Influence of Clinical Features, Serum Antinuclear Antibodies, and Lung Function on Survival of Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To evaluate the independent contribution of several clinical and laboratory variables to the mortality of a cohort of Danish patients with systemic sclerosis (SSc).

Methods. A cohort of 174 patients with incident SSc was retrospectively identified using clinical charts and study records of all new patients with SSc. Disease onset was defined as the time of onset of cutaneous sclerosis. Vital status and causes of death were determined at the end of the observation period. Data on clinical status and pulmonary function were obtained. Antitopoisomerase I (anti-topo I), anticentromere, anti-U1-RNP, anti-U3-RNP, anti-Th-RNP, and anti-RNA polymerase (anti-RNAP) antibodies were determined by means of double immunodiffusion, immunofluorescence, hemagglutination technique, radioactively labelled antisense riboprobes, and ELISA, respectively.

Results. Patients were followed for a mean period of 13.3 yrs; 16 died of an SSc related condition and 50 of other causes. Pulmonary fibrosis, DLCO reduction < 40% of the expected, diffuse cutaneous involvement, SSc nephropathy, cardiac disease, and anti-topo I and anti-RNAP antibody were related to decreased survival due to SSc. Variables that entered a Cox regression model of SSc related mortality were right heart failure (RR 12.4, 95% CI 2.5–60), diffuse SSc (RR 7.8, 95% CI 1.8–35), SSc nephropathy (RR 6.1, 95% CI 1.8–21), and DLCO < 40% (RR 4.8, 95% CI 1.1–20). The relative risk of developing right heart failure and diffuse SSc given the presence of anti-RNAP antibody was 14 (p = 0.0001) and 1.9 (p = 0.01), respectively. The corresponding figures for anti-topo I antibody were 4.6 (p = 0.02) and 2.0 (p = 0.01).

Conclusion. SSc related mortality was associated with right heart failure and diffuse SSc, both of which were also associated with the presence of anti-topo I and anti-RNAP antibody. The prognostic value of these autoantibodies may lie in the early course of the disease when specific morbidity has not yet evolved. (J Rheumatol 2001;28:2454–9)

Key Indexing Terms:
SYSTEMIC SCLEROSIS
PROGNOSIS
ANTI-RNA POLYMERASE ANTIBODIES
ANTI-TOPOISOMERASE I ANTIBODIES

It is well established that the survival rate of patients with systemic sclerosis (SSc) is reduced compared with that of the background population[1-3]. Several features have been described to be associated with poor survival of patients with SSc. Among others, these are presence of antitopoisomerase I (anti-topo I) antibodies[4], presence of anti-RNA polymerase (anti-RNAP) antibody[5], isolated severe reduction of pulmonary diffusing capacity[6], restrictive lung disease[7], diffuse cutaneous involvement[8], cardiac and renal involvement[4], male sex[9], and high age at disease onset[4]. Several of these features are interrelated, and we therefore wanted to identify the independent risk contribution of several demographic, clinical, and laboratory variables to the mortality of our cohort of Danish patients with SSc.

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MATERIALS AND METHODS

Patients. A total of 174 patients with SSc were selected from a prospective cohort[3]. Patients were selected if they met the classification criteria for SSc proposed by the American College of Rheumatology in 1980[10], serum was still available from the first visit, and pulmonary function test results were available from the beginning of the observation period. Accurate clinical charts with detailed skin evaluations were available for all patients, since systematic recordings were performed by Dr. Asboe-Hansen, the late chief of the Department of Dermatology at Rigshospitalet in Copenhagen. Later all patients had been followed up by Dr. Ullman. Disease onset was defined as the time of first physician observed cutaneous sclerosis (CS), which was also the beginning of the observation period. The mean duration of patient observed CS prior to the first visit was 3.4 years. The observation period was terminated ultimo 1996 or at the time of death.

Clinical features. History and physical and laboratory findings were entered consecutively into a cumulative database. Clinical events that

occurred either before inclusion or during the observation period were entered. If the observation period was terminated by death, the final event was not categorized as a preexisting symptom. Patients were classified according to the maximum extent of skin thickening by means of a 2 subset model[11] into limited or diffuse SSc; the dividing lines were at the elbows and knees.

Inner organ involvement was defined as follows: (1) pulmonary fibrosis: bibasilar interstitial fibrosis on chest radiograph; (2) esophageal dysfunction: distal esophageal hypomotility judged by radiological examination of esophagus and/or esophageal manometry; (3) myositis: muscle weakness and elevated serum creatine phosphokinase, a myopathic electromyogram and/or characteristic biopsy findings. Renal scleroderma crisis defined as rapidly decreasing renal function combined with increasing blood pressure was also accounted for. Right heart failure was not systematically investigated in all patients. However, when clinically suspected, the condition was confirmed by echocardiography.

Serological tests. The serological tests comprised the determination of IgG antinuclear antibody (ANA) by an indirect immunofluorescence (IIF) technique using commercially prepared slides of monolayer HEp-2 cells (ImmunoConcepts, Sacramento, CA, USA) as substrate and FITC labelled rabbit immunoglobulins against human Fcγ (Dako, Glostrup, Denmark). Sera were screened at a dilution of 1:40, and positive reactions were categorized according to the nuclear immunofluorescence pattern[12]. Titers were determined by 2-fold endpoint titration. Anticentromere and nucleolar antibodies were reported on the basis of the IIF pattern. Anti-topo I (Scl-70) antibodies were detected by double immunodiffusion against a thymus-spleen extract (Auto ID, ImmunoConcepts) and reference sera. Anti-U1-RNP antibodies were detected by passive hemagglutination technique[13]. The presence of the antinucleolar antibody anti-Th-RNP and anti-U3-RNP was determined using radioactively labelled antisense riboprobes[14]. Antibodies against RNAP I, II, and III were determined in a single ELISA. RNA polymerases I and II were purified from calf thymus as described[15-17]. RNA polymerase III was purified from HeLa cells[18-20]. For ELISA, a mixture of RNA polymerase I and II at a concentration of 2 μg/ml and polymerase III at 1 μg/ml were coated on Immulon 1B microtiter plates (Dynex Technologies, Chantilly, VA, USA) overnight at 4°C and then subjected to blocking with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS)-Tween for 1 h at ambient temperature, followed by washing with PBS-Tween. Volumes of 100 μl/well of positive and negative control sera and patient sera diluted 1:100 in PBS-Tween-BSA were added to the wells in duplicates and incubated 1 h at room temperature under shaking. After 4 washes with PBS-Tween, 100 μl portions of alkaline phosphatase labelled goat anti-human IgG were added to each well and incubation took place for 1 h. After 4 washes, 100 μl of TMB substrate buffer was added to each well and 10 min allowed for color development. The plates were read at 450 nm on a Molecular Devices ELISA reader linked to the Softmax computer program, and concentration calculated toward a calibration curve constituted from the standards. Arbitrary units were derived from a dilution curve using a strong positive patient serum. The cutoff for the assay is 10 units/ml in a normal control population. However, it is our experience that using cutoff levels derived from a normal population often leads to identification of false low concentration positive findings in patients with autoimmune diseases. For this reason, the cutoff was set to 20 units/ml, leading to increased specificity and delineation of the top quartile of the patient group.

Pulmonary function tests. Pulmonary function tests (PFT) were performed in all patients at the initial evaluation. All recordings were made with computerized Jaeger lung function equipment. The largest of 3 technically acceptable efforts was used to determine forced expiratory volume in the first second (FEV_1). Slow inspiratory vital capacity (VC) and the ratio between FEV_1/VC were also determined. A single breath helium–CO dilution technique was performed in 145 patients to determine their diffusing capacity of CO (DLCO). PFT results were given as percentages of the predicted values according to Quanjer[21].

VC and DLCO were considered abnormal if the values were < 80% of predicted values. An FEV_1/VC value < 70% was considered abnormal. Four mutually exclusive groups of patients were identified by means of the first PFT: patients with (1) isolated reduction of DLCO (≤ 80% of predicted) in whom VC and FEV_1/VC were within normal limits; (2) restrictive ventilatory pattern (VC ≤ 80% and FEV_1/VC ≥ 70%); (3) obstructive ventilatory pattern (FEV_1/VC < 70%); and (4) normal lung function, i.e., all values within normal limits[6].

Outcome variables. The outcome variables of this study, vital status, and the causes of death were determined prior to the specific serological tests, whereas presence and type of IIF ANA were known. The vital status at the end of the study ultimo 1996 was established by reviewing the clinical charts and by contacting the Danish Central Person Register (CPR) in which all Danish citizens are registered. The Danish CPR is a reliable source for vital status and dates of death. Causes of death were based on information obtained from hospital charts and autopsy reports (80%) or from death certificates (20%). A death was considered to be due to SSc when caused by either progressing active SSc manifestations or terminal organ failure caused by SSc, documented by the clinical charts. Patients with unknown cause of death were assumed to have died from causes not directly related to SSc since they had not presented signs of severe SSc related morbidity during the clinical followup before death.

Statistical analyses. The basic statistical processing of the study data was performed using the Epi Info 6 software package[22]. Comparison of categorical variables was performed by Fisher's exact test. Different strata of continuous variables were described and compared using nonparametric methods. Statistical significance was defined as $p < 0.05$. Estimation of survival and mortality rates was performed by lifetable analyses by calculating cumulative survival and hazard rates with their corresponding 95% confidence intervals (CI). Univariate comparison of survival was by log-rank test, multivariate survival analysis was by stepwise conditional Cox regression. Sex and age at onset (< or ≥ 50 years) were included in the model to correct for these demographic variables.

RESULTS

The basic characteristics of the 174 patients with SSc are shown in Table 1. The cohort represents 2315 patient-years of observation. During this period 66 patients died, equalling a crude mortality rate of 2.9% per year. The number of patients surviving 5, 10, and 20 years of observation were 139, 102, and 39, equalling survival rates of 90, 81, and 50% (Figure 1). Sixteen patients died of causes that could be linked directly to SSc (Table 2). Of the 16 patients, 6 died of right heart failure caused by primary pulmonary hypertension (n = 1) or secondary pulmonary hypertension due to pulmonary fibrosis (n = 5). Four more patients had right heart failure. These 4 also died, but due to other causes, i.e., acute myocardial infarction, pneumonia, respiratory insufficiency due to pulmonary fibrosis, and pulmonary embolism. Cardiovascular morbidity was the most common cause of death, accounting for 24 of 50 non-SSc deaths.

Table 3 shows how univariate analysis of various demographic, clinical, and laboratory features revealed that old age at disease onset, radiographic signs of pulmonary fibrosis, reduced DLCO below 40% of the expected value, diffuse cutaneous sclerosis, SSc renal crisis, right heart failure, anti-topo I antibody, and anti-RNAP antibody were associated with increased SSc related mortality. The all-cause mortality was associated with male sex, old age at onset, radiographic signs of pulmonary fibrosis, restrictive ventilatory pattern, reduced DLCO, esophageal dysfunction, myositis, right heart failure, and anti-RNAP antibody.

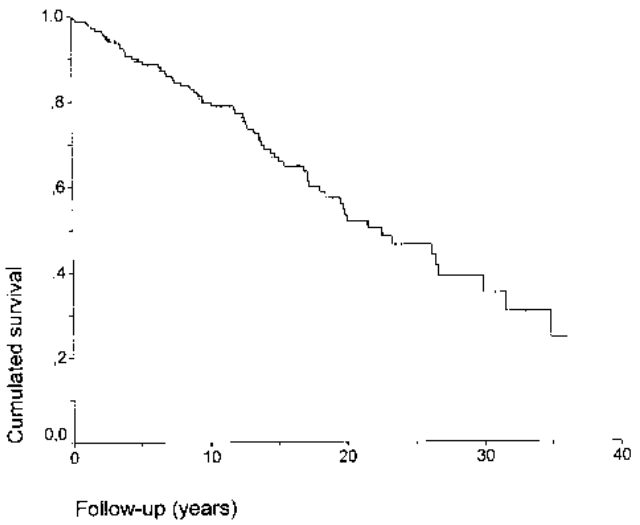

Figure 1. Survival among 174 Danish patients with systemic sclerosis from the time of first physician observed cutaneous sclerosis. Vertical bars indicate censored observations.

Conditional multivariate regression analysis of mortality correcting for sex and age using the variables from Table 3 as possible covariates showed that right heart failure, diffuse SSc, SSc renal crisis, and severely reduced DLCO independently contributed to increased risk of SSc related mortality (Table 4). Using the same approach with all-cause mortality, we found that this was related to right heart failure and a restrictive ventilatory pattern. None of the serological features were associated with either SSc related or all-cause mortality in these models. However, since anti-topo I antibody and anti-RNAP antibody were associated with increased SSc related mortality, we investigated the risk associations between these antibodies and the clinical features that had independent prognostic value with regard to SSc related mortality. We found that both antibodies were associated with right heart failure and diffuse SSc, the highest association being between right heart failure and anti-RNAP antibody (Table 5). There was also a highly increased relative risk of developing SSc renal crisis given the occurrence of anti-RNAP antibody; however, statistical significance was not achieved due to the small number of patients.

DISCUSSION

These patients make up the most recent and the most extensively examined part of a cohort consisting of 344 patients with incident SSc since the beginning of 1960[3]. The yearly mortality rate of the present cohort was lower than the one we found in our primary cohort of SSc patients, 2.9% versus 4.3%, respectively. This may indicate improved survival of Danish patients with SSc within the last 2 decades. However, the proportion of SSc related deaths was unchanged (24% versus 25%), although the number of deaths caused by renal crisis was reduced from 10.6% to 4.5%, probably as a consequence of treatment with angiotensin converting enzyme inhibitors, as noted by others[23]. As emphasized by Silman[9], it may not always be possible to distinguish between the SSc related and unrelated mortality. This assumed uncertainty was also discussed in our study of the survival of patients with SSc[3], which showed that the expected SSc unrelated mortality was twice that of the background population. However, it may still be of pathogenetic interest to focus on the part of the mortality generally regarded as primarily related to SSc. Thus, in this study the predictive value of risk factors for early death was calculated for the presumed SSc related mortality as well as for the total mortality.

As previously found, mortality increased with male sex and increasing age at the time of disease onset[4,9]. However, such factors need to be adjusted for effects carried over from the corresponding background population. We previously found that the standardized mortality rates (SMR) were not significantly different for men and women, and that the SMR actually were highest for patients with disease onset at an early age[3]. Several studies have confirmed that diffuse SSc is predictive of increased mortality[9], but in the present study this finding was significant only with regard to the SSc related

Table 1. Basic demographic data of 174 patients with systemic sclerosis.

	All Patients	Surviving Patients	Deceased Patients	p*
No. of patients	174	108	66	—
Sex, no. (%)				
Male	28 (16)	13 (12)	15 (23)	
Female	146 (84)	95 (88)	51 (77)	0.10
Age at onset of CS, yrs	50	44	57	
Median (range)	(12–80)	(12–76)	(27–80)	0.00
Duration of CS, yrs	12.0	12.2	12.0	
Median (range)	(0.1–36)	(1.2–36)	(0.1–35)	0.12
Skin involvement, no. (%)				
Limited	117 (67)	78 (72)	39 (59)	
Diffuse	57 (33)	30 (28)	27 (41)	0.10

* Chi-square test. CS: cutaneous sclerosis.

Table 2. Primary causes of death in 66 patients with systemic sclerosis.

	No. of Patients
Directly SSc related mortality (n = 16)	
Right heart failure due to pulmonary fibrosis	5
Right heart failure due to primary pulmonary hypertension	1
Cachexia due to malabsorption	4
SSc renal crisis	3
Esophageal dysfunction	2
Respiratory insufficiency due to pulmonary fibrosis	1
Other mortality (n = 50)	
Infections	
Pulmonary	5
Septicemia	1
Malignancy	
Lung cancer	7
Hematological malignancy	2
Breast cancer	2
Other	3
Cardiovascular	
Heart failure due to ischemic heart disease	6
Acute myocardial infarction	5
Pulmonary embolism	5
Cerebral thrombosis	6
Lower extremity gangrene	2
Other	5
Unknown	1

mortality. Yet in a larger cohort of patients with SSc we also observed a significant effect on the all-cause mortality[3].

The predictive value of pulmonary involvement was estimated by means of 5 variables. In this study, patients with restrictive disease had increased mortality. However, patients with radiographic signs of pulmonary fibrosis had an even worse prognosis, particularly with regard to SSc related mortality. These findings are most likely explained by the fact that patients with radiographic pulmonary fibrosis have more severe restrictive disease than those without, and that the poor prognosis of patients with restrictive disease is mainly borne by those having severe restrictive disease[7]. This is also indicated by our findings of severely reduced DLCO being a marker of poor prognosis. Only 2 of the 11 patients with severely reduced DLCO had an isolated DLCO reduction. Isolated reduction of DLCO, which also included milder cases, was not found to influence mortality. Neither was an obstructive ventilatory pattern found to be of importance for mortality. In the general population, one would expect obstructive lung disease to predict decreased survival. However, in this cohort of SSc patients other types of pulmonary involvement in the remaining patients may mask the influence of obstructive lung disease.

Ten patients had signs of right heart failure, which was highly associated with the SSc related mortality as well as with total mortality in this study. In 8 cases the right heart failure was due to pulmonary fibrosis and in 2 cases most likely due to primary pulmonary hypertension, as these patients had an isolated reduction of DLCO. Several studies[4,24,25] have stressed the importance of cardiac disease in SSc. These studies have also shown that esophageal

Table 3. Univariate analysis of mortality by demographic, clinical, and laboratory features in 174 patients with systemic sclerosis.

	All	Surviving	SSc Related Mortality		Mortality, all causes	
	no. (%)	no. (%)	no. (%)	p*	no. (%)	p*
No. of patients	174	108	16		66	
Male sex	28 (16)	13 (12)	3 (19)	NS	15 (23)	0.04
Age at onset of CS < 50 yrs	83 (48)	38 (35)	13 (81)	0.0003	45 (68)	0.0000
Pulmonary variables						
Pulmonary fibrosis on radiograph	37 (21)	11 (10)	10 (63)	0.0000	26 (39)	0.0000
Restrictive ventilatory pattern	48 (28)	22 (20)	5 (31)	NS	26 (39)	0.003
Obstructive ventilatory pattern	25 (14)	14 (13)	3 (19)	NS	11 (17)	NS
Isolated reduction of DLCO	62 (36)	41 (38)	7 (44)	NS	21 (32)	NS
DLCO reduced to < 40% of predicted	11 (6.3)	1 (0.9)	4 (25)	0.0001	10 (15)	0.0000
Other preexisting organ involvement						
Diffuse SSc	57 (33)	30 (28)	11 (69)	0.001	27 (41)	NS
Esophageal dysfunction	67 (39)	31 (28)	10 (63)	NS	36 (55)	0.05
Myositis	13 (7.5)	7 (6.5)	2 (13)	NS	6 (9.1)	0.05
SSc renal crisis	3 (1.7)	1 (0.9)	1 (6.3)	0.05	2 (3.0)	NS
Right heart failure	10 (5.7)	0	7 (44)	0.0000	10 (15)	0.0000
Antibody serology						
Antitopoisomerase I	22 (13)	11 (10)	5 (31)	0.04	11 (17)	NS
Anticentromere	64 (37)	45 (42)	6 (38)	NS	19 (29)	NS
Anti-U1-RNP	7 (4.0)	3 (2.8)	0	NS	4 (6.1)	NS
Anti-U3-RNP	8 (4.6)	7 (6.5)	0	NS	1 (1.5)	NS
Anti-Th-RNP	4 (2.3)	3 (2.8)	0	NS	1 (1.5)	NS
Anti-RNAP	39 (22)	17 (16)	9 (56)	0.0002	22 (33)	0.01

* Log-rank test using disease duration as time factor. NS: no statistically significant difference, p > 0.05. CS: cutaneous sclerosis.

Table 4. Conditional Cox regression analyses of mortality using variables from Table 3 as possible covariates corrected for sex and age.

	SSc Related Mortality		All-Cause Mortality	
	RR	95% CI of RR	RR	95% CI of RR
Covariates included in model				
Right heart failure	12.4	2.5–60	6.0	2.8–13
Diffuse SSc	7.8	1.8–35	—	
SSc renal crisis	6.1	1.8–21	—	
DLCO < 40%	4.8	1.1–20	—	
Restrictive ventilatory pattern	—		2.0	1.2–3.4

RR: relative risk.

Table 5. Association between selected autoantibodies and development of clinical features found to carry independent prognostic information with regard to SSc related survival.

	Antitopoisomerase I, n = 22			Anti-RNAP, n = 39		
	No. (%)	RR	p	No. (%)	RR	p
Right heart failure, n = 10	4 (18)	4.6	0.02	8 (21)	14	0.0001
Diffuse SSc, n = 57	13 (59)	2.0	0.01	20 (51)	1.9	0.01
SSc renal crisis, n = 3	0	0	NS	2 (5.1)	6.9	NS
DLCO < 40%, n = 11	3 (14)	2.6	NS	4 (10)	2.0	NS

RR: relative risk.

dysmotility and myositis were associated with early death, just as in the present study.

We previously showed how mortality not directly related to SSc was about twice the expected in patients with SSc[3]. As in our previous study, the most frequent cause of non-SSc death was cardiovascular disease, which again was of ischemic, atherothrombotic type in 19 out of 24 cases. Further, symptomatic macrovascular disease has been reported to occur with increased prevalence in SSc[26]. Atherothrombotic risk factors to be considered in SSc are oxidized low density lipoproteins (LDL)[27] as a result of increased oxidative stress in SSc[28]. Although a causal role of oxidized LDL in atherosclerosis has not been established, it is hypothesized that oxidized LDL may contribute to the progression of atherosclerosis by inducing foam cell generation and smooth muscle proliferation[29].

The prognostic value of 2 different ANA regularly found in sera from patients with SSc was also examined. The presence of circulating antibodies against RNAP I, II, and/or III was associated with both SSc related and all-cause mortality. Anti-topo I antibodies were also associated with the SSc related mortality, but less so than anti-RNAP antibodies, which is in agreement with a study[30] in which antibody against RNAP was particularly related to cardiac and renal death. In that study, the prevalence of anti-RNAP antibody was 5%. In our study, the prevalence of anti-RNAP was 22%. The differing prevalences may readily be explained by differences in laboratory methods of measuring anti-RNAP, namely, that our assay measured the presence of anti-RNAP I, II, and/or III. Also, the clinical profiles of the anti-RNAP positive patients differed somewhat between the 2 studies. In our cohort, about half the patients with anti-RNAP had diffuse SSc, whereas others have described prevalences up to 80–90%[30,31]. However, our study did confirm the previously described association between anti-RNAP and diffuse SSc. The total number of patients with SSc renal crisis in our study was only 3, and we did not find a statistically significant association between anti-RNAP and SSc renal crisis. However, the relative risk of developing SSc renal crisis was 6.9 given the presence of anti-RNAP, which is also in accord with previous findings[30,31]. The association between anti-RNAP and right heart failure was highly significant, but remains unexplained, since we did not find statistically significant associations between anti-RNAP and signs of restrictive pulmonary disease, the most common cause of right heart failure in this study. However, the results do comply with the previously described association between anti-RNAP and cardiac death[30].

Others have shown the predictive value of anti-topo I antibodies for survival[4,5,25,32] — they are associated with diffuse SSc, pulmonary fibrosis, and restrictive lung disease[5,32-35]. In contrast to anti-RNAP and anti-topo I antibody, the presence of anticentromere antibody had no predictive importance for mortality, which agrees with previous studies[5,30,32]. In one study, patients with anticentromere antibody even had a better survival rate than patients without these antibodies[25]. We could not determine any mortality related significance of anti-U1-RNP or anti-Th-RNP antibodies.

No novel associations to mortality in SSc have been found in these univariate analyses, but several previously described associations have been confirmed, and we therefore proceeded with multivariate analyses to identify factors of particular importance for survival in the present cohort. First, none of the serological markers seemed to carry any independent prognostic information in the presence of certain clinical features. Neither did all the significant clinical features from the univariate analyses emerge as significant in the multivariate analyses. The risk contribution of radiographic signs of pulmonary fibrosis seemed to be overcome by the presence of severely reduced DLCO and/or right heart failure. However, this only shows us that severely reduced DLCO and/or right heart failure had a greater influence on mortality than radiographic signs of pulmonary fibrosis, which still are clinically significant findings in the absence of the other pulmonary variables. This also applied for the serological variables, anti-topo I and anti-RNAP

antibody. The prognostic strength of these antibodies may therefore lie in the early course of disease, when specific morbidity has not yet evolved. Further, as suggested by Table 5, the serological findings may guide the clinician to focus on particular risk manifestations during clinical followup in the individual patient, and thus provide an opportunity to treat such manifestations early.

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REFERENCES

1. Abu-Shakra M, Lee P. Mortality in systemic sclerosis: a comparison with the general population. J Rheumatol 1995;22:2100-2.
2. Bryan C, Howard Y, Brennan P, Black C, Silman A. Survival following the onset of scleroderma: results from a retrospective inception cohort study of the UK patient population. Br J Rheumatol 1996;35:1122-6.
3. Jacobsen S, Halberg P, Ullman S. Mortality and causes of death of 344 patients with systemic sclerosis. Br J Rheumatol 1998; 37:750-5.
4. Altman RD, Medsger TA, Bloch DA, Michel BA. Predictors of survival in systemic sclerosis (scleroderma). Arthritis Rheum 1991;34:403-13.
5. Kuwana M, Okano Y, Kaburaki J, Tojo T, Medsger TA. Racial differences in the distribution of systemic sclerosis related serum antinuclear antibodies. Arthritis Rheum 1994;37:902-6.
6. Steen VD, Graham G, Conte C, Owens G, Medsger TA. Isolated diffusing capacity reduction in systemic sclerosis. Arthritis Rheum 1992;35:765-70.
7. Steen VD, Conte C, Owens GR, Medsger TA. Severe restrictive lung disease in systemic sclerosis. Arthritis Rheum 1994;37:1283-9.
8. Barnett AJ, Miller MH, Littlejohn GO. A survival study of patients with scleroderma diagnosed over 30 years (1953-1983): The value of a simple cutaneous classification in the early stages of the disease. J Rheumatol 1988;15:276-83.
9. Silman AJ. Scleroderma and survival. Ann Rheum Dis 1991;50:267-9.
10. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
11. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol 1988;15:202-5.
12. Humbel RL. Detection of antinuclear antibodies by immunofluorescence. In: van Venroij WJ, Maini RN, editors. Manual of biological markers of disease – A2. Dordrecht: Kluwer Academic Publishers; 1993:1-16.
13. Høier-Madsen M, Andersen MG. Microhaemagglutination test for detection of antibodies to "extractable nuclear antigen" (ENA). Ugeskr Laeger 1980;142:2627-30.
14. Verheijen R, Wiik A, De Jong BAW, et al. Screening for autoantibodies to the nucleolar U3- and Th(7-2) ribonucleoproteins in patients' sera using antisense riboprobes. J Immunol Methods 1994;169:173-82.
15. Dahmus MF. Purification and properties of calf thymus casein kinases I and II. J Biol Chem 1981;256:3319-25.
16. Hodo HGI, Blatti SP. Purification using polyethylenimine precipitation and low molecular weight subunit analyses of calf thymus and wheat germ DNA-dependent RNA polymerase II. Biochem 1977;16:2334-43.
17. Guifoyle TJ, Hagen G, Malcolm S. Size heterogeneity of the largest subunit of nuclear RNA polymerase II. J Biol Chem 1984;649-53.
18. Jaehning JA, Woods PS, Roeder RG. Purification, properties and subunit structure of deoxyribonucleic acid-dependent ribonucleic acid polymerase III from uninfected and adenovirus 2-infected KB cells. J Biol Chem 1977;8762-71.
19. Kovelman R, Roeder RG. Sarkosyl defines three intermediate steps in transcription initiation by RNA polymerase III: application by stimulation of transcription by EIA. Genes Development 1990;4:646-58.
20. Gottlieb E, Steitz JA. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. EMBO J 1989;8:851-61.
21. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report of Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J 1993; 16 Suppl:5-40.
22. Dean AG, Dean JA, Coulombier D, et al. Epi Info, Version 6: a word processing, database, and statistics program for epidemiology on microcomputers. Atlanta: Centers for Disease Control and Prevention; 1994.
23. Langevitz P, Buskila D. Scleroderma hypertensive renal crisis and the changing pattern of mortality in systemic sclerosis (scleroderma). Nephron 1991;57:111-2.
24. Czirják L, Nagy Z, Szegedi G. Survival analysis of 118 patients with systemic sclerosis. J Intern Med 1993;234:335-7.
25. Nishioka K, Katayama I, Kondo H, et al. Epidemiological analysis of prognosis of 496 Japanese patients with progressive systemic sclerosis. J Dermatol 1996;23:677-82.
26. Veale DJ, Collidge TA, Belch JJF. Increased prevalence of symptomatic macrovascular disease in systemic sclerosis. Ann Rheum Dis 1995;54:853-5.
27. Bruckdorfer KR, Hillary JB, Bunce T, Vancheeswaran R, Black CM. Increased susceptibility to oxidation of low-density lipoproteins isolated from patients with systemic sclerosis. Arthritis Rheum 1995;38:1060-7.
28. Herrick AL, Cerinic MM. The emerging problem of oxidative stress and the role of anti-oxidants in systemic sclerosis. Clin Exp Rheumatol 2001;19:4-8.
29. Holvoet P. Endothelial dysfunction, oxidation of low-density lipoprotein, and cardiovascular disease. Ther Apher 1999;3:287-93.
30. Kuwana M, Kaburaki J, Okano Y, Tojo T, Homma M. Clinical and prognostic associations on serum antinuclear antibodies in Japanese patients with systemic sclerosis. Arthritis Rheum 1994;37:75-83.
31. Harvey GR, Butts S, Rands AL, Patel Y, McHugh NJ. Clinical and serological associations with anti-RNA polymerase antibodies in systemic sclerosis. Clin Exp Immunol 1999;117:395-402.
32. Steen VD, Powell DL, Medsger TA. Clinical correlations and prognosis based on serum antibodies in patients with systemic sclerosis. Arthritis Rheum 1988;31:196-203.
33. Jacobsen S, Halberg P, Ullman S, et al. Clinical features and serum antinuclear antibodies in 230 Danish patients with systemic sclerosis. Br J Rheumatol 1997;37:39-45.
34. Jacobsen S, Halberg P, Ullman S, et al. A longitudinal study of pulmonary function in Danish patients with systemic sclerosis. Clin Rheumatol 1997;16:384-90.
35. Catoggio LJ, Bernstein RM, Black CM, Hughes GRV, Maddison PJ. Serological markers in progressive systemic sclerosis: clinical correlations. Ann Rheum Dis 1983;42:23-7.