Increased levels of amino terminal propeptide of type III procollagen are an unfavourable predictor of survival in systemic sclerosis

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Abstract

Objective

Investigation of the impact on survival of inflammatory parameters (C-reactive protein, ESR), markers of immune activation (serum soluble IL-2 receptor, soluble CD30), and N-terminal propeptide of type III procollagen levels (PIIINP) in systemic sclerosis (SSc).

Methods

In a prospective follow up study, clinical and laboratory data of 80 patients with SSc were evaluated. Kaplan-Meier survival curves and Cox proportional hazards model were used. Eighty cases with SSc were evaluated. Female/male ratio was 8/72. The mean (±SD) age was 49.3 (±12.3) years, 16 patients died during our mean follow up of 58.1 months.

Results

In the univariate analysis, the presence of a C-reactive protein level above 20 mg/l was an unfavourable prognostic sign ($p<0.001$). Increased level of PIIINP level also caused an unfavourable outcome of disease ($p<0.001$). Conversely, increased ESR, soluble IL-2 receptor, soluble CD30 levels, presence of anaemia, did not influence the prognosis. Male gender ($p<0.005$), diffuse cutaneous SSc, clinically significant lung involvement ($p<0.001$), kidney ($p<0.0001$), cardiac ($p<0.05$) manifestations including pericarditis ($p<0.02$) were unfavourable prognostic signs by univariate Kaplan–Meier method. Multivariate analysis by Cox proportional hazards model showed that the increased level of PIIINP (RR: 6.98), and presence of diffuse cutaneous SSc (RR: 5.14) were independent unfavourable prognostic signs.

Conclusions

An increased collagen metabolism unfavourably influences the outcome of SSc. This parameter may also be a potential candidate as a disease activity marker.

Key words

Prognosis, survival, systemic sclerosis, C-reactive protein, aminoterminal type III collagen propeptide.

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Introduction

Systemic sclerosis (SSc) is characterised by capillary vascular abnormalities, fibrosis, inflammatory changes, and atrophy affecting the skin and several internal organs. Previous studies including our works (1, 2) indicated that the presence of diffuse skin involvement (3), internal organ manifestation including the heart, kidney, lung, and the gastrointestinal tract (1, 3-7) caused a bad prognosis of the disease. More extensive skin involvement coincides with more severe internal organ manifestation(s) resulting in an unfavourable outcome of disease (1,3,6-8). When only independent prognostic factors are investigated by Cox survival analysis, the older age (8,9), renal involvement (2,5,9-11), severe lung involvement (2, 5, 9-11), clinical signs of right heart failure (5,8,11), the presence of diffuse scleroderma (2,11), the extent of skin involvement (12) were unfavourable prognostic signs.

Increased ESR was described as an unfavourable indicator of the outcome of disease (2, 4, 6, 13-15), although others did not find any effect on mortality of sedimentation rate (3). The ESR was found to be significantly higher in patients with diffuse SSc as compared with cases with limited disease (16,17). In a recent study, high levels of soluble CD30 were found in the peripheral blood of patients with SSc, and a significant correlation was detected with skin score, and ESR (18). ESR was found to be significantly higher in elderly males (4), in cases with fatal outcome within two years during the follow up (4, 5), in patients with interstitial inflammatory lung disease (19), and in the presence of anti-calpastatin (20) anti-endothelial cell antibodies (21). An inverse relationship was also described between the reduced ACE levels and ESR (22).

Acute phase proteins including plasma orosomucoid (16) and haptoglobin (16, 23) concentrations were found to be significantly higher in patients with diffuse SSc compared to cases with limited disease indicating that patient with more pronounced skin involvement may also exhibit an acute phase response. Other acute phase proteins like fibrinogen showed conflicting results either increased (24) or normal levels (17) were described. The elevated level of CRP may be present up to 42.4% of the patients (25) but the relationship between this most widely used acute phase response test, and disease activity markers seems to be also conflicting. In an early study, a disturbed acute phase response was described (26). In another paper, CRP response was found to be normal in the 20 investigated scleroderma cases (23).

As a sign of the activation of immune system an elevated level of soluble IL-2 receptor has been described by several groups (27-33). Serum soluble CD30 level which is a marker of T helper 2 mediated immune response can also be elevated in SSc, and the elevation seems to correlate with the increased ESR (18).

The altered collagen metabolism is a hallmark of SSc (25, 34-48). Increased PIIINP (25, 34-43), and type I procollagen carboxyterminal peptide (38, 44) levels have been extensively studied in SSc (25, 34-43). The serum levels of PIIINP appear to reflect the extent of skin involvement (34-36, 39, 42, 43, 45), and the disease progression (38, 39), and seem to decrease following immunosuppressive treatment (19, 34). It has been reported that the serum levels of PIIINP may have some predictive value on mortality in SSc, because the highest concentration was found in cases with rapidly progressive fatal systemic sclerosis (41). In recent studies increased serum concentrations of cross-linked carboxyterminal telopeptide of collagen I (ICTP) have also been found to be higher in dcSSc than in lcSSc, and high serum ICTP has been correlated with skin and lung involvement (39,44,46). Furthermore a correlation was also found with CRP levels (44), although there is no sufficient data to support the use the serum markers of collagen turnover in the assessment of scleroderma activity (47).

Vascular injury is one of the major abnormalities in SSc. Decreased plasma ACE activity and increased levels of von Willebrand factor antigen are found in patients with SSc, although the proportion of abnormal von Wille-

brand factor in SSc may be be low (25). There is inverse relationship between the reduced ACE levels and the ESR. It is likely that the ACE levels reflect the inflammatory aspect of the disease. Another study has revealed a significant negative correlation between the anti-endothelial cell antibodies and ACE plasma levels in both limited and diffuse cutaneous SSc (21). Presence of capillary loss was also a bad prognostic sign (9).

The inflammatory and acute phase response markers (ESR, CRP, haptoglobin, IL-6), decreased complement levels, markers of immune activation (sIL-2, sCD30, neopterin levels, etc.), parameters of increased collagen metabolism (collagen C aminopeptid terminal levels /PIIINP/, cross-linked carboxy-terminal telopeptide of collagen I, soft tissue pyridinoline), markers of vascular endothelial cell activation (angiotensine convertase enzyme: ACE; von Willebrand factor antigen: vWFAg), hyperprolactinemia, and anti-topoisomerase antibody titers seem to be potential candidates for detection of the disease activity, and may also have some predictive value for the outcome of disease, because activity producing a later damage can influence the prognosis of SSc (2-6, 13-49).

The aim of the present study was to determine how the survival is influenced by inflammatory parameters (CRP, ESR), markers of immune activation (sIL-2 receptor, sCD30), and PIIINP. We found that an increased CRP level or an elevated serum PIIINP were unfavourable prognostic signs.

Patients and methods

Eighty patients were evaluated from April 1997 until the end of November 2003 by the same investigators (LC, ZN). Patient enrolment was ended in December 1999. The minimum and maximum follow-up times of our uncensored patients at the end of November 2003 were 5 and 79 months, respectively. Classification into diffuse or limited cutaneous SSc subgroups was performed at the entry into the follow-up (50) according to the standard method (51). Symptoms which might be loosely associated to the SSc were not excluded, and only those events were taken as censored data which showed absolutely no relationship to the SSc.

The following items were recorded at the time of entry: sex, presence of anti-centromere or anti-Scl 70 antibodies, subcutaneous calcinosis, age at the onset of disease, and duration of the disease at entry into the study, subcutaneous calcinosis, Raynaud's syndrome and hand deformity with contractures. Patients exhibiting scleroderma renal crisis were considered to have a kidney manifestation. Cardiac involvement was evaluated via the clinical symptoms, and ECG. In cases with cardiac symptoms, echocardiography, and Holter monitoring were routinely performed. Dysmotility and stricture/dilatation of the lower part of oesophagus were distinguished by barium swallowing as the oesophageal manifestation of SSc. Lung manifestation of SSc was acknowledged in the presence of lung fibrosis in the chest roentgenogram and/or a forced vital capacity between 50-80%. Severe lung involvement was indicated in the presence of diffuse lung fibrosis/honeycombing on chest roentgenogram, and/or forced vital capacity was below 50% for the age, gender and body surface matched controls.

Patients exhibiting both decreased lachrymal (Schirmer's test) and salivary secretion were categorised as having sicca syndrome. An elevated serum creatinine kinase level with proximal muscular weakness was ascribed to myositis.

Anti-centromere antibody was detected on HEp-2 cells by the indirect immunofluorescence method. Anti-Scl 70 autoantibody was determined with an ELISA kit (Progen Biotechnik GMBH, Germany). Anaemia was recorded when the haematocrit level was < 33%, and no blood loss or other disease causing anaemia could be found. The ESR was graded as increased if it was >40 mm/hr for at least 3 months without any

Table I. Clinical profile of 80 patients with systemic sclerosis at entry into the follow-up,

Age at the onset of SSc (year, mean ± SD, min-max.)	49.3±12.3	(19-79)
Duration of disease at entry (year, median, min-max.)	7	(0-21)
Number of deaths due to SSc	16	
Number of deaths for reasons other than SSc	1	
Follow-up time (months, mean ± SD, min, max)	58.1±17.5	(5-79)
Females	72	(90%)
Diffuse cutaneous SSc	17	(21.3%)
Exposure to chemicals	13	(16.2%)
Subcutaneous calcinosis	11	(13.8%)
Skin pigmentary changes	9	(11.3%)
Teleangiectasia	47	(58.8%)
Raynaud's phenomenon	66	(82.5%)
Cardiac involvement	27	(33.8%)
Pericarditis	5	(6.3%)
Oesophageal manifestation	41	(51.3%)
Lung involvement (bibasilar fibrosis and/or < 80% of expected FVC)	69	(86.3%)
Lung involvement (diffuse fibrosis and/or < 50% of expected FVC)	13	(16.3%)
Scleroderma renal crisis	6	(7.5%)
Decreased lacrymal secretion	25	(31.3%)
Increased creatine kinaze level + muscle weakness	10	(12.5%)
Anaemia (> 40 mm/hr)	11	(13.8%)
Increased erythrocyte sedimentation rate	12	(15%)
Increased CRP level (> 20 mg/l)	16	(20%)
Increased sIL-2 receptor level	22	(27.5%)
Increased sCD30 level	42	(53%)
Increased serum levels of PIIINP	29	(36.3%)
Anti-Scl-70 antibody positivity	27	(33.8%)
Anti-centromere antibody positivity	12	(15%)

other known cause. Serum CRP level was determined by nephelometry (Turbox Plus Instrument, Orion Diagnostica, Espoo, Finland). In patients with no detectable infection, a doubled CRP level (> 20 mg/l) was regarded as an elevated value.

The soluble IL-2 receptor ELISA kit was purchased from Immunotech (France), the soluble CD30 kit from DAKO (Denmark), and the PIIINP /125/ RIA kit from Orion Diagnostica (Finland). For ACE detection the Sigma Diagnostics reagent (USA) was used.

Data analysis

Correlation between parameters were evaluated by Spearman's correlation coefficient. Kaplan-Meier survival curves (52) and the Cox proportional hazards model were calculated. Backward stepwise analysis was also performed to present the minimum set of variables influencing the survival (53).

Results

The clinical findings at the entry to the study are depicted in Table I. Sixteen patients died during the follow-up period of 58.1 months (min. 5, max. 79 months). One patient died for a reason other than SSc. The correlation between clinical-laboratory findings is demonstrated in Table II.

Survival analysis

Female gender ($p < 0.005$), and the presence of limited cutaneous SSc ($p < 0.001$) indicated a significantly favourable survival (Fig. 1). With regard to internal organ involvements, cardiac ($p < 0.05$), renal ($p < 0.0001$), and severe pulmonary ($p < 0.001$) involvement were found to be significantly negative prognostic factors (Fig. 2). The presence of pericarditis ($p < 0.02$) also exerted a significant effect on the survival. Oesophageal involvement and myositis did not affect the survival (data not shown). The Kaplan-Meier curve for the 80 patients is demonstrated in Figure 2.

A significantly unfavourable prognosis was also detected in patients with an elevated CRP level ($p < 0.001$) (Fig. 3). Increased serum levels of PIIINP likewise indicated a poor prognosis ($p < 0.001$) (Fig. 3). The presence of giant capillaries, or avascularity on capillaroscopy, anti-Scl70, anticentromere antibody positivity, anti-nuclear antibody positivity, anti-phospholipid antibody positivity, rheumatoid factor, a polyclonal increase in immunoglobulin G levels, increased ESR, and anaemia did not significantly influence the outcome of the disease (data not shown). An increased sIL2R level and the presence of sCD30 did not have any impact on

Table II. Correlation of clinical symptoms and clinical findings.

	Death due to SSc	dcSSc	Heart	Lung	Renal	Pericarditis
dcSSc	**0.351**	1				
Heart	**0.238**	**0.275**	1			
Severe lung inv.	**0.373**	**0.268**	**0.331**	1		
Renal	**0.451**	**0.316**	0.098	0.132	1	
Pericarditis	**0.258**	**0.371**	**0.253**	0.166	0.123	1
PIIINP ↑	**0.403**	0.053	0.012	**0.232**	0.18	0.128
CRP > 20 mg/l	**0.375**	0.199	0.172	0.203	0.214	0
ESR > 40 mm/h	0.14	0.039	0.218	**0.479**	0.013	0.036
sIL-2R ↑	0.182	-0.046	-0.144	0.032	**0.25**	-0.043
sCD30 ↑	0.163	0.127	0.044	-0.056	**0.271**	-0.065
	PIIINP ↑	CRP > 20	ESR > 40	sIL-2R ↑	sCD30 ↑	
PIIINP ↑	1					
CRP > 20 mg/l	**0.273**	1				
ESR > 40 mm/h	**0.339**	**0.315**	1			
sIL-2R ↑	**0.234**	0.112	-0.024	1		
sCD30 ↑	-0.012	-0.088	-0.021	0.193	1	

Non-parametric Spearman's correlation coefficient was calculated. Bold fields indicate the 2-tailed significant values at 5% level.
dcSSc: diffuse cutaneous systemis sclerosis, heart: cardiac involvement, severe lung inv.: severe lung involvement with diffuse pulmonary fibrosis (for details, see *Methods*), renal: scleroderma renal crisis. PIIINP ↑: increased amino terminal propeptide of type III procollagene level, ESR: red blood cell sedimentation rate, sIL-2R ↑: increased serum soluble IL-2 receptor, sCD30 ↑: increased serum soluble CD30 level.

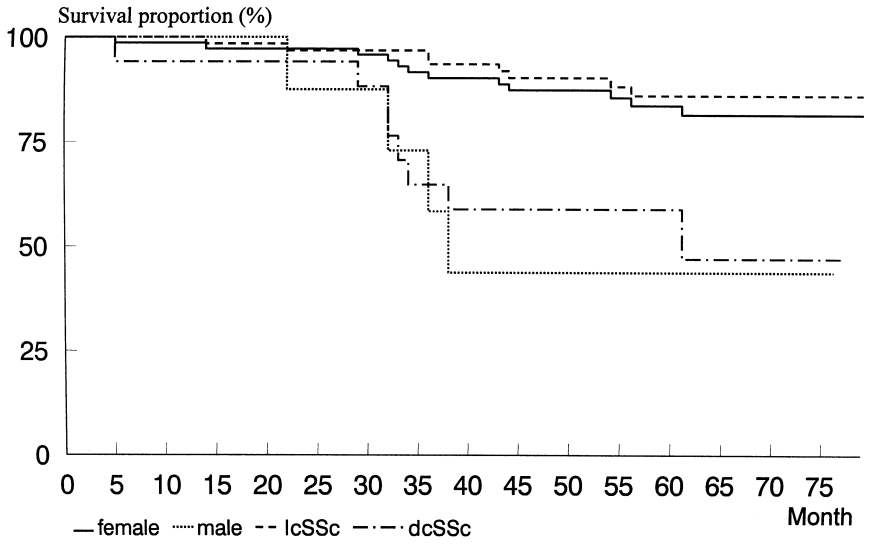

Fig. 1. Kaplan-Meier survival curves for gender, and for patients with diffuse and limited cutaneous systemic sclerosis.
Female: female patients (n = 72); male: male patients (n = 8); lcSSc: limited cutaneous systemic sclerosis (n = 17); dcSSc: diffuse cutaneous systemic sclerosis (n = 63).

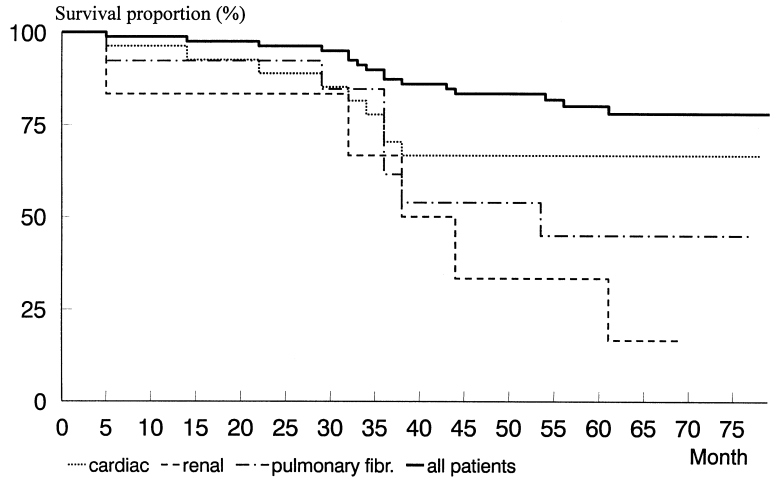

Fig. 2. Kaplan-Meier survival curves for total mortality of 80 patients with systemic sclerosis, and for patients with cardiac, renal and pulmonary involvement.
Cardiac: cardiac involvement (n = 27); renal: scleroderma renal crisis (n = 6); pulmonary fibr.: diffuse lung fibrosis (n =13); all patients: survival function of the 80 patients with systemic sclerosis.

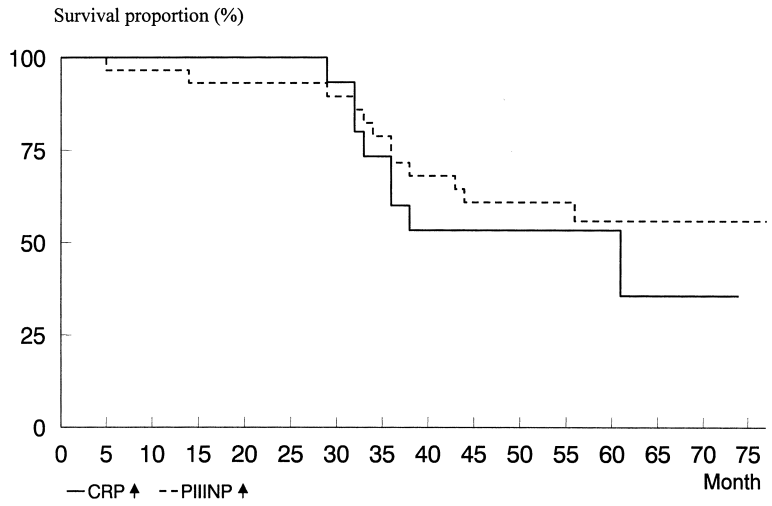

Fig. 3. Kaplan-Meier survival curves for patients with increased C-reactive protein and aminoterminal propeptide of type III procollagen levels. CRP↑ elevated C-reactive protein level (n = 16); PIIINP↑ elevated aminoterminal propeptide of type III procollagen level (n = 29).

Table III. Cox proportional hazards regression analysis with data measured at entry into the study*.

	Pr > Chi-square	Risk ratio (95% CI)
Age at onset	0.498	-
Duration of SSc at study entry	0.401	-
Cardiac involvement	0.728	1.33 (0.27-6.53)
Scleroderma renal crisis	0.281	2.27 (0.51 -10.11)
Diffuse lung fibrosis	0.889	1.12 (0.24-5.28)
Pericarditis	0.337	2.31 (0.42-12.74)
Increased CRP	0.294	1.98 (0.55-7.13)
PIIINP	**0.016**	**5.65 (1.39-23.04)**
Diffuse SSc	0.085	3.53 (0.84-14.84)
Gender	0.872	0.84 (0.09-7.26)

*Model contains covariates showing significant effect on survival with univariate method with addition of age at onset and duration of SSc at study entry.

the survival. Either a decreased or increased ACE did not prove to be a prognostic sign in SSc (data not shown).

The results of the Cox proportional hazards model is demonstrated on Table III. Increased serum levels of PIIINP exerted a significant effect in the multivariate model (risk ratio: 5.65).

The model with the gender, and the age at onset of the SSc added to the variables selected by the backward stepwise method for the Cox proportional hazards model revealed that diffuse SSc and increased serum levels of PIIINP were independent unfavourable prognostic signs (Table IV).

The presence of increased CRP levels was significantly correlated with death due to SSc, increased ERS and the presence of giant capillaries by capillaroscopy. Elevated PIIIP levels showed a correlation with increased severe lung involvement, elevated CRP, ESR, sIL-2R levels, and decreased ACE (Table V).

Discussion

Not surprisingly our investigation with the univariate Kaplan-Meier model confirmed that the presence of male gender ($p < 0.005$), diffuse SSc, cardiac manifestation including pericarditis ($p < 0.02$), elevated (> 20 mg/l) C-reactive protein level ($p < 0.001$), and elevated PIIINP also caused an unfavourable outcome of disease ($p < 0.001$) were unfavourable prognostic signs (Figs. 1 and 2). Conversely, an increased ESR, anaemia, elevated sIL-2 receptor, and sCD30 levels did not influence the prognosis. In a former study, the serum sIL-2 was higher in patients with diffuse cutaneous SSc compared to limited cutaneous SSc cases (33). In another study (10) sIL-2 level was a bad prognostic marker. In our study, we did not experience any major impact of sIL-2 levels on the prognosis of patients. In contrast to a previous finding (18) we did not demonstrate that soluble CD30 may be correlated with diffuse skin involvement, and we demonstrated that an elevated soluble CD30 level did not influence the survival. The discrepancy may be partially explained by different definition of CD30 elevation, and by

Table IV. Stepwise Cox proportional hazards regression analysis with data measured at entry into the study*.

	Pr > Chi-square	Risk ratio (95% CI)
Diffuse SSc	**0.007**	**5.14 (1.56-16.91)**
PIIINP	**0.002**	**6.98 (2.08-16.91)**
Scleroderma renal crisis	0.198	2.42 (0.63-9.26)
Gender	0.612	0.66 (0.14-3.21)
Age at onset	0.429	-

*Set of variables selected by stepwise selection analysis with addition of sex and age at onset. For details, see *Methods.*

Table V. Comparison of clinical – laboratory parameters of cases with increased and normal CRP and PIIINP levels.

	CRP > 20 mg/l		Normal CRP		p*
No of cases	16		64		
Age (yr)	51.3	(SD: 16.3)	48.8	(SD: 11.1)	NS
Death due to SSc	**8**	**(50%)**	**8**	**(12.5%)**	**p < 0.001**
PIIIP	**10**	**(62.5%)**	**19**	**(29.7%)**	**p < 0.015**
Decreased ACE	2	(12.5%)	5	(7.8%)	NS
Increased sCD30	7	(43.8%)	35	(54.7%)	NS
We < 40 mm/h	**6**	**(37.5%)**	**6**	**(9.4%)**	**p < 0.005**
DcSSc	6	(37.5%)	11	(17.2%)	NS
Renal involvement	3	(18.8%)	3	(4.7%)	NS
Cardiac	8	(50%)	19	(29.7%)	NS
Mild lung involvement**	14	(87.5%)	55	(85.9%)	NS
Avascularity	0	(0%)	11	(17.2%)	NS
Giant capillaries	**0**	**(0%)**	**16**	**(25%)**	**p < 0.025**
	Elevated PIIINP		Normal PIIINP		
No of cases	29		51		
Age (yr)	50.7	(SD:13.7)	48.8	(SD 11.1)	NS
Death due to SSc	**11**	**(37.9%)**	**5**	**(9.8%)**	**p < 0.001**
CRP > 20 mg/l	**10**	**(34.5%)**	**6**	**(11.8%)**	**p < 0.015**
Increased sIL-2R	**12**	**(41.4%)**	**10**	**(19.6%)**	**p < 0.036**
Decreased ACE	**5**	**(17.2%)**	**2**	**(3.9%)**	**p < 0.043**
Increased sCD30	15	(51.7%)	27	(52.9%)	NS
We > 40mm/h	**9**	**(31.0%)**	**3**	**(5.8%)**	**p < 0.002**
DcSSc	7	(24.1%)	10	(19.6%)	NS
Renal involvement	4	(13.8%)	2	(3.9%)	NS
Cardiac involvement**	10	(34.5%)	17	(33.3%)	NS
Mild lung involvement**	26	(89.7%)	43	(84.3%)	NS
Diffuse lung fibrosis**	**8**	**(27.6%)**	**5**	**(9.8%)**	**p < 0.038**
Giant capillaries	3	(10.4%)	13	(25.5%)	NS

* For age (yrs.) the t-test was used, in the other comparisons the Chi-square test was used, NS: not significant at $p < 0.05$ level.
**For details, see *Methods.*

the fact that in our study the disease duration was longer.

The sign of increased collagen metabolism was a relevant factor influencing the survival (Table II). The extents of skin involvement, and the presence of lung fibrosis strongly influence the outcome of SSc (1-7, 10, 12). It has been described that serum levels of PIIINP may have a predictive value on mortality in systemic sclerosis (41). Patients with shorter disease duration had higher serum levels of PIIINP than patients with a longer disease duration. The highest serum concentrations of PIIINP were found in seven patients who died within 2 years indicating that in rapidly progressive systemic sclerosis PIIINP may have a significant predictive value (41). A positive correlation was also described between skin score and the PIIINP (35,45). Increased levels of PIIINP in serum seems to correlate with skin involvement and the clinical course (34-36, 39, 42, 43, 45), especially with a rapid disease progression (38, 42, 45). In other recent studies an increased serum concentration of cross-linked carboxyterminal telopeptide of collagen I (ICTP) was found in SSc (39, 44, 46). Distinctly higher levels of ICTP were observed in dcSSc than in lcSSc (39,44), and high serum ICTP was also correlated with acute phase reactants (44). Conversely, the serum PIIINP concentration was elevated not only in dcSSc but in lcSSc as well (39). These findings correlate with our results by Kaplan-Meier method that both increased collagen metabolism and acute phase reaction may be associated with disease severity, and therefore may be important factors in prognosis (39).

Cox proportional hazard model indicated a poor disease outcome in patients with pulmonary (2, 12) and cardiac (2, 5, 8, 10-12) involvements. The extent of skin involvement (12), an older age at onset (8), hypertension (12) and the presence of an abnormal urine sediment (10) were also poor prognostic signs. The presence of diffuse cutaneous SSc (2, 11), pericarditis, and an increased ESR are also defined as poor prognostic signs (2).

With Cox proportional hazards model an increased ESR (6, 7), age, anaemia, trunk involvement (6), cardiopulmonary abnormalities, abnormal urine sediment were reported to be unfavourable prognostic signs (6, 10). Furthermore, proximal muscle weakness, certain alterations in urine or blood cell counts, a reduced arterial PO_2, a decreased carbon monoxide diffusing capacity, left ventricular enlargement, older age, azotaemia and a decreased serum total protein level exerted a significant negative effect on the survival (5).

Our present study indicated that in the backward selection of the Cox propor-

tional hazards model an increased level of PIIINP (Risk ratio: 6.98), and the presence of diffuse cutaneous SSc (risk ratio: 5.14) are independent unfavourable prognostic factors (Tables III-V).

Only a few studies indicated an increased CRP response in a certain subgroup of patients with SSc (19, 26, 39), although others did not show such a response (23). Our investigation also demonstrated the importance of acute phase response in SSc indicating that this was a parameter that substantially influenced the survival (Fig. 3, Table V).

Previous studies revealed an unfavourable outcome of the disease in patients with an elevated ESR (2,4,6,10,13,14) suggesting that the inflammatory activity of the disease may influence the survival, although others had not find any effect on mortality of sedimentation rate (3). We also described in our previous work that increased ESR was an unfavourable indicator of the outcome of disease (2). Comparing our patients demographic data in the two independent cohorts, the age was higher and the disease duration was shorter at the beginning of the follow up, and the recruitment of the patients for the two investigations was in different period (1982-1993 and 1997-2003), and in a different geographic area. Patients with a longer disease duration, and a persisting increased ESR may have a certain extent of unfavourable predictive value, although other explanations including geographical/enviromental differences may not be excluded.

Our study indicates that altered collagen metabolism, an acute phase reaction measured by CRP level may be used as prognostic markers. With regard to the signs of immune activation and vascular injury further investigations are required so as to identify useful surrogate markers for disease outcome in SSc.

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