phase protein secreted by hepatocytes, is the consequence in arthritis of the release of the proinflammatory cytokines IL-1, IL-6, and tumor necrosis factor-α.

We have found that YKL-40 is correlated with T cell activation. This point could confirm that YKL-40 is involved in the immune response, with some HLA-DR4 peptide-binding motifs recognized by T cells, as found in patients with RA[27].

We were unable to find significant differences in the levels of serum YKL-40 among patients from different clinical subsets. In addition, we were unable to find significant differences in the levels of serum YKL-40 among patients stratified according to involvement of heart, lung, gastrointestinal tract, and kidney. On the contrary, significantly higher levels of serum YKL-40 values were found in patients with arthralgias/arthritis.

It is notable that we found no correlation between either YKL-40 and PIIINP or between joint involvement and PIIINP, which is considered a reliable marker of fibroblast function. Therefore our results point out a significant relationship between serum YKL-40 levels and arthropathy and suggest YKL-40 to be a reliable marker of articular involvement in SSc.

In our study, YKL-40 levels did not correlate with radiological joint changes. Other reports also suggested that in RA, YKL-40 cannot be used as a marker of progression of joint damage[28-30]. Finally, no differences were found concerning drug use.

Our study has some weaknesses. In addition to the limited number of patients, the most important one relates to its cross-sectional design that did not allow us to evaluate this biochemical marker as a predictor of joint change progression. In addition, other points need to be addressed

Table 2. SSc patients with abnormal levels of serological variables.

Serological Variable	n	Median (range)	Normal Values	Prevalence of Abnormal Values, %
ESR, mm/h	40	15 (2–80)	≤ 20	20
CRP, mg/dl	33	0.4 (0.3–7.5)	≤ 0.5	42.4
sIL-2Rα, pg/ml	40	1124.8 (167–9618)	670–2030	20
PIIINP, µg/l	16	3.4 (1.89–5.28)	0.96–3.19	50
vWF, % of activity	16	98.7 (60–180)	60–180	12.5
RF, UI/ml	33	1.4 (21–219)	≤ 14	18.2

RF: rheumatoid factor.

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for the epidemiological characteristics of our patients. The high prevalence of anti DNA-topoisomerase positive patients is a well established feature of our series[14,31] and may relate to unestablished ethnic or environmental factors.

Our preliminary study shows that an increased YKL-40 serum concentration occurs in many SSc patients, and is related to T cell activation, as shown by IL-2Rα levels and joint involvement. This could indicate that YKL-40 serum changes reflect cartilage and/or synovial fibroblast activity. A longitudinal study is needed to address the value of YKL-40 as a serological marker of joint disease in systemic sclerosis, and its use in monitoring clinical changes and drug influence.

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