

**SUMMARY OF THESIS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (PH.D.)**

**ASSOCIATED DISEASES OF SJÖGREN'S SYNDROME – CLINICAL AND
IMMUNOLOGIC FEATURES**

by

SZÁNTÓ ANTÓNIA M.D.



University of Debrecen Medical and Health Science Center

3rd Department of Internal Medicine

Division of Clinical Immunology

DEBRECEN

2008

I.Introduction

Sjögren's syndrome

Sjögren's syndrome (SS) is a chronic autoimmune exocrinopathy. Primary (glandular and extraglandular symptoms-pSS) and secondary (association with other autoimmune diseases -sSS) form can be distinguished.

Most frequent glandular symptoms include keratoconjunctivitis sicca, xerostomia and bilateral asymmetric parotid gland swelling. Extraglandular symptoms are represented by non-erosive polyarthritis, myositis, Raynaud's syndrome, tubulointerstitial nephritis, polyneuropathia and cutan vasculitis. Most severe complication of Sjögren's syndrome is the increased risk of malignant lymphoma.

Sjögren's syndrome is diagnosed according to the American-European Consensus classification criteria created in 2002. Beside the sicca symptoms, the typical small salivary gland biopsy result or presence of antibodies to Ro/SS-A and/or La/SS-B is needed to prove the disease.

Pathogenesis of Sjögren's syndrome is still not understood correctly, but there is no doubt that Sjögren's syndrome is caused by the combination of multiple agents: the selective damage of the exocrine glands is the end of a multi-step process. Beside genetic predisposition (HLA-DR and -DQ alleles), a disturbance in the selection procedures of the thymus plays an important role, and that can be triggered by viruses. As a result, autoimmune responses are generated against the changed autoantigens of the glandular epithel cells. This leads to chronic inflammation, fibrosis and the loss of the physiologic function. Lymphocytic infiltration of the affected glands and polyclonal B-cell activation is assured by increased expression of cell-adhesion molecules.

Antibodies to Ro/SS-A and La/SS-B are characteristic to Sjögren's syndrome. Their presence supposes longer disease duration and more frequent occurrence of extraglandular symptoms. Prevalence of anti-Ro/SS-A and anti-La/SS-B in primary Sjögren's syndrome are 95%, and 87%, respectively. Their specificity is much lower: both antibodies occur in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and also in other autoimmune diseases.

Fodrin is a ubiquitous protein which is located at the margin of the chromaffin cells and takes part in the process of the secretion. Fodrin forms a part of the cytoskeleton as a heterodimeric actin-binding protein which might be cleaved by calcium-activated proteins (calpain) and caspase family cysteine proteases during Fas-induced apoptosis. The result of the cleavage is a 120 kd fragment called alfa-fodrin.

The role of antibodies to alfa-fodrin (aAF) in different autoimmune diseases (primary and secondary SS, SLE, RA, autoimmune pancreatitis) has been widely investigated in the past few years, although the outcome was contradictory. IgA and IgG isotype antibodies are known, prevalence is 55-64% in pSS and 40-86% in sSS. These antibodies appear at younger age and occur significantly more often in primary than in secondary SS. Recent data suggest that aAF antibodies do not have an important diagnostic role in SS because of their low specificity and sensitivity.

However, there is a consensus about the relationship existing between fodrin and apoptotic processes.

Sjögren's syndrome associated to SLE

Lupus is a systemic autoimmune disorder causing autoimmune inflammation of multiple organs through the deposition of autoantibodies and immune complexes. It is characterized by diverse clinical symptoms, remissions and exacerbations and the presence of numerous autoantibodies, most characteristic is the antibody to double-strand DNA. It is often associated with secondary sicca-symptoms, but this is different from the association of the two diseases: the former doesn't need the serological alterations of SS, but needs the presence of the subjective sicca-symptoms and 2 criteria of objective xerostomia, keratoconjunctivitis sicca and positive result of small salivary gland biopsy, while the latter means that the diagnostic criteria of both diseases are present simultaneously. Prevalence of SS between lupus patients is about 8-30% according to the results of different studies.

Sjögren's syndrome associated with Hashimoto's thyroiditis

Hashimoto's thyroiditis (HT) is an organ-specific autoimmune disorder characterized by bilateral swelling and lymphoplasmocytic infiltration of the thyroid gland, presence of antibodies to thyroid peroxidase (aTPO) and thyroglobulin (aTG),

eventually resulting in hypothyreosis. Prevalence of HT between SS patients is 14.6%, according to a long-term follow-up study.

Aims

While studying the clinical and pathogenetic highlights of SS, my aims were to find answers for the following questions:

1. What is the prevalence of the association of SS and SLE among the patients followed-up in our center?

2. Are there characteristic clinical, laboratory or serologic signs for the association of SS and SLE which might influence the follow-up and/or treatment of the patients?

3. Is there a characteristic immunogenetic feature differentiating the association of SS and SLE from SS or SLE alone?

4. What is the prevalence of the different isotypes of antibodies to α -fodrin between patients with primary and secondary Sjögren's syndrome?

5. What is the specificity and sensitivity of antibodies to α -fodrin regarding primary Sjögren's syndrome?

6. How often are present antibodies to α -fodrin in patients with Hashimoto-thyreoiditis with and without Sjögren's syndrome?

7. Is there a relationship between the pathogenesis of the secretory disorders in SS and HT and the presence of antibodies to α -fodrin?

II. Methods

Patients and methods

II/a. Association of Sjögren's syndrome and systemic lupus erythematosus

Data of 362 SLE and 670 SS patients were studied to evaluate patients with the association of the two diseases (SS-SLE). Diagnosis was established according to the European-American Classification Criteria for SS and to ARA criteria for SLE. Fifty consecutively selected patients with SS, 50 with SLE and 50 healthy control persons served as controls. SLE patients with sicca complaints were excluded from the control group of the study. We analyzed demographic data, clinical features (Raynaud's syndrome, skin symptoms, thyroiditis, renal and pulmonary involvement, myositis, peripheral and central nervous system involvement, serositis, antiphospholipid syndrome), immunoserologic data (anti-Ro/SS-A, anti-La/SS-B, rheumatoid factor, antibodies to ds-DNA, β_2 glykoprotein-I and cardiolipin, antinuclear factor, cryoglobulinaemia), hematologic features (anemia, leukopenia, lymphopenia, thrombocytopenia).

Genetic evaluation

Genomic DNA was isolated from buffy coats of EDTA-anticoagulated blood. Polymerase chain reaction- (PCR-) based HLA-DRB typing was performed. The determination of HLA-DQ subtypes was performed using DQB1*02, DQB1*03, DQB1*04, DQB1*05 and DQB1*06 kits. HLA genotypes were determined on the basis of the PCR pattern obtained by electrophoresis with a 2% agarose gel. DNA bands were detected using Alpha Imager MultiImage Light Cabinet.

II/b. Antibodies to α -fordin in primary and secondary Sjögren's syndrome

Presence of IgA and IgG isotypes of antibodies to anti- α -fodrin (aAF) was determined in 67 patients with primary SS, 20 patients with SS secondary to RA and 17 patients with SS secondary to SLE. Sera of 20 patients with RA alone, 21 patients with

SLE alone and 30 healthy control persons were analyzed, too. RA and SLE were diagnosed according to the relevant ACR classification criteria. Since these measurements were performed before 2002, diagnosis of SS was established based on the European Community Study Group (ECSG) classification criteria.

II/c. Sjögren's syndrome and Hashimoto thyroiditis

Serum samples of 61 patients with primary SS (male to female ratio 3/58), 27 (3/24) with Hashimoto thyroiditis (HT), 31 (2/31) with SS and HT (SSHT) and 77 (7/70) healthy blood donors were analyzed. Patients were selected consecutively.

HT was considered if antibodies to thyroglobuline (aTG) and/or to thyroid peroxidase (aTPO) were present in the sera. This was proved with thyroid biopsy in 18 out of the 27 patients with HT and in 4 out of the 31 patients with SSHT. Each patient with thyroiditis was euthyroid with or without thyroid hormone substitution. SS was diagnosed according to the revised American-European classification criteria.

Serologic analysis of autoantibodies

IgA and IgG isotypes of antibodies to α -fodrin were evaluated using commercial ELISA kits, just like anti-Ro/SS-A and anti-La/SS-B antibodies. Cut-off values were the followings: 15 U/ml for antibodies to α -fodrin, 10 U/ml for anti-Ro/SS-A and anti-La/SS-B.

Antibodies to TG and TPO were detected with commercial ELISA kits, as well. Cut-off value was 225 IU/ml for aTG and 35 IU/ml for aTPO.

Statistical analysis

Data were analyzed using Microsoft Excel. Statistical analyses were performed using SPSS software, version 15.0. Kolmogorov-Smirnov test was used for the examination of normality. With continuous parameters showing normal distribution, an independent sample t-test was performed, whereas with the other continuous parameters not showing normal distribution, a Mann-Whitney test was carried out. With discrete

parameters, Fisher's exact test and chi-square test were used. Spearman's rho test was used to correlation analysis. Values of $p < 0.05$ were considered statistically significant.

III. Results

III/a. Association of Sjögren's syndrome and systemic lupus erythematosus

Among the 362 SLE and 670 SS patients followed in the 3rd Department of Internal Medicine, the association of SS and SLE was proved in 56 cases. This means 15.46% of lupus patients and 8.35% of SS patients.

Clinical, serologic and laboratory data observed at the different groups is shown on table 1, 2 and 3.

Table 1. Differences in clinical parameters between the different groups

Parameter observed	Value			P-value		
	<u>SS-SLE</u>	<u>SS</u>	<u>SLE</u>	<u>SS-SLE vs SS</u>	<u>SS-SLE vs SLE</u>	<u>SS vs SLE</u>
Age (years)	50.8	59.7	43.6	<0.01	0.01	<0.01
Raynaud's phenomem (%)	35.71	30	28	0.532	0.396	0.826
Renal involvement (%)	57.14	2	66	<0.01	0.312	<0.01
Pulmonary involvement (%)	28.57	8	24	<0.01	0.594	0.029
Myositis (%)	1.78	8	4	0.186	0.601	0.400
Perypheral nervous system involvement (%)	17.85	16	12	0.799	0.400	0.564
Central nervous system involvement (%)	25	4	36	<0.01	0.218	<0.01
Thyroiditis (%)	21.42	10	6	0.109	0.023	0.461
Serositis (%)	37.5	4	42	<0.01	0.636	<0.01
Skin involvement (%)	50	12	52	<0.01	0.837	<0.01
Antiphospholipid syndrome (%)	17.85	2	20	<0.01	0.778	<0.01

Table 2. Serologic alterations in the studied groups

Parameter observed	Value			P-value		
	<u>SS-SLE</u>	<u>SS</u>	<u>SLE</u>	<u>SS-SLE vs SS</u>	<u>SS-SLE vs SLE</u>	<u>SS vs SLE</u>
Rheumatoid factor (U/ml)	120.39	133.24	31.65	<0.01	0.126	<0.01
Cryoglobulinemia (%)	3.57	8	2	0.418	1.0	0.169
Anti-Ro/SS-A (%)	94.64	70	74	<0.01	<0.01	0.656
Anti-La/SS-B (%)	73.21	40	44	<0.01	<0.01	0.685
Anti-Ro/SS-A (U/ml)	125.71	86.71	77.7	<0.01	<0.01	0.848
Anti-La/SS-B (U/ml)	55.12	31.9	24.47	0.013	<0.01	0.435
Anti-ds-DNA (U/ml)	132.51	25.39	223.35	<0.01	<0.01	0.025
Antinuclearis factor (%)	94.64	70	94	<0.01	1.0	<0.01
Anti C1 IgG (U/ml)	60.71	10	44	<0.01	0.085	<0.01
Anti C1 IgM (U/ml)	64.28	20	56	<0.01	0.146	<0.01
Anti β_2 GPI IgG (U/ml)	41.07	8	46	<0.01	0.609	<0.01
Anti β_2 GPI IgM (U/ml)	30.35	16	30	0.082	0.968	0.096

Table 3. Hematologic alterations in the different groups

Parameter observed	Value			P-value		
	<u>SS-SLE</u>	<u>SS</u>	<u>SLE</u>	<u>SS-SLE vs SS</u>	<u>SS-SLE vs SLE</u>	<u>SS vs SLE</u>
Anemia (%)	53.57	18	72	<0.01	0.051	<0.01
Leukopenia (%)	66.07	44	76	0.022	0.262	<0.01
Lymphopenia (%)	42.85	18	58	<0.01	0.120	<0.01
Thrombocytopenia (%)	25	16	36	0.254	0.218	0.023

Male to female ratio was 4/52 in the SS-SLE group, 2/48 in the SS group and 5/45 among SLE patients.

Patients in the SS group were significantly older (59.7 ± 8.6 years) than both in the SLE (43.6 ± 15.2 years) and in the SS-SLE group (50.8 ± 13.5 years) (Table 1).

Out of the 56 patients with SS and SLE, at 53 patients it was identifiable, whether it was SLE or SS to appear first. At 23 patients (43.39%) the two diseases started simultaneously between 1972 and 2003; the mean duration of the disease was 8.1 years. In 8 cases (15.0%), SS preceded SLE with 1-18 years. In 22 cases (41.5%) SLE was the first presenting, 1-19 years before SS (mean 6.7 years).

Regarding Raynaud's phenomenon, myositis, peripheral nerve involvement and cryoglobulinaemia, there were no significant differences among the three groups, however, myositis was tendentially more frequent in the primary SS group. The frequency of thyroiditis was significantly higher in the SS-SLE group than in the SLE group, while compared with the SS group, the difference was not significant. Antiphospholipid syndrome, renal, lung, central nervous system and skin involvement was significantly less frequent than in the other two groups, but the difference between the SLE and the SS-SLE group was not statistically significant.

Both the prevalence and the titer of the Ro/SS-A, La/SS-B autoantibodies and rheumatoid factor were the highest in the SS-SLE group. Antinuclear antibodies occurred with the lowest frequency in the SS group, while the occurrence was significantly higher in the other two groups, but the difference between them was not significant.

Double-stranded DNA antibodies presented with the highest concentration in the SLE group, significantly higher than in patients with SS-SLE, while in the latest group the concentration was still significantly higher than in the SS group where the mean value did not reach the upper limit of the normal range. Concerning hematological deviations, the SS-SLE group did not differ statistically from the SLE group, while in the SS group, these alterations were significantly less frequent. Three of the antiphospholipid autoantibodies (IgG and IgM-type anti-cardiolipin; IgG type anti- β_2 GPI) showed similar results: there was no significant difference between the SS-SLE and the SLE groups, but the presence of these autoantibodies was significantly less frequent in the SS group, compared with the other two groups. The presence of the IgM isotype anti- β_2 GPI autoantibodies did not differ in any of the groups.

Immunogenetic analysis did not show significant difference among the three patient groups, regarding HLA-DRB1*1501, DRB1*0301, DQB1*0602, DQB1*0502, DQB1*0201, DQB1*0303, DQB1*0304, DQB1*0402, DQB1*0301 and DQB1*0501 alleles (Table 4).

HLA allele	<u>SS-SLE</u>	<u>SS</u>	<u>SLE</u>	P value		
	(n=33)	(n=41)	(n=28)	<u>SS-SLE</u> <u>SS</u> vs	<u>SS-SLE</u> <u>SLE</u> vs	<u>SS</u> vs <u>SLE</u>
DRB1*1501	1	1	0	1.000	-	-
DQB1*0602	5	4	7	0.501	0.335	0.106
DRB1*0301	1	1	1	1.000	1.000	1.000
DQB1*0502	5	5	6	0.744	0.525	0.334
DQB1*0201	15	18	12	0.894	0.839	0.931
DQB1*0303	2	2	1	1.000	1.000	1.000
DQB1*0304	1	0	0	-	-	-
DQB1*0402	2	3	1	1.000	1.000	0.641
DQB1*0301	12	17	7	0.655	0.340	0.159
DQB1*0501	4	8	3	0.391	1.000	0.505

Table 4: Result of the immunogenetic analysis in the groups of patients

However, there was a remarkable correlation between the levels of anti-Ro/SS-A and HLA-DQB1*0201 alleles ($r=0.464$, $p=0.01$), and so was the correlation between the levels of anti-La/SS-B antibody and HLA-DQB1*0301 alleles ($r=0.408$, $p=0.038$). In the SS-SLE and in the SLE group, also anti-La/SS-B correlated with HLA-DQB1*0201 alleles ($r=0.574$, $p<0.001$ and $r=0.342$, $p=0.001$, respectively).

III/b. Antibodies to α -fodrin in primary and secondary Sjögren's syndrome

Out of the 67 patients with primary Sjögren's syndrome, anti- α -fodrin IgA was present in 25 (38%), whereas anti- α -fodrin IgG was present in 26 (39%). Regarding the control group, both IgA and IgG isotypes were present in two patients (Figure 1).

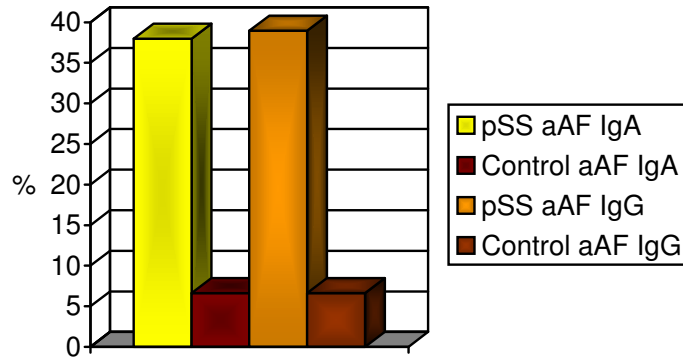


Figure 1. Prevalence of antibodies to α -fodrin in primary Sjögren's syndrome and in the control group

We observed the presence of IgA antibodies to α -fodrin in 13 (65%) and IgG antibodies in 11 (55%) among patients with SS secondary to RA. Between patients with SS secondary to SLE, both IgA and IgG antibodies were present in 9 cases (53-53%).

In RA, 2 (10%) of the 20 sera were positive to IgA and 3 (15%) to IgG isotype aAF. aAF IgA was present in 2 (9%), aAF IgG in 3 (14%) patients with SLE.

Summary of the results can be seen on Table 5.

	Number of cases	Percentage of anti α -fodrin IgA positive sera (Number of cases)	Percentage of anti α -fodrin IgG positive sera (Number of cases)
SS	67	38 (25)	39 (26)
SSS(RA)	20	65 (13)	55 (11)
RA	20	10 (2)	15 (3)
SSS(SLE)	17	53 (9)	53 (9)
SLE	21	9 (2)	14 (3)
Control	30	6 (2)	6 (2)

Table 5. IgA and IgG positive sera in pSS, SSS, RA and SLE

Statistical analyses showed the followings: prevalence of aAF was significantly higher in primary and secondary SS than in the control group. Both isotypes were significantly more frequent in secondary SS than in RA and SLE presenting alone.

Level of aAF antibodies in the sera of patients is shown in figure 2:

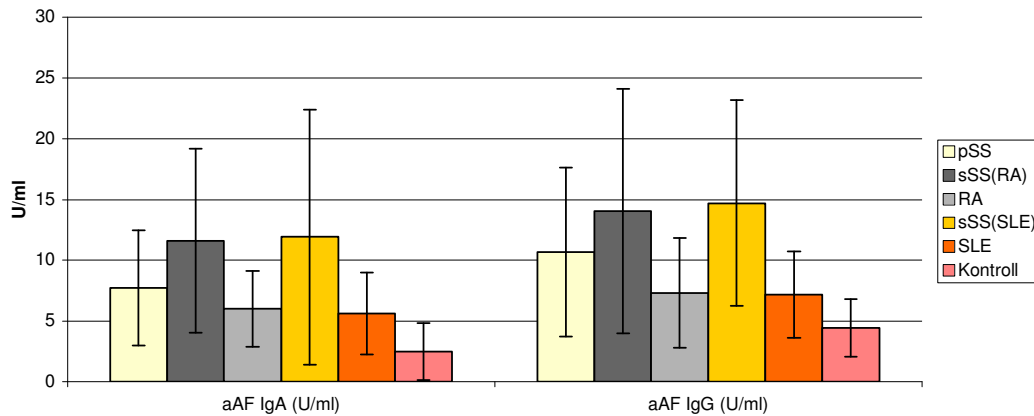


Figure 2. Serum level of antibodies to α-fordin in the different patient groups

Related to the control group, each patient group presented a higher level of aAF. Serum concentration of aAF was significantly higher in secondary SS than in SLE or RA presenting alone.

Sensitivity of antibodies to α-fodrin for primary SS was 37.3% regarding IgA and 38.8% regarding IgG isotypes. Specificity was 93.3% at both isotypes. Calculation was later re-made with strengthening the diagnostic criteria according to the latest classification criteria created in 2002 (46 out of 67 patients still fulfilled these criteria), and this resulted 17.3% for sensitivity of IgA, while 28.2% for sensitivity to IgG. Specificity was 93.3 and 100% for IgA and IgG isotypes, respectively.

III/c. Sjögren's syndrome and Hashimoto thyroiditis

Mean age of patients was 57.75±12.65 years in the SS group, 47.78±13.70 in HT group, 59.87±10.73 years in the SSHT group and 43.79±11.05 years in the control group.

In SS, 14 patients were positive for aAF IgA (22.9%) and 24 for IgG (39.3%). 29 patients (49.5%) had at least one of the two isotypes.

2/27 HT patients were positive for IgA (7.4%) and 4/27 for IgG type aAF (14.8%), 6/27 (22.2%) had one of the two isotypes in the serum.

Concerning the SSHT group, 6/31 patients presented with IgA (19.3%), 10/31 with IgG (32.2%) and 12/31 (38.7%) with either aAF isotypes. One of the 77 healthy blood donors were positive for IgA (1.29%) and 2 for IgG (2.59%) aAF (3/77, 3.89% had at least one sort of antibody) (Figure 3).

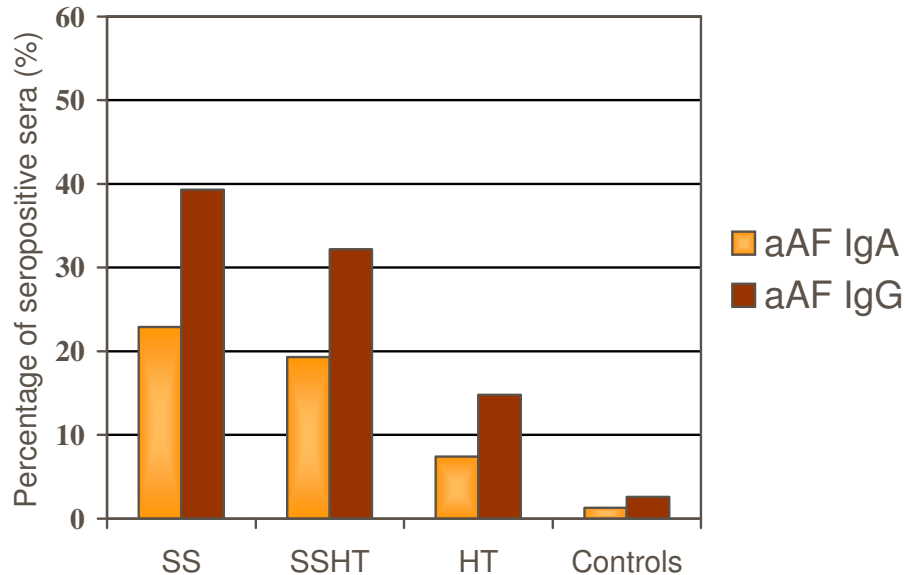


Figure 3. Occurrence of aAF IgA and aAF IgG in the different patient groups

Statistical analysis showed that in all patient groups, the level of both isotypes of aAF was present in a significantly higher level than in the control group. There was no significant difference in the level of aAF neither between HT patients with or without SS, nor in IgA type aAF between SS and HT patients.

When examining the occurrence of aAF, we found that both isotypes of aAF were significantly more often present in SS and SSHT than in the control group. IgG type aAF was more often found in HT than in the control group, but there was no significant difference neither in the occurrence of aAF between SSHT and HT group, nor in that of SS and HT regarding IgA type antibodies (Figure 4).

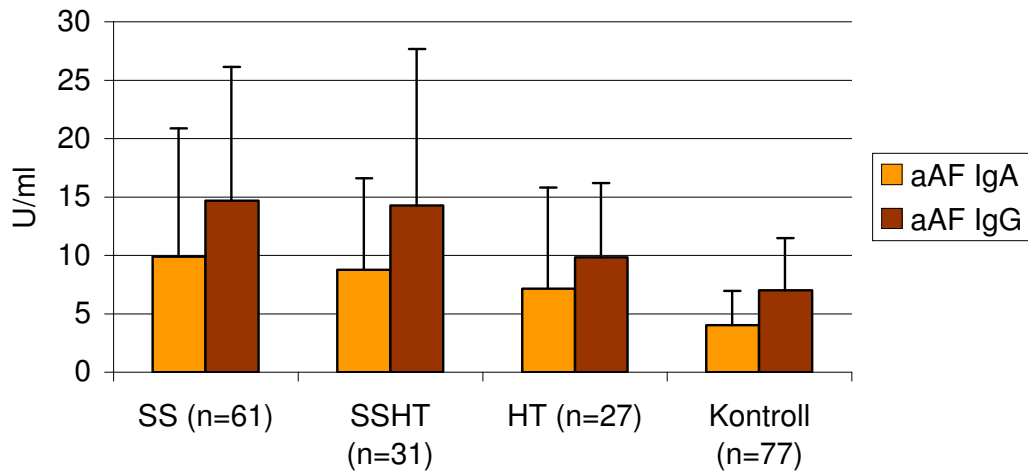


Figure 4. Mean values of aAF antibodies in the studied groups

We found correlation between the level of IgG type aAF and aTG ($R=0.43$, $p=0.028$).
None of the other antibodies showed correlation with each other.

IV. Discussion

IV/a. Association of Sjögren's syndrome and systemic lupus erythematosus

Based on our findings, when SS followed the diagnosis of SLE, the average time interval between the diagnoses was about as much as the difference between the mean ages of the SLE and the SS-SLE group (6.7 years vs. 7.2 years). The mean age of the primary SS group was much higher (almost 9 years) than the SS-SLE group. The presence of SLE seems to accelerate the development of SS. French authors reviewed the charts of 55 patients with SS, where four of the patients developed SLE. According to their conclusions, the main clinical events suggesting the progression into SLE were pleuropericarditis, glomerulonephritis and focal nervous system disease. Manoussakis et al. examined patients with secondary SS to SLE: in 69.2% of the cases, sicca-complaints preceded the appearance of SLE with 1-15 years.

Concerning the quality of life and target organ lesions, no difference has been found between patients with SS, SLE and SS-SLE regarding neuropsychiatric, cardiovascular, gastrointestinal gonadal, pulmonary and skin involvement. Based on our findings, Raynaud's syndrome, myositis, peripheral nerve involvement, thrombocytopenia and cryoglobulinaemia occurred with a similar frequency in the three groups. However, we found a remarkable difference in respect of central nerve involvements in the SS group compared to patients with SS-SLE or SLE alone. Some authors did not find a difference between patients with SS and SLE, while others reported contradictory findings.

Autoimmune thyroiditis occurred most frequently in patients with SS-SLE (21.42%), while in patients with SS the difference was not significant. These findings can be explained with the fact that autoimmune thyroiditis often accompanies SS.

Both anti- β_2 GPI and anti-cardiolipin IgG antibodies, as well as IgM type anticardiolipin antibody and secondary antiphospholipid syndrome presented the least frequently in the SS group, while the other two groups did not differ significantly. In another study, the frequency of secondary antiphospholipid syndrome was found to be higher than in ours.

Our results support the idea that there are distinct clinical, laboratory and serological features that distinguish the association of SS and SLE from SS and SLE

alone. The presence of rheumatoid factor is less frequent, while anti-Ro/SS-A, anti-La/SS-B, ANA, anti-DNA and antiphospholipid autoantibodies, antiphospholipid syndrome, anemia, leucopenia and lymphopenia occur more frequent than in SS. The mean age is lower, too. In the group of patients with SS and SLE, pulmonary, renal, skin and central nervous system involvement and serositis occur more often than in patients with SS alone. In the SLE group, patients are significantly younger than in the SS-SLE group. The presence of thyroiditis, and autoantibodies to Ro/SS-A, La/SS-B and ds-DNA are more frequent in the SS-SLE group than in patients with SLE alone. Accordingly, in patients with SS and SLE, the complications mentioned above require a more stringent follow-up, in order to start with the adequate treatment as soon as possible.

The characteristic features of the associated form reflect the different pathogenesis of the two diseases. SS is a systemic autoimmune disorder in its pathology because the production of autoantibodies is directed against general nuclear constituents (Ro and La antigens). In the other hand it has organ specific features, leading to the damage of exocrine glands. Both SS and SS-SLE are characterized by the common co-existence of autoimmune thyroiditis, another organ specific autoimmune disease. However, antiphospholipid syndrome is characteristic for SLE (its prevalence was 20% in our study), which suggests that antiphospholipid syndrome in patients with associated SS and SLE follows the pathomechanism of the autoimmune coagulopathy characteristic for SLE.

In earlier investigation we described the characteristic features of secondary SS (secondary SS associated with SLE). We showed higher frequency of certain clinical symptoms (e.g. myositis, arthritis), compared to patients with SLE. Thus, we concluded that the accumulation of the symptoms could be explained with the interaction of distinct pathogenic factors.

The more frequent representation of the SS specific DRB1*0301 allele has been described, instead of the SLE-specific DRB1*1501 and DQB1*0602 alleles in secondary SS associated to SLE. Our results did not show significant difference in the occurrence of the investigated alleles in the three patient groups.

In our opinion, it is of great importance to distinguish between SS secondary to SLE (secondary SS) and SS associated with SLE. In this study, all SS associated with SLE (SS-SLE) patients had circulating anti Ro/SS-A and/or anti La/SS-B autoantibodies, 55 out of the 56 patients had objective keratoconjunctivitis sicca and 22

out of the 56 had objective xerostomia besides their subjective sicca symptoms. Minor salivary gland biopsy was performed only at 3 patients from this group, because at the patients in whom SS preceded SLE, it was unnecessary for the diagnosis of primary SS. Based on the current criteria for secondary SS, in these patients, even after 1-18 years of having primary SS, if SLE develops, the diagnosis of SS becomes uncertain.

Our findings support the idea that although all 56 patients fulfilled the diagnostic criteria for primary SS, together with the diagnosis of SLE, only 22 had secondary SS. On the other hand, based on the present classification criteria for SS, an SLE patient having hypertension as a result of chronic steroid use and therefore taking antihypertensive drugs, can be diagnosed as secondary SS, based on clinical symptoms (xerostomia, keratoconjunctivitis sicca) caused by the well-known antihypertensive drug side-effects. There is no need for histological signs or for the specific serological alterations to the diagnosis of secondary SS: subjective sicca symptoms and objective symptoms, such as xerostomia and keratoconjunctivitis sicca are enough to consider an SLE patient as having secondary SS without the pathogenetic background of this autoimmune exocrinopathy. Therefore, it would be necessary to revise the classification criteria for secondary SS and make it more specific.

IV/b. Antibodies to α -fodrin in primary and secondary Sjögren's syndrome

There are several studies examining the presence of aAF in primary and secondary SS and in other autoimmune diseases.

In childhood SS, sicca symptoms present rarely, but the histologic manifestations of the disease are the same as in adult patients. Antibodies to 120 kDa α -fodrin appear at younger age than antibodies to Ro/SS-A and/or La/SS-B, thus supporting the early diagnosis of SS.

Watanabe et al found the prevalence of aAF significantly higher than in SLE. Sensitivity of aAF was 67%, its specificity was 93%. Presence of aAF correlated with hypergammaglobulinemia, presence of RF and antibodies to La/SS-B. German colleagues observed findings similar to ours: they showed the presence of aAF IgA in 64% of pSS, 47% of SS secondary to SLE and 86% of SS secondary to RA. These percentages were 55, 40 and 43% regarding IgG isotypes, respectively. In SLE and RA

presenting alone, both IgA and IgG isotype occurred quite rarely (1/50 patients with SLE and 2/12 patients with RA for IgA and 1/50 and 5/12 for IgG).

Several working groups published data similar to ours regarding the relatively high specificity and low sensitivity of aAF. This suggests that evaluation of aAF is worth doing first of all in the lack of anti-Ro/SS-A and anti-La/SS-B autoantibodies in the diagnosis of primary and secondary SS.

IV/c. Sjögren's syndrome and Hashimoto thyroiditis

We found that serum level of aAF was significantly higher not only in SS and SSHT patients, but also in HT patients than in the control group. The finding that there is no significant difference neither between the level nor between the occurrence of aAF in the different patient groups (except for the IgG isotype aAF between SS and HT patients) allows us to suppose that fodrin has a possible role in the secretion not only in exocrine but also in endocrine processes and that the presence of aAF can serve as a marker of secretory disorders. Even if it is probably not the marker of all secretory disturbances, it might show one of the numerous existing pathways.

Alfa-fodrin antibodies have been widely studied in the past years, and seemed to be promising novel markers in the diagnosis of SS. It has been showed that antibodies to alfa-fodrin appear at younger age than anti-Ro/SS-A and anti-La/SS-B in SS patients. Numerous studies have revealed that aAF presents significantly more often in SS than in SS associated with SLE or RA. Later, growing research results suspected that in fact, aAF is neither specific, nor sensitive enough for being useful in the diagnosis of SS itself in the every-day rheumatology practice.

Both in SS and in HT, the main pathogenetic event is lymphocytic infiltration with the predominance of T cells. Recent findings support the idea that B-cells play a pivotal role in the pathogenesis of SS by autoantibody production and act as antigen presenting cells. Therefore, they contribute to the perpetuation of the humoral autoimmune machinery in the disease. Miyazaki et al described that the N- terminal fragment of alfa-fodrin mediated in vivo immunoregulation of autoimmune responses in SS.

In the general population, aAF is proved to be associated with objective sicca-symptoms, even if the patient does not fulfill the criteria for Sjögren's syndrome. This suggests that fodrin is in connection with secretion and not only in autoimmune

processes, but also in dry eye and dry mouth caused by different effects (e.g. drugs, elderly glandular dysfunction, e.t.c.).

Ishimura et al demonstrated that fodrin played a role in the secretory activities of the colloid in rats, and that is known as a key event in the thyroid hormone secretion. These data raise the possibility that fodrin is associated with glandular secretion both in the endocrine and in exocrine type. The fact that we did not find significant difference in the presence and particularly in the level of aAF between almost each patient groups, can be another factor supporting this hypothesis. The correlation between aTG – which is an antibody to the main protein of the colloid - and IgG-type aAF also suggests that these antibodies might have a role in the pathogenesis of autoimmune thyroid disease concerning the „final common effector pathway”, secretion. Our results support the idea that antibodies to AF are markers for secretion disorders, both for the exocrine, and for the endocrine type. We believe that the assessment of these autoantibodies help in the diagnosis and follow-up of patients with impaired secretory capability, such as SS and HT. Even if they are not capable to serve as diagnostic criteria, their significance is in the assessment of the irreversibility of secretion disorders and aids in the initiation of a proper substitution therapy.

V. Summary

New results obtained in my work are the followings:

1. Out of the 362 SLE and 670 SS patients followed-up in our Center, an association of SS and SLE was shown in 56 cases (15.46% of lupus and 8.35% of SS patients).

2. Based on the assessment of a large cohort of patients, we were the first to conclude that SS-SLE had characteristic differences compared to SS or SLE alone. These features include both laboratory alterations and clinical symptoms.

3. There was no characteristic immunogenetic feature of SS-SLE in the examined population. However, positive correlation was detected between antibodies to Ro/SS-A, La/SS-B and HLA-DQB1*0201.

4. Antibodies to α -fodrin in the sera of patients suffering from Hashimoto's thyroiditis were detected by us. Moreover, we proved that there was no significant difference between patients with Sjögren's syndrome and Hashimoto's thyroiditis and patients having both diseases simultaneously, regarding the occurrence of IgA isotype antibody to α -fodrin.

5. There was a middle-grade correlation between the serum level of IgG isotype antibody to α -fodrin and antibodies to thyroglobulin. This suggests a relationship between the presence of antibodies to α -fodrin and the pathogenesis of Hashimoto's thyroiditis, since they influence the hormone-secreting activity of the colloid.

6. According to my results, fodrin is likely to play a role not only in the exocrine, but also in the endocrine secretory processes. Antibodies to α -fodrin might serve as markers of secretory disorders. The clinical significance of these antibodies is in the assessment of the irreversibility of secretion-disorders and aids in the initiation of a proper substitution therapy.

Publications:

List of publications of which the thesis are based on:

1. **Szanto A**, Szodoray P, Kiss E, Kapitany A, Szegedi Gy, Zeher M: Clinical, serological and genetic profiles of patients with associated Sjögren's syndrome and systemic lupus erythematosus Hum Immunol 2006 Nov;67(11): 924-30.

IF: 2.605

2. **Szanto A**, Csipo I, Horvath IF, Biro E, Szodoray P, Zeher M: Autoantibodies to alfa-fodrin in patients with Hashimoto thyroiditis and Sjögren's syndrome: possible markers for a common secretory disorder. Rheumatol Int. 2008; 28(11):1169-72.

IF: 1.27

3. **Szántó A.**, Csípő I., Zeher M.: Alfa-fodrin elleni autoantitestek előfordulása Sjögren-szindrómás betegeinknél. Magyar Immunológia 2003;2(2):40-44.

4. **Szántó A**, Kiss E, Sas A, Szegedi Gy és Zeher M: Szisztémás lupus erythematosus és Sjögren-szindróma társult eseteinek elemzése (Orv Hetil 2005;146 (50): 2533-8.

4. Zeher M, Csípő I, Bacskó Gy, **Szántó A**. Autoantibodies against Alpha-fodrin in patients with Sjögren's syndrome. Advances in immunopathology & respiratory allergy 2005;113-115. ISBN 88-7587-166-3

5. **Szántó A.**, Csípő I. és Zeher M: Sensitivity and specificity of anti-alfa-fodrin antibodies in primary Sjögren's Syndrome. J Rheumatol. 2005 Jan;32(1):197; author reply 197-8.

IF: 3.010

Impact factor: **3.875 (+3.010)**

Other publications:

1. **Szántó A.**, Szegedi A., Zeher M.: Az autoimmun urticariáról – esetismertetések tükrében. *Allergológia és Klinikai Immunológia* 2003; 6:172-176.
2. **Szántó A.**: Biológiai terápia vasculitisekben – cikkreferátum. *Magyar Immunológia* 2003; 2(4):46-7.
3. **Szántó A**, Veisz R, Krenács L, Csiki Z, Griger Z, Zeher M: Korai fázisban felismert Takayasu-arteritis. *LAM* 2006;16(8-9):762-767.
4. Centola M, Frank MB, Bolstad AI, Alex P, **Szanto A**, Zeher M, Hjelmervik TO, Jonsson R, Nakken B, Szegedi G, Szodoray P. Genome-scale assessment of molecular pathology in systemic autoimmune diseases using microarray technology: a potential breakthrough diagnostic and individualized therapy-design tool. *Scand J Immunol.* 2006 Sep;64(3):236-42. Review.
IF: 2.090
5. Szodoray P, Koczok K, **Szanto A**, Horvath IF, Nakken B, Molnar I, Zeher M: Autoantibodies to novel membrane and cytosolic antigens of the lacrimal gland in primary Sjögren's syndrome. *Clin Rheumatol* 2008 Feb;27(2):195-9.
IF: 1.644
6. Szabo N, Csiki Z, **Szanto A**, Danko K, Szodoray P, Zeher M: Functional and morphological evaluation of hand microcirculation with nailfold capillaroscopy and laser Doppler imaging in Raynaud's and Sjögren's syndrome and poly/dermatomyositis. *Scand J Rheumatol* 2008 Jan-Feb 37(1):23-9.
IF: 2.640
7. Horvath IF, **Szanto A**, Csiki Z, Szodoray P, Zeher M: Intrapulmonary rheumatoid nodules in a patient with long-standing rheumatoid arthritis treated with leflunomide. *Pathol Oncol Res* 2008; 14(1):101-4.
IF: 1.272

Cumulative impact factor: **11.521**

Posters, lectures

Szántó A, Zeher M. Vasculitisek Sjögren-szindrómában. MAKIT Kongresszus, Sopron, 2002

Szántó A, Horváth I, Szodoray P, Zeher M. Sjögren-szindróma és cryopathiás vasculitis. Magyar Belgyógyász Társaság Észak-Kelet-Magyarországi Szakcsoport Nagygyűlése, Eger, 2002.

Szántó A, Csípő I, Zeher M. Alfa-fodrin elleni antitestek Sjögren-szindrómás betegeinkben. MAKIT Eger, 2003.

Szántó A, Csípő I, Zeher M. Az alfa-fodrin elleni autoantitestek jelentősége Sjögren-szindrómában. TAIM Alapítvány Tudományos Kongresszusa, Debrecen, 2003.

Szántó A, Csípő I, Zeher M. Alfa-fodrin elleni autoantitestek Sjögren-szindrómás betegeinkben. Magyar Belgyógyász Társaság Észak-Kelet-Magyarországi Szakcsoport Nagygyűlése, Debrecen, 2003.

Szántó A, Csípő I, Zeher M. Alfa-fodrin elleni autoantitestek jelentősége Sjögren-szindrómában. Magyar Reumatológusok Egyesületének 75. Vándorgyűlése, Szeged, 2003. Poszter.

Szántó A, Csípő I, Zeher M. Alfa-fodrin izotípusok összefüggése a Sjögren-szindróma tüneteivel. MAKIT Kongresszus, Keszthely, 2004.

Szántó A, Csípő I, Zeher M. Alfa-fodrin izotípusok meghatározásának jelentősége: összefüggésük a Sjögren-szindróma tüneteivel. MRE Vándorgyűlés, Budapest, 2004.

Szántó A, Kiss E, Szegedi Gy, Zeher M. Szisztémás lupus erythematosus és Sjögren-szindróma társulása. MAKIT Kongresszus, Debrecen, 2005.

M Zeher, I Csípő, G Bacskó, **A Szántó**. Autoantibodies against alpha-fodrin in patients with sjögren's syndrome. III. World Congress on Immunopathology, Thailand, Pattaya, 2005. Poster.

Szántó A, Szodoray P, Kapitany A, Kiss E, Szegedi Gy, Zeher M. Association of Sjögren's syndrome and systemic lupus erythematosus. International Symposium on Sjögren's syndrome, Washington, 2006 (poster)

Szántó A, Veisz R, Krenacs L, Csiki Z, Griger Z, Zeher M. Korai fázisban felismert Takayasu-arteritis. MAKIT Kongresszus, Gyula, 2006.

Szántó A, Zeher M. A Takayasu arteritistről, egy esetismertetés kapcsán. III. Debreceni Belgyógyászati Napok, 2006.

Szántó A, Veisz R, Krenacs L, Csiki Z, Griger Z, Zeher M. Korai fázisban felismert Takayasu arteritis. MRE Vándorgyűlés, Debrecen, 2006. Poszter

Szántó A, Horváth I, Csípő I, Szodoray P, Zeher M. Alfa-fodrin elleni antitestek Sjögren-szindrómában és Hashimoto-thyreoiditisben. MAKIT Kongresszus, Balatonalmádi, 2007.

Szántó A, Csípő I, Szodoray P, Zeher M. Alfa-fodrin elleni antitestek: közös marker a szekréciós rendellenességekre? MRE Vándorgyűlés, Szeged, 2007.

M Zeher, IFanny Horvath, **A Szanto**, P Szodoray. Autoimmune thyroid diseases in 479 Hungarian patients with primary Sjögren's syndrome. International Congress of Immunology, Rio De Janeiro, 2007