Survival, risk assessment and causes of death in a series of 439 patients with systemic sclerosis

Ph.D. thesis

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1. BAKGROUND

Systemic sclerosis (SSc) is a connective tissue disease characterised by autoimmune phenomena, generalised vasculopathy and fibrosis. As a hallmark of SSc the skin involvement is characterized by thickening, hardening and tightening of the skin. Heterogeneous clinical manifestations include skin, musculoskeletal and internal organ involvement. The cardiopulmonary and renal manifestations are the main causes of mortality, while skin, gastrointestinal and musculoskeletal involvement mainly cause disability and reduce quality of life. The onset of SSc is usually in the forties, therefore the typical cases are middle aged or elderly women, but in all age groups it can develop. In these particular age groups the presence of certain comorbidities may also substantially influence the outcome of the disease.

Previous studies showed that older age at onset, male gender, diffuse cutaneous SSc subset, interstitial pulmonary involvement (ILD), decreased forced vital capacity (FVC) and/or diffusing capacity of carbon monoxide (DLCO), primary pulmonary arterial hypertension (PAH), cardiac and renal involvement were associated with poor outcome. Certain gastrointestinal manifestations may also be associated with poor prognosis. Elevated erythrocyte sedimentation rate (ESR), anemia, and hypoalbuminemia were also found to predict a poor outcome. Furthermore, presence of anti-topoisomerase autoantibody (anti-Scl70) was associated with poor survival. Conversely anti-centromere antibody (ACA) positivity was associated with a rather favorable outcome. In general, involvement of the internal organs including the lungs, heart and kidney are the major determinants of the outcome of the disease, thus several further factors have been discovered as additional determinant of the outcome of SSc. Furthermore, certain comorbidities including arterial hypertension, and coexistent malignancy have also been identified as poor prognostic factors of SSc. Our previous study also showed that a synchronous appearance of a malignancy was an independent predictor of mortality.

Because of the extreme variability of disease course at individual patient level, it is crucial to perform risk assessment and define prognostic groups. The most important element of the risk assessment is to define subsets within SSc with high risk to develop certain organ involvement. This is the only approach to start a therapeutic intervention as early as possible. SSc is an orphan disease, therefore both single center and multicentre databases are required to achieve the sufficient number of patients to obtain reliable data for risk assessment.

There are three major SSc-specific autoantibodies which are mutually exclusive. Out of these three particular autoantibodies, the impact on the clinical outcome is well characterized in cases with either ACA or anti-Scl70. The presence of ACA was found to be protective against interstitial lung involvement whereas anti-Scl70 was correlated with digital ulcers and the development of ILD. In general, ACA is a favorable and anti-Scl70 is a poor prognostic marker of survival. Anti-RNA polymerase III antibody (anti-RNAP3) is the third “classic” scleroderma specific autoantibody which may be associated with PAH, scleroderma renal crisis (SRC), gastric vascular ectasia (GAVE) and tumor development, joint pain and puffy hands. There is a remarkable variability in both the clinical presentation and the geographic distribution of SSc cases with this particular autoantibody, therefore it is an unmet need to better characterize the anti-RNAP3 antibody associated clinical manifestations. To further clarify the clinical importance of the presence of anti-RNAP3, large multicentre studies

should be performed because of the small number of patient with anti-RNAP3+ cases in the individual databases.
Autopsy finding still remains important because in many cases only autopsy can reveal or verify organ involvements, and remarkable discrepancies have been described between clinical and autopsy findings. This particular discordance between clinical findings and autopsies was up to 63%, and regarding the main diagnosis leading to death up to 33.6%. Unfortunately, autopsies are recently rarely performed, therefore the only approach to analyse autopsy findings is to analyse data of large multicentre databases.
There is an unmet need for introducing new serum biomarkers which are useful for the risk assessment of the different organ manifestations and prediction of disease outcome. Regarding the heart involvement, the diagnostic and prognostic value of B-type natriuretic peptides (NT-proBNP) is well established in SSc. Elevated level high sensitivity troponin and NT-proBNP are associated with poor outcome.
There is a need to develop new serum biomarkers useful for risk assessment in patients with SSc. Recent clinical studies suggest that galectin-3 may be related to the development of skin and organ sclerosis as well as to the aberrant activation of angiogenesis in SSc.

2. AIMS

2.1 Our major aim was to analyze the survival, causes of death and risk factors affecting mortality in a large series of patients with SSc followed up in a university tertiary care center in Hungary. Our further aim was also to investigate whether there is an improvement in the survival in the last decades compared to the previous Hungarian and international follow up studies. Furthermore, we also aimed to compare autopsy results of SSc patients with our clinical finding in the framework of a European League Against Rheumatism Scleroderma Research and Trials (EUSTAR) study group organized for this particular purpose.

2.2 Our second aim was to identify new risk factors of poor survival in our cohort. We focused on the musculoskeletal system related risk factors and comorbidities.

2.3 We aimed to analyze the characteristics of anti-RNA polymerase III antibody positive patients with systemic sclerosis in the framework of the EUSTAR registry with a special focus on the risk of cancer development. Comparison between our local cohort cases and EUSTAR registry based patients was also performed.

2.4 Our further aim was to identify new serum biomarkers that are useful in risk assessment. We focused on the evaluation of serum galectin-3 levels. Our major question was that besides increased serum NT-proBNP levels, increased galectin-3 level is also a predictor of mortality in SSc.

3. PATIENTS AND METHODS

3.1. Investigation of patients

3.1.1. Survival and risk factor analysis in 439 patients with systemic sclerosis

The follow up investigation of the outcome was carried out in our tertiary care centre including patients who attended our tertiary center between 1995 and 2015. Baseline data were collected prospectively in our database, thus in some cases retrospective data analysis was also performed. Patients lost to follow up was defined as patients who did not appear in the center for twelve months after their last visit. In order to clarify the reason of the patients' absence in the follow up, telephone calls were made and letters were sent to patients and if possible to the local GP. The diagnosis of SSc based on American College of Rheumatology (ACR) preliminary classification criteria were found in 469 cases, and only 30 patients were considered lost to follow up (6.4%).

Cause(s) of death were defined based on last discharge papers, consultation with the patients' GP and autopsy results. Each cause of death was extensively discussed, and final agreement was achieved among the three participating investigators.

ILD was documented in case of fibrosis detected by high resolution computer tomography (HRCT) and concurrent decreased forced vital capacity (FVC<80%). Extensive interstitial lung involvement was recorded in case of either the fibrosis affected the upper and middle area of the lungs on HRCT and/or FVC<50% or honeycombing was detected by HRCT. Cardiac involvement was recorded if the patient had at least one of the following conditions: decreased ejection fraction <50% (EF), elevated right ventricular pressure detected by echocardiography (>40 mmHg - except in patient with severe pulmonary involvement), relaxation disorder (defined by the cardiologist evaluation based on E/A ratio), abnormal electrocardiogram (ECG) (arrhythmia, conduction disturbances, brady- or tachycardia; heart rate consistently <60/min or higher than 85/min confirmed by cardiologist that it is heart manifestation related). Primary pulmonary arterial hypertension (PAH) was recorded if elevated mean pulmonary arterial hypertension was verified by right heart catheterization (RHC). Patients having elevated right ventricular pressure on echocardiography were referred to RHC by the cardiologist based on a standard protocol. Esophageal involvement was documented if the patient had dysphagia and/or barium-swallowing x-ray showed hypomotility and/or strictures/dilatation. SRC (including the normotensive form) was defined by the agreement of two experts based on the available definition. Small joint contractures were recorded if range of motion was less than 75% of normal in the metacarpophalangeal and proximal interphalangeal joints evaluated by rheumatologists of our tertiary care center. Systemic arterial hypertension was documented if hypertension was diagnosed previously or diagnosis of hypertension was established simultaneously with SSc. Coexistent malignancy was documented in cases when time between malignancy and SSc onset was maximum 5 years. Abnormal nailfold capillaroscopic pattern was registered in presence of giant capillaries, hemorrhage, capillary loss or signs of neoangiogenesis.

Antinuclear, anti-centromere and anti-Scl-70 antibodies were detected by conventional enzyme linked immunosorbent assay (ELISA) method. Anti-RNAP3 antibodies were detected by immunoblot technique.

Regarding the study on comparison of clinical findings and autopsy results in July 2014, the European Scleroderma Trials and Research Group (EUSTAR) database was reviewed for deceased SSc patients. Participating centres were invited to fill out a standardised autopsy questionnaire for patients who underwent autopsy.

3.1.2. Clinical characterisation of anti-RNA polymerase3+ patients regarding tumor development

EUSTAR is a multinational unique database collecting prospectively and longitudinally SSc patients' data. Data for current study were extracted from the registry in March 31, 2014, when 11,399 patients from 118 centers fulfilling either the 1980 ACR or the 2013 ACR/EULAR classification criteria. Patients were included in the study when anti-RNAP3 status was available in at least 1 visit. Patients positive both for anti-RNAP3 and for other SSc-specific antibodies (ACA or anti Scl70 antibodies) were excluded from comparisons between anti-RNAP3+ and anti-RNAP3– patients. 4986 patients from the EUSTAR database with information on their anti-RNAP3 status were included. As a case-control study, 158 anti-RNAP3+ cases were evaluated. The participating centres were asked to provide data of the 199 local matched control cases. Some additional retrospective data were also queried in participating centers.

Our center contributed to this particular study with the data of 21 anti-RNAP3+ SSc patients (10.6% of all cases) and 21 control patients negative for anti-RNAP3.

3.1.3. Study on galectin-3 and NT-pro-BNP levels on survival

Blood samples of patients were collected for storage between 1st January 2005 and 31st December 2008. Baseline clinical, laboratory, spirometric and echocardiographic data were collected at the same time. Characterization of left ventricular diastolic function was based on mitral inflow pattern (E/A ratio), left ventricular wall thickness and left atrial size.

Follow-up time was defined in this study as the time between the date of blood sample collection and the date of death or the last clinical visit.

Significant coronary artery disease was defined as coronary artery stenosis <50% proved by invasive measurements or as history of previous myocardial infarction. The diagnosis of pulmonary arterial hypertension was based on results obtained by RHC (mean pulmonary artery pressure $\geq$ 25 mmHg and pulmonary capillary wedge pressure $\leq$ 15 mmHg). Patients with both transient and chronic atrial fibrillation were recorded. Severe pulmonary involvement was diagnosed when diffuse fibrosis or honeycombing was detected by high resolution CT and FVC <50% was measured by spirometry.

Analysis of galectin-3 levels was performed using ELISA method and NT-proBNP was measured using chemiluminescence immunoassay.

3.2. Statistical Analysis

For statistical analysis Kaplan-Meier curves and log-rank test were used to determine the survival and factors affecting the survival. Items found to be significant according to univariate analysis were further tested with to Cox proportional hazard model to examine the independent prognostic factors. Comparisons between groups were performed using independent samples t-tests or Mann-Whitney test for continuous variables while chi square test for categorical variables.

Regarding the galectin-3 studies, clinical variables that correlate with galectin-3 level were determined using bivariate Pearson correlation. As first step, age correction was performed using partial correlation analysis. In a second step, age, gender and BSA were used as correcting factors. Since concentration of galectin-3 and NT-proBNP did not show normal distribution, logarithmic transformation was performed.
Relationship between logarithmic transformed galectin-3 and mortality was investigated by using Cox proportional hazards models, including adjustment for age, gender, and BSA. Finally, lnNTproBNP was also added to the model, given its known association with outcomes in systemic sclerosis.
Receivers operating characteristic (ROC) curves were used to examine the performance of galectin-3 and NT-proBNP in predicting all-cause mortality. Area under the curve (AUC) was calculated from the ROC curve. Optimal cut-off value was chosen to maximize sensitivity and specificity. Kaplan-Meier survival curves were created and differences between groups were tested by Mantel-Cox log rank test.
Regarding the RNAP3 studies, the statistical analysis included chi-square test with Pearson correction or Fisher's exact test, and continuous variables using the Student t test, Mann-Whitney U test, or ANOVA, as appropriate. A multivariate logistic regression analysis (adjusted for sex, age, and disease duration) was also performed. Besides a priori potential confounders, variables associated with $p < 0.05$ in univariable analysis were considered. Bonferroni correction for multiple comparison was applied. The Kaplan-Meier method and the log-rank test were applied to analyse the progression of mRSS and survival.
For all our analysis, version 16.0 SPSS for Windows was used (Inc., Chicago, IL, USA).

3.3. Ethics

The study on prognostic analysis was approved by the Regional Ethics Committee (2939/2007). Analysis on the prognostic value of different heart related biomarkers was also approved by the Regional Ethics Committee 5338/2014, and analysis of patients enrolled to EUSTAR database was approved by the Hungarian National Ethics Committee (430/PI/2012 and 426/2013). Written informed consent was obtained from patients according to the Declaration of Helsinki.

4. RESULTS

4.1. Survival and risk factor analysis in 439 patients with systemic sclerosis

4.1.1. Major clinical findings of the follow up study

The median age at onset was 46 years. Diffuse cutaneous SSc (dcSSc) was present in 179 (40.8%) cases. Seventy-three (16.6%) male patients were enrolled. Anti-Scl70 positivity was found in 157 cases (35.9%) and ACA positivity in 113 cases (25.9%). Coexsistent malignancies were present in 36 cases, twelve out of these patients had anti-Scl70 positivity, 10 ACA positivity and 2 anti-RNAP3 positivity. Patients with coexistent malignancy had significantly worse survival by univariate analysis compared to those without coexistent malignancy ($p<0.0001$).

4.1.2. Mortality

The all-cause survival was 88.2% at 5 years, 80.8% at 10 years, 67.5% at 15 years and 31.6% at 20 years, respectively. When only the SSc related causes of death were taken, the survival rate showed 95.6% at 5 years, 87.5% at 10 years and 74.2% at 15 years, respectively. When fatal outcome caused by overlap syndrome and/or coexistent malignancy was added to the strictly SSc-related causes of death we found 88.1% survival at 5 years, 79.9% at 10 years, 73.6% at 15 years and 63.4% at 20 years.

4.1.3. Univariate analysis

Univariate analysis showed that dcSSc, male gender, presence of small joint contractures, ILD, cardiac involvement, elevated right ventricular pressure on echocardiography, less than 50% EF and ECG abnormalities, esophageal involvement, scleroderma renal crisis, history of hypertension, anti-Scl70 positivity, low hemoglobin, hematocrit and albumin levels, elevated ESR, coexistent and current or previously diagnosed malignancies were associated with poor prognosis. The presence of ACA and lack of giant capillaries, microhemorrhages neoangiogenesis or avascularity showed a favorable outcome.

4.1.4. Multivariate analysis

Cox analysis showed that male gender, presence of topoisomerase I antibody, DLCO and FVC<70%, presence of small joint contractures, more than 40 mmHg right ventricular pressure on echocardiography, ECG abnormalities, history of arterial hypertension, low hematocrit and albumin levels and presence of malignancies predict poor outcome.

Cox analysis, performed with exclusion patients died of SSc unrelated causes of death showed similar pattern, but arrhythmias, decreased FVC were no more independent predictors of mortality, while less than 50% of ejection fraction predicted worse outcome. Further exclusion of patients who died of paraneoplastic syndrome and overlap syndromes showed on multivariate analysis that abovementioned parameters with exception of malignancies and <70% DLCO and FVC predicted poor outcome.

4.1.5. Analysis of cause of death

During the follow up 106 patients died, 77 (72.6%) because of SSc. In ten SSc related cases it was impossible to make distinction between different organ systems, multiple causes of death were declared. Sixteen patients' death was attributed to cardiac involvement, 8 to PAH, 13 to ILD, 2 to scleroderma renal crisis, 6 to gastrointestinal involvement and 5 treatment-related infections. Out of the 17 cases of death due malignancies 12 patients died of coexistent malignancy and 5 because of other tumors, but connection between the onset of malignancy and SSc was clear, mainly due to therapy used for SSc.

4.1.6. Comparison of early and late onset SSc

Thirty three patients with SSc were followed up had Raynaud symptom (RP) onset under age 20. Patients having early onset SSc had a significantly more impaired FVC (<80%, 70% and 50%), conduction disturbances detected by ECG, and low body mass index (BMI) compared to cases with a disease onset between 21 and 64 years. Kaplan-Meier analysis revealed that poor survival was associated with the presence of FVC<70% and DLCO<70%, renal involvement, elevated ESR, decreased hemoglobin and hematocrit levels.

Out of the 439 patients 38 developed RP after the age of 65. When comparing clinical features of patients having late onset SSc we found significantly more patients having cardiac involvement including elevated right ventricular pressure on echocardiography, ECG disturbances compared to patients with RP developed between age 21 and 64.

4.1.7. Comparing autopsy results to clinical findings

The entire EUSTAR investigation consisted of 11 patients' data who deceased between 2007 and 2014. Cause of death defined by pathologist and clinician were identical in nine out of eleven cases. Seven deaths were defined as SSc-related by the clinician, whereas only five deaths were classified as SSc-related by the pathologist after autopsy. Regarding this particular investigation one patients' data were sent for further EUSTAR analysis from our tertiary care center. The pathological and clinical cause of death was not identical in this particular case.

4.2. Clinical associations of anti- RNAP3+ cases based on the analysis of the EUSTAR database

4.2.1. Clinical associations of anti-RNAP3+ patients

Two hundred and twenty three patients (4.5%) were reported as anti-RNAP3+, whereas 4763 were always reported as anti-RNAP3– in the EUSTAR database. In our local registry 21 patients fit the enrolment criteria out of 317 cases followed up at that time at our center (anti-RNAP3 +, enrolled to EUSTAR). The same number of controls fitting the control criteria was also collected.

Regarding the whole EUSTAR investigation, in univariable analysis, positivity for anti-RNAP3 was associated with male sex ($p < 0.0001$), arterial hypertension ($p = 0.03$), diffuse cutaneous involvement ($p < 0.0001$), renal crisis ($p < 0.0001$), and joint contractures ($p < 0.0001$). In the multivariable model, anti-RNAP3 positivity was independently associated with renal crisis ($p < 0.0001$) and diffuse cutaneous involvement ($p < 0.0001$).

4.2.2. Association of anti-RNAP3 with cancer based on the case-control study

Thirteen EUSTAR centers participated in the case-control study, collecting retrospective data from 158 anti-RNAP3+ SSc cases and 199 anti-RNAP3– local SSc controls. The interval between diagnosis and the last visit available was shorter in anti-RNAP3+ cases than in controls.

There was no difference in the number of deaths and their causes between cases (n = 25: 13 due to SSc, 10 to cancer, 2 to other reasons) and controls (n = 31: 15 due to SSc, 6 to cancer, 8 to other reasons, and 2 unknown). Cumulative survival was not different between the 2 groups.

Regarding our local anti-RNAP3+ and the local control cases, there was no difference in the number of death. In the anti-RNAP3+ group 5 patient deceased, causes of death were PAH in two cases, lung cancer in one case, pericardial tamponade in one case and in one case ILD. In the control group only one patient died, its cause of death was lung cancer. The 5 year survival showed no statistical difference.

Regarding the total EUSTAR investigation, in multivariable analyses, anti-RNAP3 positivity was negatively associated with gastroesophageal reflux disease (p = 0.003), but was positively associated with renal crisis (p = 0.0005) and GAVE (p = 0.0009).
The overall rate of malignancies was higher in anti- RNAP3+ patients than in controls (17.7% vs 9.0%, p = 0.015). In particular, cancers synchronous with SSc were more frequent, considering those diagnosed either between 6 months before and 12 months after SSc onset (7.0% vs 1.0%, p = 0.004), or within a larger time interval extended to 2 years before and after SSc onset (9.0% vs 2.5%, p = 0.007). Looking at the malignancy type, the frequency of solid tumors in anti-RNAP3+ patients was higher than in controls (p = 0.012), particularly for breast cancers (p = 0.03).
Regarding our local data no significant difference was observed on comparing detailed gastrointestinal symptoms and the presence of malignancies, neither on peak mRSS. In our local cohort, only three patients had malignancies, none of them had an early onset of synchronous, coexistent malignancy.

4.4. Effect of serum galectin-3 and NT-proBNP levels on the outcome of SSc

4.4.1. Correlations of galectin-3 and NT-proBNP with clinical variables

Both biomarkers showed positive correlation with age. NT-proBNP levels significantly correlated with right ventricular pressure and with the diagnosis of PAH. Both biomarkers correlated positively with the grade of left ventricular diastolic function as well as with the laboratory parameters of inflammation. Negative correlation was found between DLCO and both biomarkers. Both biomarkers showed significant correlation with the occurrence of death even after adjustments for age, gender and BSA.

4.4.2. All-cause mortality

In univariate Cox regression analysis, both galectin-3 (HR=4.611; 95% CI of HR: 2.074-10.252; p=0.000) and NT-proBNP (HR=2.109; 95% CI of HR: 1.526-2.915; p=0.000) showed significant association with all-cause mortality. In multivariate Cox regression analysis galectin-3 remained independent predictor of all-cause mortality after adjustment for age, gender and body surface area (BSA). This independent association still persisted after the inclusion of NT-proBNP.
Using ROC analysis, galectin-3>10.25 ng/ml and NT-proBNP>140.1 pg/ml were the best predictors of the all-cause mortality.

4.4.3. SSc-related mortality

The leading cause of death was related to SSc in 21 patients. In univariate Cox regression analysis, both galectin-3 and NT-proBNP were significant predictors of the SSc-related mortality. In Cox multivariate regression analysis galectin-3 remained independent predictor of SSc-related mortality.

5. DISCUSSION

5.1. Survival, causes of death and factors affecting outcome

SSc is a multisystem disorder characterized by autoimmune phenomena, vasculopathy, and tissue fibrosis. This particular systemic autoimmune disease severely affects the survival and

quality of life. In spite of the fact that there is no currently available disease modifying antirheumatic drug for the treatment of SSc, the outcome showed improvement in the last decades. One of the major causes of this particular improvement is the overall gradual progress in medical care. SSc patients experienced a substantial benefit from the improvement of cardiology/pulmonology care. The other fact that is important in the improvement is the early cytostatic treatment of extensive skin disease and lung involvement.

In the management of patients with SSc, the basic questions is the early risk assessment, and the adjusted, individualized treatment. Risk assessment and analysis of the outcome is important in patients with SSc because this particular disorder is characterized by high variability in clinical presentation. Patient at high risk for developing severe internal organ complication should be treated aggressively. Furthermore, joint involvement appear early during the disease course, there it should also be treated as early as possible.

Our major aim was to investigate the risk factors of mortality in a large series of patients followed up for a relatively long time period with low lost to follow up rate. The strength of this particular study was that due to an extensive "tracing down work", the number of patients lost to follow up was low (6.4%).

The leading cause of death was the cardiopulmonary manifestation of the disease. The frequency of SSc unrelated causes of death was increased, it is likely that patients more likely to survive SSc related complication due to therapeutic intervention and die due to an SSc unrelated disease like their contemporaries.

An improvement of survival has been observed in cases with SSc. The survival rate we found was similar to those published in recent studies, and it was also substantially better than previous decades. The survival improved compared to our previous survival analysis; in the abovementioned study the 10 years survival rate was 72.6% compared to 79.9% in the present study. This is possible due to shorter time between disease onset and enrollment or better therapeutic options.

Our results are in accordance with previous univariate analysis, we have also confirmed that dcSSc, male gender, pulmonary, and cardiac involvement, scleroderma renal, elevated right ventricular pressure, decreased EF, ECG abnormalities, decreased DLCO and FVC, esophageal involvement were associated with poor survival, as well as anti-Scl70 positivity, scleroderma capillaroscopic pattern, anemia, elevated ESR. Conversely, ACA was associated with a favorable outcome.

Joint involvement often ends up with joint contractures, which substantially influences the quality of life of patients. Although it was not clearly defined, the presence of osteoarticular involvement was found to be a risk factor of mortality in a large series of patients from Brazil. Our earlier local study on a cohort independent from this particular study already indicated that presence of hand deformity with contractures is associated with a poor survival. In our new investigation, we demonstrated that presence of small joint contractures is an independent poor prognostic marker of mortality in patients with SSc.

Parallel to our work the data showing that presence of joint contractures is associated with poor outcome was confirmed by an EUSTAR analysis.

In our series, the presence of history of hypertension was strongly associated with mortality.

In early onset SSc we found that decreased FVC was associated with poor survival similarly to the adulthood disease. In the early onset SSc subgroup we found significantly more patients

with decreased FVC, low body weight and conduction disturbances at enrollment compared to the "middle aged" group (onset of 21-64 years). Our overall conclusion is that a special focus is necessary on the appearance/presence of interstitial lung involvement during the follow up of the young SSc patients. The potential early appearance of conduction disturbances on ECG requires regular cardiology search for the presence of heart involvement.

Survival in elderly patients is significantly lower compared to patients with not elderly onset SSc patients and lcSSc is more frequently observed. Patients with elderly onset SSc have a higher risk for pulmonary hypertension, cardiac disease, pulmonary and renal involvement compared to patients with younger-age at disease onset. The higher prevalence of cardiac involvement in elderly patients may be explained by the high overall incidence of cardiac disease in the elderly population. Elderly onset SSc patients should undergo detailed cardiac investigations, not only due to their age, but due to their SSc as well.

In a few cases, there was a dissent between clinician and pathologist whether pneumonia or structural lung damage due to SSc caused death. As a complex multi-system disease, discrepancies up to 63% between the treating physician and pathologist for the cause of death are not uncommon in SSc patients. One reason for this might be the fact that vascular events occurring in SSc patients are typically multifactorial, notably in the presence of cardiovascular risk factors.

The comparison of autopsy results and clinical findings showed that in several individuals, end organ damage of SSc, notably myocardial fibrosis was only found at autopsy, but not clinically. This is important as it shows that despite modern diagnostic tools, occult organ affection in SSc is still frequent. This is in line with other studies *e.g.* showing myocardial SSc involvement in 80–90% of patients, often despite normal ECG and normal left ventricular systolic function.

Notwithstanding, this small case series demonstrates the limitations of clinical diagnostic procedures to detect end-organ damage. Autopsies might help to educate the clinicians, who treat severe SSc to better understand the disease and improve patient care.

5.2. Role of anti-RNAP3 in scrrening for early malignancies and certain internal organ manifestations

Besides the well-known internal organ alterations, the coexistence of a malignancy (paraneoplastic syndrome) also strongly influences the outcome of SSc. Anti-RNAP3 and anti-PmScl antibody positivity was found to be associated with an increased risk of cancer in patients with SSc.

In the large EUSTAR study, the clinical associations of anti-RNAP3 in SSc were analysed. The patients positive for anti-RNAP3 are at increased risk for developing cancers. This particular study showed an OR of 7.38 (95% CI 1.61–33.8) within an interval between 6 months before and 12 months after SSc onset. Our center contributed to this large study with 1/5 of the all included cases. Our local results did not show the same risk because the number of patients in our local cohort was low. It is clear that the exclusive, appropriate approach of the investigation of a small subset of SSc is the multicentre investigation.

In the EUSTAR cohort an increased overall prevalence of cancer in anti-RNAP3+ patients was also observed, thus with a low OR. In years distant from the onset of SSc, the EUSTAR investgation did not observe any increased frequency of cancer in anti-RNAP3+ patients.

Therefore, no available data thus far suggest that the risk of cancer is extended beyond an interval of a few years around SSc onset. The large majority of malignancies associated with the onset of anti-RNAP3+ SSc were solid cancers. In our experience around half of these malignancies were breast cancers. These results suggest the possible need of a cancer screening program for anti-RNAP3+ patients.
In this particular EUSTAR study, patients with older age or diffuse cutaneous involvement were particularly at risk. These risk factors may help in clinical practice to institute appropriate cancer screening at SSc diagnosis in these individuals.
It is noteworthy that single-center observational studies on the relative risk of cancer simultaneous to the onset of SSc were very similar to the results of this particular EUSTAR study. Anti-RNAP3 antibodies are associated with poor prognostic manifestations of SSc, including increased risk of malignancy diagnosis (in particular of breast cancer) close to the SSc onset.

5.3. Galectin-3 and NT-pro-BNP as a maker of cardiac disease and poor outcome in SSc

Our further aim was to describe new potential serum biomarkers, which are useful in risk assessment and prediction of disease outcome. The diagnostic and prognostic value of NT-proBNP is already well established in SSc and was confirmed in our patient cohort. In univariate Cox regression analysis NT-proBNP was significant predictor of mortality.
Our results also indicate that galectin-3 is also an independent predictor of all-cause and disease-related mortality in SSc. Added to NT-proBNP, galectin-3 provides valuable complementary prognostic information, mainly by reflecting mortality risk associated to organ sclerosis and inflammation.
Galectin-3 levels showed positive correlation with the duration of the disease. In addition, galectin-3 levels were higher in the diffuse cutaneous form of the disease, which may be the sign of the more extensive fibrosis in this form of SSc.
Galectin-3 is a regulatory molecule acting at various stages along the continuum from acute inflammation to chronic inflammation and tissue fibrogenesis. In heart failure patients with reduced and preserved ejection fraction DeBoer et al. found significant correlation between galectin-3 and various pro-inflammatory cytokines, including CRP. Likewise, in our SSc population galectin-3 levels showed significant correlation with the laboratory parameters of inflammation, such as erythrocyte sedimentation rate and CRP.
In contrast to the findings of Taniguchi et al. we have found significant negative correlation between DLCO and galectin-3 levels, suggesting, that galectin-3 may be biomarker of interstitial lung disease in SSc. This result is in line with the work of Nishi et al. reporting increased level of galectin-3 in bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with collagen vascular disease.
In our population, galectin-3 levels correlated positively with the grade of the left ventricular diastolic dysfunction. Similar finding were reported in the work of Shah at al., where higher levels of galectin-3 were related to Doppler indices of impaired myocardial relaxation and higher filling pressure in patients with heart failure.
This is the first study investigating the long-term prognostic value of galectin-3 for the prediction of all-cause and disease-related mortality in SSc patients. In Cox multivariate

regression analysis galectin-3 was proved to be independent predictor of the all-cause and disease-related mortality even after the inclusion of NT-proBNP.
The mean galectin-3 level in our population tended to be lower than those in the recent heart failure studies. One of the possible explanations of this phenomenon is that only less than a half of our patients had manifest heart failure (44.7% was treated with loop diuretics). On the other hand, a number of galectin-3 immunoassays are available, and the measured concentrations may vary between assays.

6. SUMMARY

Systemic sclerosis is a multisystem disorder characterized by autoimmune phenomena, vasculopathy, and tissue fibrosis. This particular systemic autoimmune disease severely affects the survival and quality of life.
The mortality of the different SSc subgroups is substantially different. Our major aim was to investigate the risk factors of mortality in a large series of patients followed up for a relatively long time period with low lost to follow up rate (6.4%). The Hungarian health care system is centralized for the treatment of SSc cases. Our study most probably represents the overwhelming majority of patients in the South-West Hungarian district.
Our current study showed that there was an overall improvement in survival of patients with SSc either compared to our earlier studies or international publications. The outcome showed a gradual improvement in the last decades, and we found the same in our cohort. One of the major causes of this particular improvement is the overall progress in medical care. In particular, SSc patients experienced a substantial benefit from the improvement in the early recognition and management of cardiac/lung disorders. The other fact that is important in the improvement is the introduction of early cytostatic treatment of extensive skin disease and lung involvement. The basic question is the early risk assessment and the individualized follow up of the patients with SSc. Risk assessment and analysis of the outcome is important, because this particular disorder is characterized by high variability in clinical presentation. Our patient database and prospective data collection has enabled us to improve risk assessment.
To best our knowledge we proved first in a multivariate analysis, besides the well-known risk factors, the presence of small joint contractures was associated with increased mortality risk. Furthermore, patients with a history of systemic arterial hypertension were also at higher risk for outcome. These two particular clinical findings can be used for an improved risk assessment in the future.
Besides the overall risk assessment, it is crucial to define patient subsets with different outcomes. We have confirmed the well-known fact that disease onset in elderly age causes a poor survival. Furthermore, we first described that patients with an onset of Raynaud's syndrome before the age of 20 years are likely to have a lung involvement with a decreased FVC, therefore a special focus on the signs of early interstitial lung involvement is mandatory during the follow up.
However anti-Scl70 and ACA positive patients characterize a well-known subset, anti-RNAP3 positive cases also define a diffuse SSc patient subset with characteristic clinical findings. Earlier monocenter studies showed that these patients exhibited a relative risk of cancer appearing simultaneously to the onset of SSc. As a part of the large European

multicentre study we have confirmed that the risk of the presence of simultaneous cancer was high in this particular subset of SSc cases. This multicentre investigation also proved that anti-RNAP3 was associated with GAVE and SRC development. Sub-analysis of our small case cohort did not show the same results indicating that for risk assessment of a small subset of SSc patients, large multicentre studies are required.
Beside clinical investigations it is important to define new serum biomarkers which could improve either risk assessment of a patient subset requiring special focus on one particular organ involvement. In our study galectin-3 levels showed positive correlation with the grade of left ventricular diastolic function as well as ESR and CRP. Negative correlation was found between galectin-3 and DLCO. Furthermore, the follow up study clearly indicated that galectin-3 is an independent predictor of all-cause and disease-related mortality in SSc. Added to NT-proBNP, galectin-3 provides complementary prognostic information, mainly by reflecting mortality risk associated to organ sclerosis and inflammation.

7. NEW RESULTS

Our current study shows that there is an overall improvement in survival of patients with SSc either compared to our earlier studies or international publications.

We have first described that the presence of small joint contractures at onset of SSc is associated with increased mortality risk; this is an independent risk factor for mortality in SSc. This particular finding can be used for risk assessment in patients with SSc.

Patients with hypoalbuminemia have a poor survival; it is an independent risk factor of mortality.

Patients with onset of Raynaud's syndrome before the age of 20 years are likely to have a decreased FVC. Young patients with SSc need a special focus on interstitial lung involvement during the follow up.

Anti-RNAP3 positive cases show a relative risk of cancer appearing simultaneously to the onset of SSc in the large European multicenter study. The same was not found in our small cohort. For this kind of risk assessment, a large multicenter study is the appropriate investigation.

Galectin-3 levels showed positive correlation with the grade of left ventricular diastolic function as well as ESR and CRP. Negative correlation was found between galectin-3 and DLCO.

Galectin-3 is an independent predictor of all-cause and disease-related mortality in SSc. Added to NT-proBNP, galectin-3 provides complementary prognostic information, mainly by reflecting mortality risk associated to organ sclerosis and inflammation.

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9. LIST OF PUBLICATIONS RELATED TO THE SUBJECTS INCLUDED IN THE THESIS

9.1.Papers

Nagy G, Minier T, Varjú C, Faludi R, Kovács KT, Lóránd V, Hermann V, Czirják L, Kumánovics G. The presence of small joint contractures is a risk factor for survival in 439 patients with systemic sclerosis. Clin Exp Rheum 2017; 35:61-70. **IF: 2.634**

Lazzaroni MG, Cavazzana I, Colombo E, Dobrota R, Hernandez J, Hesselstrand R, Varju C, Nagy G, Smith V, Caramaschi P, Riccieri V, Hachulla E, Balbir-Gurman A, Chatelus E, Romanowska-Próchnicka K, Araújo AC, Distler O, Allanore Y, Airò P; and EUSTAR co-authors. Malignancies in Patients with Anti-RNA Polymerase III Antibodies and Systemic Sclerosis: Analysis of the EULAR Scleroderma Trials and Research Cohort and Possible Recommendations for Screening. J Rheumatol 2017; 44: 639-647**. IF: 3.150**

Faludi R, Nagy G, Tőkés-Füzesi M, Kovács K, Czirják L, Komócsi A. Galectin-3 is an independent predictor of survival in systemic sclerosis. Int J Cardiol 2017; 233: 118-124. **IF: 6.189**

Sandmeier B, Jäger VK, Nagy G, Carreira PE, Tzankov A, Widuchowska M, Antic M, Distler O, Reichert H, Distler JH, Walker UA, Hügle T. Autopsy versus clinical findings in patients with systemic sclerosis in a case series from patients of the EUSTAR database. Clin Exp Rheumatol 2015; 33: S75-9. **IF: 2.495**

9.2. Published Abstracts

Nagy G. Systemas sclerosisban szenvedő betegek túlélési vizsgálata. XXI OTDK Orvos-és Egészségtudományi Szekció: Előadás kivonatok p.464 (ISBN 978-963-306-203-6) (2013)

Nagy G. Survival of patients with systemic sclerosis Arch Hung Med Assoc Am 2013; 21: 61.

Czirják L, Nagy G, Kumánovics G. Systemic Sclerosis as a paraneoplastic syndrome CEJMed 2014; 126: S218.

Nagy G, Minier T, Varjú C, Kovács KT, Lóránd V, Hermann V, Czirják L, Kumánovics G. Prognosis and survival are different in early and late onset systemic sclerosis: Observations of 340 Hunagrian patients of a single center. JSRD 2016; 1: 92.

Lazzaroni MG, Cavazzana I, Colombo E, Dobrota R, Hernandez J, Hesselstrand R, Varju C, Nagy G, Smith V, Caramaschi P, Riccieri V, Hachulla E, Balbir-Gurman A, Chatelus E, Romanowska-Próchnicka K, Araújo AC, Distler O, Allanore Y, Airò P. Increased frequency of malignancies, and in particular breast cancer, synchronous to the onset of systemic sclerosis in anti-RNA Polymerase III antibodies positive patients: A EUSTAR multicenter study JSRD 2016; 1: 33.

Faludi R, Tokes-Fuzesi M, Nagy G, Czirjak L, Komocsi A. Galectin3 is an independent predictor of survival in systemic sclerosis European Heart J 2016; 37: 443.

9.3. Presentations

Nagy G. Systemas sclerosisban szenvedő betegek túlélési vizsgálata, Kari TDK Konferencia, 2013.02.07.

Nagy G. A túlélés értékelése szisztémás sclerosisban XI. Reumatológus Rezidens és Szakorvosjelölt Fórum, 2013. 03.09.

Nagy G. Systemas sclerosisban szenvedő betegek túlélési vizsgálata XXXI. OTDK Elméleti Epidemiológia szekció, Szeged. 2013.04.05.

Nagy G, Minier T, Varjú C, Kovács KT, Lóránd V, Hermann V, Czirják L, Kumánovics G. Prognosis and survival are different in early and late onset systemic sclerosis: observations of 439 Hungarian patients of a single center" IV Scleroderma World Congress, Lisszabon, Portugália 2016 February 12-17.

Nagy G. A korai és késői kezdetű systemas sclerosis jellemzői XV. Pécsi Reumatológus Rezidens és Szakorvosjelölt Fórum 2016. május 3-4.

10. LIST OF PUBLICATIONS NOT RELATED TO THE SUBJECTS INCLUDED IN THE THESIS

10.1. Papers

Varjú C, Péntek M, Lóránd V, Nagy G, Minier T, Czirják L. Musculoskeletal involvement in systemic scelrosis: an unexplored aspect of the disease JSRD 2017; 1: 19-32.

10.2. Published Abstracts

Nagy G. Rare antibodies in systemic sclerosis and their clinical associations Poszter absztrakt Arch Hung Med Assoc Am 2014; 22: 80.

Gulyás K, Nagy G, Lóránd V, Minier T, Kumánovics G, Simon D, Varjú C, Berki T, Czirják L. Az RNS polimeráz III és egyéb ritkább antinucleoláris antitest-pozitív szisztémás sclerosisos betegek klinikai jellemzői a Pécsi Tudományegyetem Klinikai Központ Reumatológiai és Immunológiai Klinika beteganyagában Magyar Reumatológia, 2014; 55: 141

Nagy G, Kumanovics G, Czirják L. Scleroderma overlap syndromes CEJMed 2014; 126: S202

Nagy G, Minier T, Tuba É, Varjú C, Kumánovics G, Czirják L. Raynaud-szindróma gyakoriságának vizsgálata szisztémás autoimmunbetegek körében Magyar Reumatologia, 2015; 56, 146.

Kumanovics G, Nagy G, Czirjak L. Raynaud-szindróma és tüdőfibrosis összefüggésének vizsgálata szisztémás autoimmun betegek körében. Magyar Reumatológusok Egyesülete Vándorgyűlés 2017. szeptember Magyar Reumatologia, 2017; 58: 166.

Nagy G, Czirjak L, Kumanovics G. Kapillármikroszkópos mintázat vizsgálata szisztémás autoimmun betegeknél Magyar Reumatológusok Egyesülete Vándorgyűlés 2017. szeptember Magyar Reumatologia, 2017; 58: 176.

Kumánovics G, Nagy G, Czirják L. Evaluation of nailfold capillaroscopic pattern and capillary density in 318 patients with connective tissue diseases. V Scleroderma World Congress Bordeaux, France 2018 February JSRD 2018; 3: 184

Nagy G, Czirják L, Kumánovics G. Evaulation of capillaroscopic pattern in SLE patients with and without Raynaud syndrome. 11. European Lupus Meeting Düsseldorf, Germany2018 March LUPUS 2018; 5: A57.

10.3. Presentations

Nagy G. Ritka scleroderma antitest specificitásokhoz (RNA Pol III, Th/to) társuló klinikai jellemzők. XII. Reumatológus Rezidens és Szakorvosjelölt Fórum, 2014. 03.09.

Nagy G, Kumánovics G, Czirják L. Scleroderma overlap syndromes. Central European Congress of Rheumatology, Wien 2014.12.06-07.

Nagy G. A kapillármikroszkópia értékelési szempontja szisztémás szklerózisban. XIII. Pécsi Reumatológus Rezidens és Szakorvosjelölt Fórum, 2015. 04.17.

Nagy G, Minier T, Tuba É, Varjú C, Kumánovics G, Czirják L. Raynaud-szindróma gyakoriságának vizsgálata szisztémás autoimmun betegek körében. Magyar Reumatológsok Egyesülete Vándorgyűlés 2015. szeptember 12-16.

Nagy G, Czirják L, Kumánovics G. 1st CE Young Researcher Forum, Bratislava, Szlovákia 2016. november első szerzős előadás cím: „Evaulation of capillaroscopic pattern in systemic sclerosis- preliminary results"

Nagy G. XIV. Pécsi Reumatológus Rezidens és Szakorvosjelölt Fórum 2017. április első szerzős előadás cím: „Kapillármikorszkópos mintázat szisztémás lupus erythematosusban és antifoszfolipid szindrómában”

Nagy G. Magyar Reumatológusok Egyesülete Vándorgyűlés 2017. szeptember első szerzős előadás cím: „Kapillármikroszkópos mintázat vizsgálata szisztémás autoimmun betegeknél”

Sum of impact factor of original publications: 14.468