Ph.D.THESES

CLINICAL OBSERVATIONS IN SYSTEMIC SCLEROSIS

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INTRODUCTION

Systemic sclerosis

Systemic sclerosis is a rare polysystemic autoimmune disorder. The first symptoms ususally appear over the age of forty years with female dominance. There are two main groups to distinguish: diffuse cutaneous systemic sclerosis (dcSSc) and limited cutaneous sytemic sclerosis (lcSSc). These two groups are different in their clinical symptoms and prognosis as well. The diagnosis is based on the criteria of the American College of Rhematology created in 1980.

The etiology is complex and not fully known by now. There were three main factors found in the pathogenesis. Vasculopathy is a result of endothelial damage, which, throughout an inflammational cascade, leads to the progressive stenosis and obstruction of the blood vessels. Pathological immune responses develop both on the cellular and the humoral lines. Leukocytes of the peripheral blood, CD4+ lymphocytes in the skin and alveolar CD8+ cells all produce Th2 type cytokines. The alterations of the humoral immune response also play an important role in the pathogenesis of SSc. High specificity autoantibodies can be detected in the blood samples of patients with SSc. The autoantibody against Scl-70 antigene is produced against the DNA topoisomerase I enzyme and, likely to the anti-RNA polymerase I-III and anti-fibrillarin antibodies, specific for the diffuse cutaneous form bearing severe clinical manifestations. Anti-centromere antibodies are detected usually in the lcSSc group. B-cells contribute to the fibrogenesis not just by producing autoantibodies, but by secreting interleukines eg. Il-6 that directly stimulates fibroblasts. These changes will result in fibroblast proliferation and re-structurization of the extracellular matrix. TGF-beta is one of the key molecules of fibroblast activation, but some other cytokines and growth factors also play an important role. The fibrotic process mainly develop in the skin and some other inner organs, but a diffuse perivascular fibrosis can be observed as well. The fibrotic damage of the inner organs is responsible for the morbidity and mortality of scleroderma. Besides these three main mechanisms, gene polymorphisms, environmental factors and foeto-maternal microchimerism may also contribute to the pathogenesis of the disease.

Skin symptoms dominate the patients' complaints: the skin of the fingers becomes thick and stiff, sclerodactylia develops. Almost all patients have stiff skin in the acral regions. Diffuse cutaneous systemic sclerosis can be diagnosed if the trunk and/or the proximal parts of the extremities are affected as well. Other skin symptoms might be present eg. teleangiectasias, subcutaneous calcinosis that are rather specific for lcSSc, and ulcers on the fingertips that heal with digital pitting scars. The severity of skin symptoms determine the patients' quality of life.

Raynaud's phenomenon develop in 90-95 % of the patients. The specific pattern of the small vessels can be detected by capillary microscopy on the fingertips. It is the inner organ manifestations that are mainly responsible for the mortality of the disease, pulmonary complications are the leading causes of death. The two main pathological entities are pulmonary fibrosis and pulmonary hypertension. The functional damage of the esophagus might be also present. Skin manifestations appear either in diastolic dysfunction of the left ventricle or in arrhythmias. Besides endothelial dysfunction, accelerated atherosclerosis can be detecetd by most of the patients.

Real disease modifying therapy that will cure scleroderma is not known. The aim of treatment would be to influence the three main pathological factors. Recently, there has been a significant improvement in the treatment of vascular symptoms by the introduction of phosphodiesterase inhibitors and endothelin-1 receptor antagonists. Immunosuppressive therapy is administered in rapidly progressive skin symptoms, fibrotising alveolitis or overlap syndromes. The treatment of inner organ complications is an important task for rheumatogists, however, a thorough cooperation would be needed with colleauges representing different disciplines.

Characteristics of juvenile systemic sclerosis

SSc is a rare disease in childhood, the onset of the disease usually occurs at the age of 40. In 2001 there was a consensus to establish the classification criteria for jSSc by the first International Workshop on Juvenile Scleroderma in Italy. This classification system is a little bit different form the ACR criteria adapted for adult patients and currently being validated in pediatric rheumatology. Parallel to the development of classification criteria of jSSc and increasing information on survival and prognostic factors for survival in adult SSc, our knowledge of the prognosis and survival of juvenile SSc is currently limited. In adult SSc populations the presence of cardiac, pulmonary, renal involvements, similarly to the presence

of the diffuse skin symptoms lead to poor outcome. SSc confers a high mortality risk, but there is a considerable heterogenity across settings. The age of onset may be important factor for the outcome as jSSc and the adult onset SSc may have different organ manifestations and thus differences in prognosis.

Systemic sclerosis and rheumatoid arthritis overlap syndrome

Rheumatoid arthritis is a chronic progressive polyarthritis. It is usually involving peripheral joints in a symmetric distribution. The potential of the synovial imflammation to cause cartilage destruction and bone erosions. The presence of rheumatoid factor (RF) is not specific for RA, but anti cyclic citrullinated peptide (anti-CCP) antibody has got over 95% of specificity.

Features of two systemic autoimmune diseases may develop in some patients, where each component fulfills the diagnostic criteria of the American College of Rheumatology (ACR).

The prevalence of SSc-RA overlap is 4,3% to 5,2% among SSc patients. In addition, higher incidence of RA is found within SSc patients than in the general population.

The diagnosis of RA in SSc is not always obvious as arthralgia and arthritis is commonly associated in SSc patients as well. Symmetric polyarthritis and joint contractures can occur in both diseases. The radiological destruction is more pronounced in RA, than in SSc. Not only the diagnosis, but the treatment is also challanging as steroids can provoke renal crisis in scleroderma.

Macrovascular disorders, the role of plasma homocysteine levels and MTHFR gene polymorphism in systemic sclerosis

SSc is associated with endothelial cell dysfunction, where classically the microvasculature is affected. In recent years increased attention has been paid to the importance of large vessel involvement in SSc Increased plasma homocysteine concentration is an independent risk factor for macrovascular disorders and it may be associated with an increased risk of small-vessel thrombosis also. It has been suggested that homocysteine is involved in the promotion of platelet activation, hypercoagulability, oxydative stress, endothelial dysfunction, smooth muscle cell proliferation and oxydation and peroxydation of lipids. The sulfur-containing amino-acid homocysteine is formed during the metabolism of

metionine. Methylentatrahydrofolate reductase (MTHFR), a key enzyme in homocysteine metabolism, seems to play a role in both hypertension and cardiovascular disaese. The T-allele of the 677C/T MTHFR polymorphism causes a thermolability of the enzyme, reduces its activity and inhibits the formation of 5-methyltetrahydrofolate, which serves as a methyl donor during the remethylation of homocysteine to methionine.

AIMS OF OUR WORK

Investigating the clinical features and pathogenesis of SSc, our aims were as follows:

1. A retrospective study was performed to investigate the frequency and clinical relevances of gastrointestinal involvement in Hungarian scleroderma patients. These results were compared to the data obtained from large rheumatology centers in Europe.

2. We wanted to learn more about jSSc, a rare disease form of scleroderma. Analyzing the clinical and laboratory characteristics of jSSc patients currently undergoing follow-ups in our institution we supposed a possible role of them in the prognosis of jSSc patients.

3. We assessed 22 patients with SSc-RA overlap. Clinical and immunological features, as well as their HLA-DR genotypes of these overlap patients were analysed supposing that overlap syndrome of SSc and RA may be a distinct clinical entity.

4. We investigated plasma homocysteine levels in patients with systemic sclerosis and studied the association between plasma Hcy, C677T polimorphism of 5,10-methylentetrahydrofolate reductase (MTHFR), and the clinical manifestations in SSc.

PATIENTS AND METHODS

Gastrointestinal manifestations in SSc

We assessed the charts of 246 patients with SSc undergoing regular follow-ups at our institution. Patients were recruited between 1994 and 2004. All patients fulfilled the ACR criteria for the classification of SSc. Data referred as GI complications were collected regarding the patients' charts. All patients underwent radiographic studies including barium swallow as a non-invasive screening test for esophageal dysfunction. In accordance to the patients' complaints and symptoms, 146 patients also underwent upper panendoscopy to evaluate GERD, as well as esophageal, gastric and duodenal mucosal inflammation and ulcers. Involvement of the colon and the anorectum was examined by colonoscopy. Laboratory tests including ESR, blood count, chemistry, kidney and liver function were routinely performed. In addition, immunolaboratory analysis of antinuclear antibodies (ANA), anti-centromere (ACA), anti-topoisomerase (Scl70), antimitochondrial (AMA), anti-smooth muscle (SMA) and liver-kidney membrane (LKM) antibodies were also performed.

Juvenile systemic sclerosis

Eight juvenile onset SSc patients were enrolled in the study from the pool of 230 SSc patients undergoing regular follow-ups in the Division of Rheumatology, 3rd Department of Internal Medicine and Division of Pediatric Immunology in the year of 2005. Patients had limited or diffuse cutaneous form of SSc, as defined by LeRoy et al, and fulfilled the ACR criteria for the classification of SSc.

At the time of the analysis, 2/8 patients were still under 18 years while 6/8 patients were adults. Seven females and one male were enrolled, mean age was 30.1 years, range 13-47. Disease duration ranged from 3 to 37 years, the mean age at disease onset was 10.87 years. Clinical symptoms, internal organ manifestations and immunolaboratory tests were analyzed. These included the assessment of Raynaud-phenomenon, pulmonary function testing, echocardiography, esophageal radiography, as well as the determination of autoantibodies in the sera of patients.

SSc-RA overlap syndrome

RA-SSc overlap patients were selected from 477 SSc patients diagnosed in the Department of Rheumatology in Debrecen and Pécs University Center between 1991 and 2007. Altogether 22 SSc-RA overlap patients were recruited from Hungary. Their mean age was 53±14.6 yrs. All pateints fulfilled the ACR criteria for both SSc and RA. Clinical features including pulmonary, cardiac, esophageal manifestations and others were analyzed.

Immunological analyses included tests for antinuclear antibodies (ANA), anti-centromer antibody (ACA), anti-topoisomerase I antibody (anti-Scl 70), IgM rheumatoid factor (RF) and anti cyclic citrullinated peptide (anti-CCP) antibody.

HLA-DR genotyping described later in detail was also performed in these SSc-RA overlap patients, as well as in 38 other patients with SSc (31 females and 7 males) and 100 other petients with RA (75 females and 25 males). Blood was also obtained form 50 matched healthy controls. The control group underwent the same procedures as the patient groups.

Macrovascular disorders, serum homocysteine level and MTHFR gene polymorphism

152 SSc patients (131 lcSSc, 21 dcSSc) were included in our study, 133 females and 19 males. All fulfilled the ACR criteria for SSc. The mean age was 54.2 yrs, mean disease duration was 9.61 yrs. We investigated the plasma homocysteine levels, C677T polymorphism of 5,10-methylentetrahydrofolate reductase, and the clinical manifestations in patients with SSc. For comparison, we studied 58 age and sex matched healthy controls (mean age:49.9 years, 46 females and 12 males).

Evaluation of autoantibodies and serum homocysteine level

ANA and ACA were determined by indirect immunofluorescence on Hep2-cells. ANA positivity was assessed at 1:40 dilution. Anti-Scl70, anti-CCP and anti-U1RNP were determined in all patients by ELISA. IgM RF was assessed by nephelometry. The normal values for IgM RF and anti-CCP were <50 U/ml and <25 U/ml, respectively.

Anti-Ro/SS-A and anti-La/SS-B autoantibodies were determined by ELISA. The cut-off values were10 U/ml for both.

Total homocysteine levels were measured with high performance liquid-chromatography method. The upper limit of the normal range of total plasma homocysteine was 12,5 μmol/l.

Genetic assays

Genomic DNA was isolated from buffy coats of EDTA anticoagulated blood using QIAamp Blood Mini Kit. PCR-based HLA-DRB typing was performed. All samples were processed according to the instructions of the manufacturer using recombinant Taq DNA polymerase. HLA-DRB genotypes were determined on the basis of the PCR pattern obtained using 2% agarose gel electrophoresis. DNA bands were detected using Alpha Imager MultiImage Light Cabinet.

The C677T mutation of the MTHFR gene was assessed by DNA-fragmentation using specific restriction endonuclease enzyme followed by PCR amplification and agarose gel electrophoresis. Patients and controls were genotyped as homozygous for the mutation (TT), heterozygous (CT) or wild type (CC).

Statistical analysis

Data were processed with the help of Microsoft Excel spreadsheet program. Statistical analysis was carried out using SPSS softver (15.0 version). Statistical analysis included paired t test, Mann-Whitney U-test, calculation of Spearman's correlation coefficient and Chi-square test was applied to compare parameters in different groups. P values <0.05 were considered significant.

RESULTS

Gastrointestinal manifestations in SSc

Patients included 206 women and 40 men with a mean age of 54.2±9.6 years. The mean duration of the disease at the time of the study was 9.1 years. The patients were subclassified into dcSSc (n=50) and lcSSc (n=196) subtypes.

All internal organ manifestations are listed in Table 1. Altogether 177 (71.9%) SSc patients developed significant clinical involvement of the alimentary tract based on the diagnostic procedures we have performed.

Table 1: Prevalence of internal organ involvement in 246 SSc patients

Site	*Prevalence*
Lung	182/246 (74.0%)
Gastrointestinal tract	177/246 (71.9%)
Heart	133/246 (54.1%)
Kidney	17/246 (6.9%)

Table 2: Frequency of the symptoms of gastrointestinal involvement in 246 SSc patients

	Number of patients/ SSc subsets	
Gastrointestinal symptoms	*dcSSc n=50*	*lcSSc n=196*
sicca symptoms	14 (28%)	67 (34.2%)
heartburn	28 (56%)	121 (61.7%)
dysphagia	32 (64%)	124 (63.2%)
nausea/vomiting	31 (62%)	110 (56.1%)
abdominal pain/dystension	30 (58.3%)	84 (42.8%)
weight loss	32 (62.5%)	67 (34.1%)
postprandialis fullness/epigastric dyscomfort	22 (44%)	85 (43.3%)
diarrhea	34 (66.6%)	85 (43.3%)
constipation	10 (20 %)	70 (35.7%)
fecal incontinence	3 (6.0%)	1 (0.5%)

Patient history was taken with special regards to GI involvement. The results are shown in Table 2.

The main structural and functional manifestations are included in Table 3.

3. táblázat: Functional and morphological manifestations of the gastrointestinum in 246 SSc patients

Localization	*Functional and morphological features*	*Number of patients /SSc subsets* *dcSSc n=50*	*lcSSc n=196*
Esophagus		**154 (62.6%)**	
	dysmotility	18 (36%)	111 (56.6%)
	reflux esophagitis	10 (20%)	48 (24.4%)
	dilatation	5 (10%)	27 (13.7%)
	stenosis	2 (4%)	3 (1.5%)
	ulcus esophagei	1 (2%)	1 (0.5%)
	Barrett metaplasia	0 (0%)	1 (0.5%)
Stomach		**78 (31.7%)**	
	chronic gastritis	17 (34%)	28 (14.2%)
	H.pylori pozitivity	10 (20%)	30 (15.3%)
	ulcus ventriculi	5 (10%)	15 (7.6%)
	hiatus hernia	3 (6%)	8 (4%)
	cardiac stenosis	1 (2%)	2 (1%)
Small intestine		**2 (0.8%)**	
	malabsorption	2 (4%)	1 (0,5%)
Colon, anorectum		**26 (11%)**	
	diverticulosis coli	4 (8%)	6 (3 %)
	Crohn's disease	0 (0%)	3 (1.5%)
	anal sphincter dysfunction	2 (4%)	1 (0.5%)
Pancreas and biliary tract		**24 (10%)**	
	primary biliary cirrhosis	0 (0%)	4 (2%)
	chronic cholangitis	2 (4%)	7 (3.5%)
	fibrosis of bile ducts	2 (4%)	3 (1.5%)
	sclerosis of the papilla Vater	1 (2%)	2 (1%)
	chronic pancreatitis	2 (4%)	1 (0.5%)

The esophagus was the most frequently affected as esophageal impairement was found in 63% of SSc patients. Reflux oesophagitis was present in 23.5% of the patients evaluated with gastroscopy, and we found squamous cell carcinoma of the esophagus in one patient. Gastric involvement was present in 31,7%, but we found no neoplastic alterationin that region. Severe malabsorption occured in three cases, where total parenteral nutrition (TPN)

were also administered. Functional and morphological alterations of the anorectum occurred in 11% of the investigated patients. Associated diseases of the pancreas and the biliary tract were present in 10% of the patients. The evaluation of dysfunction of the anal sphincter was not routinely performed, only in some cases, where manometric studies showed absent recto-anal inhibitory reflex and decreased resting and squeeze pressure in the anal canal.

Juvenile systemic sclerosis

Table 4 shows the patients' demographical data.

Table 4: Clinical and demographic characteristics, follow-up time of eight jSSc patients

Patient	*Sex*	*Age*	*Onset of SSc (age)*	*Subset*	*R/U*	*Lung*	*Heart*	*GI*	*secunder Sjögren* syndrome
1.	F	47	10	lcSSc	R/U	-	+	-	+
2.	F	46	17	lcSSc	R/U	+	+	+	-
3.	M	34	3	lcSSc	R	-	-	-	+
4.	F	29	13	lcSSc	R	+	+	+	-
5.	F	40	16	lcSSc	R	-	-	+	-
6.	F	13	7	dcSSc	R	-	-	-	-
7.	F	14	11	dcSSc	R	-	-	-	-
8.	F	18	10	lcSSc	R	-	-	-	-

R: Raynaud syndrome, U: ulcers

Only two patients were affected by diffuse cutaneous SSc (dcSSC) and six by limited cutaneous SSc (lcSSc). Raynaud pnenomenon was present in all cases, but ulcers of the fingers developed only in two cases. Analysis of internal organ manifestations revealed that pulmonary fibrosis developed in only two patients during the follow-up period (alveolitis was

found in only one case by HRCT). Three patients had cardiac manifestations with diastolic function abnormalities of assessed by echocardiography and three patients had GI manifestations, such as esophageal dismotility.

During the analysis of autoantibody profile of our patients, 7/8 patients showed ANA positivity. We found ACA positivity in only two cases, however none of these patients ever carried anti-Scl70 autoantibody (Table 5).

Table 5: Autoantibody profile of eight jSSc patients

Patient	*ANA*	*ACA*	*anti-Scl70*	*Other autoantibodies*
1.	+	-	-	RF, ENA, CL, β2GP, SS-A
2.	+	+	-	-
3.	+	-	-	SS-A, SS-B, β 2GP
4.	+	-	-	β 2GP
5.	-	+	-	RF
6.	+	-	-	dsDNA
7.	+	-	-	-
8.	+	-	-	dsDNA

Clinical, serological and genetic characteristics of SSc-RA overlap syndrome

Five of the 22 patients (23%) had dcSSc and 17 patients (77%) had lcSSc. The diagnosis of RA followed that of SSc in 19 patients (86.4%), and preceded that of SSc in three patients (13.6%). Regarding organ manifestations, 17 of the 22 patients (77.3%) had pulmonary fibrosis, 12 (54.5%) had esophageal dysmotility, while 11 (50%) had cardiac and five (22.7%) had renal involvement. All patients with renal involvement had chronic renal failure; however none of them had scleroderma renal crisis or renal hypertension. Two out of the 22 overlap patients (9.1%) had pulmonary arterial hypertension (PAH). Three overlap patients with lcSSc (13.6%) fulfilled the criteria for CREST syndrome and three patients (13.6%) had secondary Sjögren's syndrome. Marked articular destructions and erosions were observed ont he proxymal interphalangeal (PIP), metacarpophalangeal (MCP), carpal joints or ulnar heads in 18 (81.8%) SSc-RA patients.

ANA positivity was detected in all SSc-RA overlap patients (100%), anti-topoisomerase I antibody positivity in 5 (22.7%), IgM RF positivity in 16 (72.7%), anti-CCP positivity in 18 (81.8%) and ACA positivity in only 2 (9.1%) SSc-RA patients. The mean values for IgM RF and anti-CCP were 81.8 U/ml and 105.1 U/ml, respectively.

We compared HLA-DR genotypes of the overlap patients with genotypes of SSc, RA patients and healthy subjects (Table 6).

Table 6: HLA-DR genotype of SSc-RA, SSc, RA patients and healthy controls

HLA-DRB1 genotype	*SSc-RA (n=22)*	*SSc (n=38)*	*RA (n=100)*	*Control (n=50)*
DRB1*1	**7 (32%)**	**4 (10,5%)**	**46 (46%)**	**8 (16%)**
DRB1*3	**8 (36%)**	**18 (47%)**	**5 (5%)**	**9 (18%)**
DRB1*4	**6 (27%)**	**6 (16%)**	**31 (31%)**	**7 (14%)**
DRB1*7	2 (9%)	7 (18%)	4 (4%)	13 (26%)
DRB1*8	2 (9%)	2 (5,3%)	1 (1%)	3 (6%)
DRB1*11	**8 (36%)**	**16 (42%)**	**7 (7%)**	**10 (20%)**
DRB1*12	1 (4.5%)	2 (5.3%)	0 (0%)	8 (16%)
DRB1*13	4 (18%)	3 (7.9%)	3 (3%)	9 (18%)
DRB1*14	1 (4.5%)	1 (2.6%)	1 (1%)	1 (2%)
DRB1*15	2 (9%)	4 (10.5%)	2 (2%)	6 (12%)
DRB1*16	2 (9%)	6 (16%)	1 (1%)	9 (18%)

Allele frequencies of HLA-DR3 and HLA-DR11 were significantly increased in SSc-RA overlap patients (36% and 36%, respectively) compared with RA patients (5% and 7%, respectively) or to healthy controls (18% and 20%, respectively) ($P<0.05$). HLA-DR3 and HLA-DR11 frequency was also significantly higher in SSc patients (47% and 42%, respectively) compared with RA patients (5% and 7%, respectively) and to the control group (18% and 20%, respectively) ($P<0.05$). On the other hand, HLA-DR1 and HLA-DR4, namely the 'shared epitope' genotypes, exerted significantly higher frequencies in SSc-RA (32% and 27%, respectively) and RA patients (46% and 31%, respectively) compared with either SSc patients (10.5% and 16%, respectively) or controls (16% and 14%, respectively) ($P<0.05$).

Macrovascular disorders, serum homocysteine level and MTHFR gene polymorphism in SSc

78% of SSc patients had pulmonary, 57% had esophageal and 39% had myocardial involvement, while 9.8% of the patients had pulmonary hypertension. PAH was screened by doppler echocardiography, and PAH was considered present, if estimated right ventricular systolic pressure exceeded 45 mmHg. 20% of SSc patients had macrovascular manifestations (24 lcSSc, 7 dcSSc). 26/31 patients (84%) had obliterative arteriosclerosis of lower extremities, 8 (26%) had coronary heart disease, 2 (6.4%) had stroke and 3(9.7%) had deep venous thrombosis.

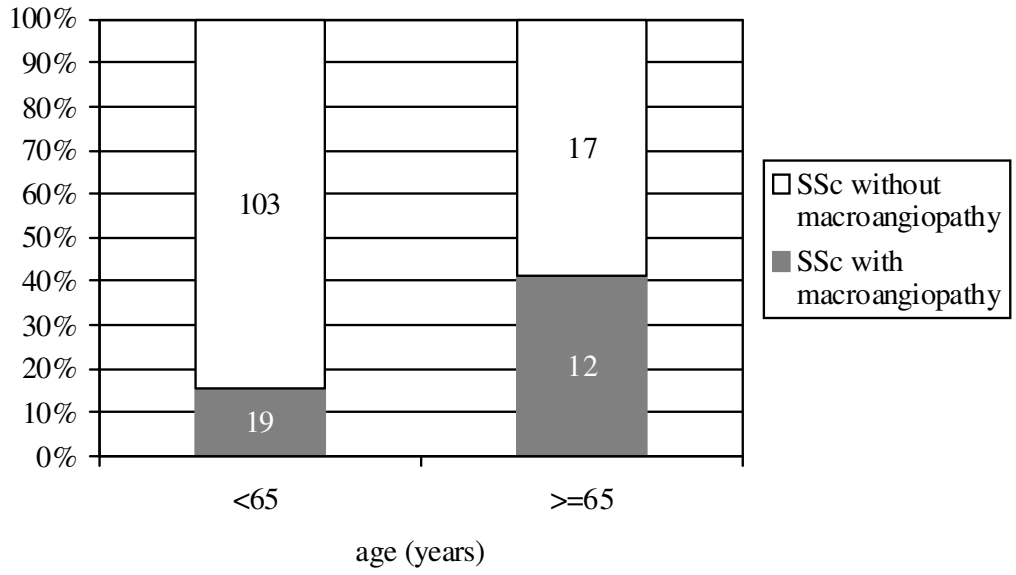
The mean plasma homocysteine levels were 9.3 μmol/l in SSc and 10.1 μmol/l in controls. There were no significant differences int he homocysteine levels between SSc and controls or between lcSSC or dcSSc subtypes.

Analyzing the MTHFR genotypes, no statistcal differences were found between SSc patients and controls. 49% of patients showed wild, 36% heterozygous, 15% homozygous MTHFR genotypes while 40% of controls had wild, 47% heterozygous and 13% homozygous genotype. There were no significant differences in homocyteine levels between homozygous, heterozygous and wild genotype within the SSc and control group.

Analyzing correlations between homocysteine levels and macroangiopathic events in SSc we found significantly higher homocyteine concentrations in patients with macroangiopathic events (10.5±7.1 μmol/l) compared to patients without such clinical manifestations (9.1±7.6 μmol/l). 6/31 patients with macroangiopathic disorders (19%) had homozygous (TT) MTHFR variants, 16 (52%) had heterozygous (CT) and 9 (29%) had wild type (CC).

A significant correlation was found between the age of SSc patients and the existance of macrovascular disease (Figure 1).

Figure 1: Relations of age with macroangiopathy in SSc patients



Finally, a positive correlation was observed between plasma homocysteine levels and the disease duration of SSc (r= 0,164, p=0,043). In addition, the presence of macrovascular abnormalities in SSc patients was associated with longer disease duration.

Analyzing the clinical parameters, prevalence of pulmonary hypertension was elevated in our patients who had higher than 15 μmol/l plasma homocysteine concentration. 10 of 22 patients with Hcy concentration >15 ± μmol/l had pulmonary hypertension (45%).

DISCUSSION

Gastrointestinal manifestations in SSc

This is the first cohort study on GI involvement of SSc in Central-Eastern Europe. Scattered recent publications addressed the same issue. Results of these studies were similar to ours: the esophagus was the most commonly affected organ of the GI tract. In our present study, barium swallow test revealed that distrurbed esophageal peristalsis was frequently associated with SSc, mostly with lcSSc subtype. The longer disease duration may account for this observation. The main consequence of the impairement of esophageal function is the development of GERD, which could further evolve into peptic erosive esophagitis. In our experience, one-third of the patients with esophageal dysfunction progressed to GERD or ulceration. Before the advent of proton-pump inhibitors, esophageal strictures were a common complication in patients with SSc.

Small intestinal involvement is also frequent among SSc patients and it often leads to life-threatening complications. The true prevalence of small bowel dysfunction is unknown and the diagnosis is often delayed as the clinical symptoms are non-specific and radiological tests are not sensitive enough. We found higher incidence of abdominal dystension, weight loss, increased flatulence and at least intermittant episodes of diarrhea in our cohort. We observed severe malnutrition in three patients with dcSSc who finally underwent TPN support. Finally two of them died in consequence of malabsortion with cachexia and other secondary complications. Involvements of the anorectum and the colon was present only in 11% of SSc patients. Our results are not in accordance with other reports. As a screening test, in general, we used radiological studies, barium enemas to detect colonoc involvement and invasive methods were only performed in appropriate indications. In addition, complaints like diarrhea, flatulence or fecal incontinence may not reflect objective changes as patients when questioned frequently shame these symptoms.

In conclusion, the early evaluation of particular symptoms and early detection of GI manifestations have a particular importance in the follow-up of patients with SSc. Symptomatic treatment csan improve the overall prognosis of the disease and the patients‘ sense of well-being and quality of life.

Juvenile systemic sclerosis

None of our patients developed severe pulmonary, cardiac or other internal organ manifestations during the follow-up period, so our patients are fully active in daily life at this moment. These results suggest that internal organ manifestations and the frequency of autoantibodies are far less pronounced in jSSc compared to our adult onset SSc population.

These results are similar to that ones described by Foeldvari et al, who analyzed 135 jSSc patients in a multicenter international study. It is still not fully clear, whether this differnce is a reflection of a slower disease progression or is due to the significantly less influence of co-morbidity factors in the pediatric group. To collect more evidence in this field, more multicenter international studies are required.

Clinical, serological and genetic characteristics of SSc-RA overlap syndrome

In the present study, the genetic, serological and clinical characteristics of SSc-RA overlap syndrome were assessed in the to-date largest cohort of 22 patients. The prevalence of SSc-RA overlap is 4.3% to 5.2% among SSc patients, which is similar to the incidence (4.6%) found in our cohort. In addition, higher incidence of RA is found within SSc patients than in the general population. Thus, it is clinically important to check for the occurrence of RA in patients with established SSc.

The clinical presentation of our SSc-RA patients showed a mixture of organ manifestations characteristic for SSc and RA. Thus, our patients exert a unique clinico-serological pattern as the majority of them have lcSSc with anti-topoisomerase seropositivity in 23% of the patients but ACA positivity in only 9% of them. These differences may be due to the smaller number of SSc-RA patients in other cohorts or to the geographical heterogenity of patients in various studies.

Significantly increased frequencies of HLA-DR3 and HLA-DR11 were observed in SSc-RA in comparison with RA patients and healthy subjects. In addition, allele frequencies of the HLA-DR1 and HLA-DR4 ‘shared epitope’ genes were significantly higher in SSc-RA and RA than in SSc or controls.

Regarding SSc-related HLA-DR genes, HLA-DR3, as well as anti-topoisomerase I autoantibodies have been associated with pulmonary fibrosis in SSc patients. In our present

study, we also detected the HLA-DR3 allele in onethird of SSc-RA patients and, as described earlier, the majority of our patients had lcSSc.

Our data support that SSc-RA overlap syndrome may be a distinct genetic, serological and clinical entity. As SSc-RA overlap patients in many ways differ from either RA or SSc patients, our data may have significant clinical relevance. A detailed clinical, radiological, laboratory and genetic assessment of either RA or SSc patients may reveal some overlap cases, which need special attention including individual treatment and follow-up.

Macrovascular disorders, serum homocysteine level and MTHFR gene polymorphism in SSc

In the present study, we did not find significant differences in Hcy levels and MTHFR polymorphism between SSc patients as well as healthy controls lacking any vascular disease, although impaired endothelial function in hyperhomocysteinemia is well known. The frequency of C677T variant of MTHFR gene in our results are similar to other reports where the homozygous form was found in about 10–13% and the heterozygous form in about 45% of Caucasian people.

Assessing the relationship between Hcy levels and the occurrence of macroangiopathic/thromboembolic events in SSc patients, we found significantly higher Hcy concentrations in patients with vascular/thromboembolic events in comparison to SSc patients without such manifestations. Altogether, 71% of patients with macrovascular disorders had either homozygous (TT) or heterozygous (CT) MTHFR variants. These data suggest that the existence of MTHFR C677T mutation (TT or CT form), may influence the incidence of macrovascular abnormalities in SSc. Although it is the microvasculature that is primarily affected in patients with SSc, it is recognized that largevessel disease also occurs with higher incidence and the involvement of the macrovasculature may be involved in the outcome of SSc. Considering these results and data previously reported by others, which could not identify MTHFR gene polymorphism as an independent risk factor for vascular complications in macrovascular diseases, we can conclude that there are other risk factors that may be crucial for the development of macrovascular manifestations in SSc.

We found a positive correlation between age and the existence of macrovascular manifestations in SSc. Besides there was a significant correlation between disease duration and the development of macrovascular manifestations. These results suggest that macrovascular disease is not only an age-related feature in SSc but may also depend on

disease-associated mechanisms. Finally, prevalence of pulmonary hypertension was elevated in 45% of our patients who had >15 μmol/l plasma Hcy concentration, which may have relevance for clinical practice.

SUMMARY

New results obtained in our study are as follows:

1. Out of the 246 SSc patients followed-up regularly in our institution, 177 (72%) patients developed significant clinical involvement of the alimentary tract. This was the first cohort study on gastrointestinal involvement of SSc in Hungary and even in Central-Eastern Europe.

2. In our present study, abnormalities of the anorectum and colon were observed only in 11% of our SSc patients. These results are not in accordance with other reports and suggest a particular importance on making non-invasive screening as well as extended diagnostic procedures in favour of early evaluation of gastrointestinal manifestations.

3. Associated diseases of the pancreas and biliary tract (e.g. PBC and Crohn's disease) were present in 10% of our SSc patients. A multidisciplinary collaboration is essential for early detection of these particular diseases and for designing an appropriate treatment protocol.

4. Our results suggest that the internal organ manifestations and the frequency of autoantibodies in juvenile onset SSc are far less pronounced in comparison to adult-onset SSc. Our study also suggests that the outcome of patients with jSSc may be better than that of patients with adult onset SSc.

5. In the present study, the genetic, serological and clinical characteristics of SSc-RA overlap syndrome were assesssed in the to-date largest cohort of 22 patients. The clinical presentation of our SSc-RA patients showed a mixture of organ manifestations characteristic for SSc and RA and also a mixed serological pattern resembling both SSc and RA.

6. Our SSc-RA overlap patients carried both the SSc-associated HLA-DR3 and HLA-DR11 alleles, as well as the RA-related HLA-DR1 and HLA-DR4 alleles.

7. These data support that SSc-RA overlap syndrome may be a distinct genetic, serological and clinical entity.

8. In the present study we did not find significant differences in Hcy levels and MTHFR polymorphism between SSc patients as well as healthy controls lacking any vascular disease.

9. Significantly higher plasma homocysteine concentrations were observed in patients with macroangiopathy/thromboembolic events compared to patients without such clinical

manifestations. Altogether 71% of patients with macrovascular disorders had either homozygous or heterozygous MTHFR variants. Our results suggest that besides other risk factors, hyperhomocysteinaemia and the polymorphism of MTHFR gene may be involved in the vascular damage associated with SSc.

10. Our results also suggest that macrovascular disease is not only an age-related feature in SSc but may also depend on disease duration, so it should be important to screen and follow these alterations.

Publication:

Publications that grounded this work:

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