Ph.D. doctoral theses

DIFFERENTIAL DIAGNOSIS IN SJÖGREN'S SYNDROME: LABORATORY AND CLINICAL ASPECTS

Written by

István Gál, M.D.

Supervisor

Prof. Dr. Margit Zeher

3rd Department of Internal Medicine

University of Debrecen

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Abbreviations

MHC: major histocompatibility complex

HLA: human leukocyte antigen

HHV: human herpes virus

HIV: human immunodeficiency virus

CD: cluster designation

LFA-1: lymphocyte function-associated antigen 1

Ig: immunoglobulin

kD: kilodalton

MALT: mucosa associated lymphoid tissue

HTLV: human T-cell leukemia virus

EBV: Epstein-Barr virus

HCV: hepatitis C virus

Bcl-2: B-cell lymphoma-2

ANA: antinuclear antibody

AIDS: acquired immunodeficiency syndrome

ELISA: enzyme-linked immunosorbent assay

ARA: American Rheumatism Association

CT: computed tomography

ANCA: anti-neutrophilic cytoplasmic antibody

NYHA: New York Heart Association

EMG: electromyography

ENG: electroneurography

PCR: polymerase chain reaction

Hgb: haemoglobin

IU: international unit

HbsAg: hepatitis B surface antigen

SLE: systemic lupus erythematosus

EGS: extraglandular symptoms

MGUS: monoclonal gammopathy of undetermined significance

GM-CSF: granulocyte-monocyte colonya stimulating facor

1. Introduction and aims

The principal feature of Sjögren's syndrome is an autoimmune epithelitis, which is accompanied by the T- and B-cell mediated infiltration of exocrine glands and epithelial tissue structures with consequential symptoms: keratoconjunctivitis sicca and stomatitis sicca. The disease is present worldwide, affecting all age groups, with a peak incidence of onset between 40-50 years. It shows a strong predominance for the female gender, with a female:male ratio of 9:1. The calculated prevalence of Sjögren's syndrome for the overall population is approximately 0,6% (1).

There are at least 5 systems of classification criteria available for Sjögren's syndrome (2, 3, 4, 5, 6). Until the recent past, the most commonly used one was the European system (6), which, in 2002, has undergone revision in order to increase its accuracy, specificity and sensitivity (7). However, in our practice, we often see patients presenting the clinical picture and meeting the criteria of Sjögren's syndrome, but being diagnosed with a different disease, which only mimics this disorder. *Our goal was to highlight the drawbacks of the European system of classification criteria for Sjögren's syndrome through reporting cases meeting these criteria and mimicking Sjögren's syndrome. We offer recommendations for the correction of the most commonly used European system. Applying these the diagnostic power of the criteria might increase.*

The characteristic feature of Sjögren's syndrome, a lympocytic infiltration of exocrine glands can result in a complete destruction of the fine tissue structure of these organs, leading to the appearance of one of the leading symptoms and classification criteria: the sicca symptoms (keratoconjunctivitis sicca and stomatitis sicca) (8). Nevertheless, the disease might affect other organs such as lungs, stomach, kidneys, vascular system, lymphoid system, central- and peripheral nervous system, bone marrow, musculoskeletal sytem. Accordingly, we can define the so-called extraglandular involvements of Sjögren's syndrome, with the most prevalent

clinical manifestations of arthritis, myositis and vasculitis or Raynaud's phenomenon. Vasculitis is one of the most common extraglandular manifestations of Sjögren's syndrome. Its development might lead to occurrence of severe complications. The remarkable - often leukocytoclastic – inflammation of the small vessels can result in the occlusion of the vascular lumen and a serious hypoxia and eventual necrosis of the tissues. *The aim of the present work was to study and characterize one of the most common extraglandular manifestation, vasculitis,, which is often present in a subclinical form, in a large group of patients with Sjögren's syndrome.*

Pathologic activation of B-cells is a hallmark of Sjögren's syndrome but also the potentially most dangerous phenomenon accompanying the disease. B-cells infiltrating the target organs are exposed to the long-lasting stimulating effect of certain autoantigens and exogenous antigens and are prone to present with a high proliferative activity, which can lead to the transformation of these cells, and the development of lymphoid malignancies such as lymphomas. This is supported by clinical observations: patients with Sjögren's syndrome have a high risk for the development of malignant lymphomas compared to the general population (9). Interestingly, despite of this, Sjögren's syndrome patients have similar life expectancies than that of their healthy controls (10). *The aim of this retrospective study was to analyse the occurrence of Sjögren's syndrome's most dangerous complications, haematological malignancies, in a large cohort of patients.*

In the target organs, B-cells represent one quarter of the entire cellular infiltrate and are responsible for the production of antibodies characteristic for Sjögren's syndrome. Autoimmunity developing against certain ribonucleoproteins results in the appearance of anti-Ro/SSA and anti-La/SSB autoantibodies in the sera and salivary glands of the affected patients. These antibodies represent sensitive and specific markers of the disease and are part of classification criteria established for Sjögren's syndrome. (8). *In this study, we examined*

the occurrence of anti-Ro and anti-La autoantibodies in patients with Sjögren's syndrome, subgrouped on a clinical basis. Our goal was to analyse the distribution and presence of these antibodies and therefore to try to contribute to defining novel diagnostic or prognostic factors in the disease.

2. Methods

In our case reports, we use the European classification criteria established in 1993 and revised in 1996, for the diagnosis of Sjögren's syndrome (6, 8). To determine serum titres of autoantibodies anti-Ro/SSA and anti-La/SSB we used DIALAB ELISA kit (DIALAB GmbH, Austria), calibrated according to the instructions of the manufacturer.

We studied epidemiological and clinical characteristics of peripheral vasculitis accompanying Sjögren's syndrome, paying special attention to the immunserological features of the disease. We determined frequency and age characteristics of the vasculitis. Also, we analysed the spectrum of its clinical manifestations, its association with extraglandular symptoms, and its histological properties. We studied whether there is distinctive immunserological marker between Sjögren's syndrome with and without vasculitis.

To identify cytotoxic T-cells in our histological studies, we used anti-CD8 antibody (Novocastra). As pan-T-cell marker, anti-CD3 antibody (DAKO), and for labelling B-cells, anti-CD20 antibody (DAKO) were used.

In cases of coexisting Sjögren's syndrome and sarcoidosis, we used the Kyoto diagnostic criteria to establish diagnosis of the latter disease (11).

The diagnosis of amyloidosis was based on the histological features of the disease: the amyloid deposits appear in a yellow-green double colour under polarisation microscope, when stained with Congo red. With this method, we achieved clear diagnosis in the kidney-,

rectum-, skin-, and salivary gland specimens in all cases, therefore use of electron microscopy was not necessary to further confirm the presence of amyloidosis.

In cases of myeloma multiplex and coexisting Sjögren's syndrome, the diagnosis of the haematological malignancy was based on specific laboratory markers and on histological findings on bone marrow aspirate.

Studies on serological markers (anti-Ro/SSA, anti-La/SSB antibodies) of Sjögren's syndrome

Of our 464 outpatients with Sjögren's syndrome at the time of the study, we assigned 60 to be enrolled in the study. All of them have had disease duration of more than 5 years. They had sicca symptoms, objective xerophthalmia and xerostomia, serum anti-Ro/SSA in highly elevated titres and all of them had histological findings on minor salivary gland biopsy specimens characteristic for Sjögren's syndrome. Twenty of them had non-erosive polyarthritis as extraglandular symptom. None of the patients had any other severe coexisting diseases. For the diagnosis of Sjögren's syndrome, the modified European criteria (8) were used. To diagnose systemic lupus erythematosus, we applied ARA criteria established in 1982 (12).

Ten of the 60 patients suffered from primary Sjögren's syndrome without extraglandular symptoms and without anti-La/SSB autoantibody positivity, 10 patients from primary Sjögren's syndrome without extraglandular symptoms and with anti-La/SSB autoantibody positivity, 10 patients from primary Sjögren's syndrome with extraglandular symptoms (non-erosive polyarthritis) and without anti-La/SSB autoantibody positivity, 10 patients from primary Sjögren's syndrome with extraglandular symptoms (non-erosive polyarthritis) and with anti-La/SSB autoantibody positivity, 10 patients from Sjögren's syndrome/systemic lupus erythematosus overlapping disease without anti-La/SSB autoantibody positivity and 10 patients from Sjögren's syndrome/systemic lupus erythematosus overlapping disease with

anti-La/SSB autoantibody positivity. We have taken sera from each patient shortly after the diagnosis, before any immunosuppressive treatment. All of the sera has been stored at –70C^{o} for no more than 6 months.

With DIALAB ELISA kit, we quantitatively measured the levels of the anti-Ro52-kD/SSA and anti-Ro60-kD/SSA autoantibodies at least two separate times in the sera of the patients, applying human recombinant antigens. Anti-La/SSB antibodies were determined also by DIALAB ELISA, applying highly purified antigen. To validate our ELISA assay we used ImmuBlot Anti Nuclear Antibody – Western Blot Test Kit (IMMCO Diagnostics, Buffalo, NY, USA), an immunoblot assay for the detection and confirmation of anti-nuclear antibodies (including anti-SSA and anti-SSB) in human serum, in the case of 20 patients.

We compared the distribution of the anti-Ro52-kD/SSA and anti-Ro60-kD/SSA autoantibody positivities between the 6 groups of the patients. Comparison of the frequencies of anti-Ro52-kD/SSA and anti-Ro60-kD/SSA autoantibodies was performed by using Fisher's exact test and chi-square test. Significance was considered when $p<0.05$. The statistics were calculated by SPSS 8.0 software.

3. Results

- Sjögren's syndrome and sarcoidosis

We found 8 patients meeting the criteria of primary Sjögren's syndrome *and* the criteria of sarcoidosis. Of these patients, 3 had proven to had pure sarcoidosis during the long-term follow-up. The initial symptom of their illness was parotid gland swallowing and sicca symptoms. In the sera of 2 of these patients we found anti-SS-A and anti-SS-B autoantibodies in elevated titres. None of them had extraglandular manifestations typical for Sjögren's syndrome. Lip minor salivary gland biopsies in all these cases revealed the presence of sarcoidosis, without massive focal lymphoid infiltrates characteristic for Sjögren's syndrome.

Chest CT showed pathologically enlarged mediastinal lymph nodes in all of these cases. Two of them refused performing hilar lymph node biopsy; the lymph node histology of the third patient confirmed the diagnosis of sarcoidosis. Treating with low dose oral methylprednisolon and following these 3 patients for 1-5 years, their sarcoidosis healed and sicca symptoms and anti-SS-A, anti-SS-B positivity could not been observed any more. Following up for a long time, we found 5 patients with coexisting primary Sjögren's syndrome and sarcoidosis. All of them – as contrasted with the other group – had extraglandular manifestations characteristic for Sjögren's syndrome (4 non-erosive polyarthritis and 1 vasculitis cases). The initial symptom of their disease was like in the other group. Salivary gland biopsy revealed focal lymphoid infiltrates, non-caseating granulomata, Langhans and epitheloid cells. All of them had hilar lymphadenopathy on chest CT. Treating with low dose oral steroid, sarcoidosis regressed, but sicca symptoms and autoantibody positivity are still persisting. Minor salivary gland biopsy specimens of these patients obtained after healing of sarcoidosis revealed focal lymphoid infiltrates. All of them still have episodes of extraglandular manifestations, even if the signs of sarcoidosis are missing.

- Amyloidosis mimicking Sjögren's syndrome

With the case reported below, we would like to highlight diseases potentially mimicking Sjögren's syndrome and thereby causing difficulties in the differential diagnostic process.

Our female patient, D. S., at the age of 43, presented with suffusions and livid spots at sites exposed to minor physical traumas. Analysis of skin biopsy specimens revealed allergic vasculitis. Later, severe, non-selective proteinuria developed. Kidney biopsy revealed mesangial glomerulonephritis, and c-ANCA positivity appeared in the serum of the patient. At the age of 45 years, keratoconjunctivitis and stomatitis sicca developed. Microscopic evaluation of small salivary gland specimens no focal lymphoid infiltrates characteristic for

Sjögren's syndrome were seen. Immunoserology towards autoantibodies such as anti-SSA or anti-SSB were also negative. Repeated skin biopsy sampling revealed amyloidosis: an amorphous mass was seen in dermis after haematoxylin-eosin staining, while amyloid deposits were seen around the vessels by polarisation microscopy. Small salivary gland biopsy specimens also showed the presence of amyloid. Based on these findings, we asked the revision of the kidney biopsy sample, which also confirmed our diagnosis.

Sjögren's syndrome and vasculitis

Of all the 447 Sjögren's syndrome patients we enrolled in the study, we found 52 (11.6%) cases where clinically obvious vasculitis developed. Mean age of the patients was 49.6 (20-78) years at the diagnosis of the vasculitis. The cohort was composed of 49 women and 3 men.

Majority of the vasculitis cases presented with the involvement of the skin (29 cases, 58% of all patients). However, myositis verified by EMG and histology (20 cases, 40%) and motor neuropathy verified by ENG and histology (13 cases, 26%) were also prevalent. We have seen overlapping form in several cases. In most instances, Sjögren's syndrome was followed by the onset vasculitis in 4-5 years.

Purpura was the most frequent of all the dermatological manifestations (26 cases). Maculopapulous (6 cases), urticariform (6 cases), and nodous lesions (2 cases) were less common. In two third (18 out of 29) of cutaneous vasculitis cases, histology revealed mononuclear infiltration. In the remaining (10/29 cases), we identified neutrophilic infiltration, which represents the leukocytoclastic form of vasculitis.

Of the histologically verified (sural nerve biopsy, muscle biopsy) systemic manifestations, we found mixed polyneuropathy in 26% (13/50 cases) and myositis in 40% (20/50 cases). The

clinical presentation of these disorders were paraesthesias (which necessitated the ENG examinations) and myalgia (with elevated serum creatine kinase levels), respectively.

In 100% (50/50) of the patients with vasculitis, we found autoantibodies characteristic for Sjögren's syndrome (anti-SSA and/or anti-SSB) in fairly high titres. Of serological findings, cryoglobulinaemia (18/50 cases) and immunocomplexaemia (19/50 cases) were also frequently found.

Activation of vasculitis and related symptoms occurred less then 1 time in the majority of the patients (44/50) during the follow-up. In the rest of the patients, the number of flare-ups were ranging between 2 and 3.

Sjögren's syndrome and haematological malignancies (myeloma multiplex and non-Hodgkin lymphomas)

- Sjögren's syndrome and myeloma multiplex

P.I. female patient, age 75 years, was admitted to our department for sicca symptoms, polyarthralgia, high rate of erythrocyte sedimentation, and anaemia sideropenica. We established the diagnosis of primary Sjögren's syndrome based upon the characteristic clinical, serological and histological findings, but this diagnosis was followed by the one of myeloma multiplex in a couple of years. Since the myeloma was active at this time, (high rate of erythrocyte sedimentation, high level of serum paraproteins, extreme anaemia, elevated β-2 microglobulin level), we initiated an aggressive cytostatic treatment with a maintenance therapy by α-interferon. The disease was in remission for a longer period, but worsening of the clinical status again necessitated introduction of 2 cycles of cytostatic therapy Due to the success of the treatment the disease is still in mission (by summer of 2000) and the anaemia and bone pains disappeared, paraprotein levels have not changed. Besides all these, Sjögren's

syndrome symptoms (subjective and objective xerophthalmia, xerostomia) and serological findings (anti-SSA és anti-SSB autoantibodies) were still present.

P. J. female patient was diagnosed with Sjögren's syndrome and myeloma multiplex at the same time. Histologically, from skin specimens, vasculitis was also present. Following the induction therapy, involvement of 3 lumbar vertebrae and the meningeal structures (accompanied by extreme headache, verified by MRI) were seen. Consequently, besides her usual therapy, the patient received irradiation therapy of the lumbar spine, but the onset of secondary leucopenia necessitated the administration of recombinant granulocyte-monocyte colony stimulating factor. This was followed by the introduction of α-interferon-based maintenance therapy, which yielded in a temporary improvement in the clinical status and complains. Later, interferon therapy had to be interrupted because of development of severe leucopenia. Then another cytostatic cycle with targeted irradiation of the lower spine were applied. Shortly, the clinical status remarkably impaired and the involvement of the pelvis could be seen. The scheduled irradiation had to be delayed due to the onset of pneumonia. The progression we seen necessitated of a modified chemotherapeutic regimen, which resulted in a mild improvement of the clinical status. Unfortunately, severe leucopenia appeared which led to the development of pneumonia and we have lost the patient. The symptoms of Sjögren's syndrome (objective xerostomia és xerophthalmia) were present all along the duration of the disease, but the titres of anti-SSA and anti-SSB autoantibodies were not elevated in the last 2 years of the disease.

At the time of the study, we had altogether 464 patients with Sjögren's syndrome to follow up at our outpatient clinic. Based on this and our case reports, the frequency of myeloma multiplex in Sjögren's syndrome is 0.43%, and the myeloma occurs in the general population in 4-10/100.000 (13). Seemingly, Sjögren's syndrome patients have a relative risk of 50-100x to develop myeloma multiplex.

- Sjögren's syndrome and non-Hodgkin's lymphomas

In this study, we enrolled 494 patients with primary Sjögren's syndrome. During a follow-up of 19 years, we found 14 patients (13 women and 1 man) developing non-Hodgkin's lymphoma associated with the Sjögren's syndrome. Onset of the malignant lymphomas followed the diagnosis of Sjögren's syndrome with an average of 7,6 years. In Sjögren's syndrome patients with lymphoma, of extraglandular manifestations, persistent parotid swelling was found in 7/14 cases, lymphadenopathy in 5/14 cases, non-erosive polyarthritis in 6/14 cases and cutaneous vasculitis in 2/14 cases.

In 9 of 14 cases of lymphoid tumours, we found low-malignancy B-cell lymphomas, in 3 cases highly malignant lymphomas, and in 2 cases myeloma multiplex (see above).

At the time of this study, we have lost 3 patients for sepsis as a complication of the cytostatic therapy, but 11 patients were in remission.

Based on our results, the frequency of haematological malignancies in primary Sjögren's syndrome is much higher (14/494; 2.8%) than in the general population (~7/100,000; 0,007%).

Amongst patients with coexisting Sjögren's syndrome and non-Hodgkin's lymphoma, extraglandular symptoms occurred in 100%. What's more, we found more than one 1 extraglandular symptoms in these patients (with parotid swelling and lymphadenopathy being the most prevalent ones). Those patients who had remarkable swelling of their parotid glands received long-term low-dose oral corticosteroid therapy.

Serological markers in Sjögren's syndrome: importance of anti-Ro and anti-La in determining disease type and activity

From the group of patients with primary SS, without extraglandular symptoms and without anti-La/SSB positivity, 10 of 10 were positive for anti-Ro52-kD/SSA, 5 of 10 for anti-Ro60-kD/SSA autoantibody. From patients with primary SS, without extraglandular symptoms and with anti-La/SSB positivity, 9 of 10 were positive for anti-Ro52-kD/SSA, 5 of 10 for anti-Ro60-kD/SSA autoantibody. From patients with primary SS, with extraglandular symptoms and without anti-La/SSB positivity, 10 of 10 were positive for anti-Ro52-kD/SSA, 4 of 10 for anti-Ro60-kD/SSA autoantibody. From patients with primary SS, with extraglandular symptoms and with anti-La/SSB positivity, 10 of 10 were positive for anti-Ro52-kD/SSA, 4 of 10 for anti-Ro60-kD/SSA autoantibody. Summarily, 97.5% of primary SS patients were positive for anti-Ro52-kD/SSA and 45% for anti-Ro60-kD/SSA. From patients with SS/SLE overlapping disease and without anti-La/SSB positivity, 10 of 10 were positive for anti-Ro52-kD/SSA, 8 of 10 for anti-Ro60-kD/SSA autoantibody. From patients with SS/SLE overlapping disease and with anti-La/SSB positivity, 10 of 10 were positive for anti-Ro52-kD/SSA, 9 of 10 for anti-Ro60-kD/SSA autoantibody. Accordingly, 100% of SS/SLE patients were positive for anti-Ro52-kD/SSA and 85% for anti-Ro60-kD/SSA. Regardless of presence or absence of anti-La/SSB antibody positivity, the occurrence of anti-Ro60-kD/SSA positivity looks to be significantly more frequent, when SLE complicates SS, compared to primary SS cases ($p=0.0048$, Fisher's exact test). The anti-Ro52-kD/SSA positivity was more frequent than anti-Ro60-kD positivity in primary SS ($p=2,3x10^{-7}$, chi square test). The presence of extraglandular symptoms in primary Sjögren's syndrome does not look to be in connection with the distribution of anti-Ro52-kD and anti-Ro60-kD/SSA autoantibodies.

4. Discussion

Diagnostics and differential diagnostics in Sjögren's syndrome in terms of using classification criteria

Retrospectively analyzing the data of 464 patients with Sjögren's syndrome, we found 5 patient suffering from sarcoidosis as well. Therefore, in our case, the frequency of sarcoidosis in Sjögren's syndrome is 1.07 %, which value is veryí similar to that published by others (14). As the frequency of sarcoidosis in the general population is 10-80 per 100.000, frequency of sarcoidosis looks to be higher in patients with Sjögren's syndrome. When diagnosing Sjögren's syndrome, if the patient meets European criteria, the possibility of sarcoidosis must be excluded (6, 7). As sarcoidosis can coexist, the diagnosis of Sjögren's syndrome can be problematic in these cases. Even a pure sarcoidosis can be diagnosed as Sjögren's syndrome based upon a possibly false negative histology, sicca symptoms, elevated autoimmune antibody titres, etc., altogether meeting the European criteria. To be able to exclude a possible sarcoidosis in the background of the symptoms, in the case of patients with sicca symptoms or probable Sjögren's syndrome it may be considered to obtain multiple biopsy specimens and to follow up the patients for years before the final diagnosis. Also, based on these observations, revision of certain parts of the European criteria van be recommended. Assigning Sjögren's syndrome as exclusion criteria means that coexisting Sjögren's syndrome and sarcoidosis is not an existing entity, although, as seen, it is, indeed.

Amyloidosis is a systemic disease, affecting eventually all organs, including salivary- and lachrymal glands. Therefore it can lead to concerns of the differential diagnosis between Sjögren's syndrome and amyloidosis. Sjögren's syndrome, as a systemic inflammatory autoimmune disorder, can result in the development of amyloidosis in several years. In these cases, the two diseases can coexist indeed. When diagnosing Sjögren's syndrome,

amyloidosis represents an exclusion criterium. With an appropriate approach and technique of biopsy sampling, histological evaluation of small salivary gland specimens have a relatively high sensitivity amongst the diagnostic procedures used in Sjögren's syndrome. Presence of amyloid deposits can be demonstrated in two third of minor salivary gland biopsy specimens of patients with amyloidosis. Consequently, when a patient presents with sicca symptoms, minimally invasive minor salivary gland biopsy sampling can be recommended. In these instances, a possibility of amyloidosis must be kept in mind, especially when sicca symptoms are not accompanied by immunoserological abnormalities characteristic for Sjögren's syndrome. Since 100% sensitivity cannot be guaranteed during biopsy sampling of either organ in amyloidosis, in case of negative result but high likelihood of the disease, biopsy samples have to be taken repeatedly. Taking biopsy specimens from a variety of organs can increase sensitivity. By presenting this case with amyloidosis mimicking the clinical picture of Sjögren's syndrome, we point out differential diagnostic problems caused by this condition. Despite of the characteristic symptoms, diagnosis of Sjögren's syndrome could not be established. Destruction of the exocrine glands were not the consequence of an autoimmune inflammation, rather of deposition of amyloid proteins at these sites.

Sjögren's syndrome and vasculitis

Studies on larger cohorts of patients with Sjögren's syndrome reported that of the most frequent skin manifestations of vasculitis, purpura represented ~50%, urticaria ~30%, erythematous lesions 15% and subcutaneous nodular lesions ~10% of all cases (15). Our results are comparable to those referred in the international literature. We found the involvement of muscles and the nervous sytem to be fairly prevalent. In the majority of the cases, only mild symptoms were associated with the involvement of the organs. When interpreting our results, it is worthy to mention that in the clinical picture of neuropathies

developed on the ground of the vasculitis of the motor nerves and/or of the direct, cytotoxic T-cell infiltration and consequential damage of sensory nerves. The most frequent serological abnormalities were the following: strikingly elevated anti-SSA and anti-SSB autoantibody titres in the sera of the patients, cryoglobulinemia, and immunocomplexaemia. Summarily, we found ~11% occurrence of vasculitis in a large cohort of patients with Sjögren's syndrome, which represent a somewhat lower value than that reported by the majority of references in the literature. The presence of vasculitis was suggested by characteristic clinical features and the diagnosis was verified and supported by histological findings in all cases. Considering that these vasculitis cases can be present in an asymptomatic, subclinical form, the prevalence others and we found can be much higher, indeed.

Sjögren's syndrome, non-Hodgkin's lymphomas, and myeloma multiplex

The higher prevalence of lymphoma in patients with primary SS has been known for almost 3 decades (16). This recognition was confirmed by further publications and the results suggest more frequent occurrence of malignant lymphomas in SS in comparison with the control population (17, 18, 19, 20, 21). Comparing our results with those of other authors we can unambiguously establish the higher prevalence of lymphoma in contrast with the healthy controls but this has been considerably lower than in most of the cases. In our study 2.8 percent of NHL frequency was demonstrated which are similar to the results of Vidal (21) and Kelly (17). The high occurrence of extraglandular manifestations in patients with Sjögren's syndrome and accompanying malignant lymphomas is considerable. The relation of these symptoms in the pathogenesis of lymphomas in SS is not clear. Considering that in our SS/NHL patients, the extraglandular symptoms were present for several years when diagnosing the NHL-s, it can be suggested that the presence of these symptoms mean a primary SS form of higher risk for developing secondary lymphomas. Summarizing our

results we can establish the higher frequency of the lymphoma in our 494 patients with primary SS compared to that of general population, comparably to a lot of the publications in the literature. We assume that the relatively low occurrence of MALT type NHL-s in our patients can be explained by that the corticosteroid therapy used for the treatment of salivary gland enlargement in patients with primary Sjögren's syndrome.

The occurrence of monoclonal gammopathy of undetermined significance (MGUS) in Sjögren's syndrome patients is some percent (22). In our patients, the frequency of MGFUS was ~4% (17 out of 464 patients). Likelihood of the transformation of MGUS into myeloma multiplex is thought to be ~15% (23, 24, 25, 26). This points out the necessity of careful follow-up of patients with monoclonal gammopathy. Our first patient with myeloma and Sjögren's presented with an aggressive haematological malignancy with severe symptoms and pathological laboratory findings. It was necessary to use radical cytostatic protocols from the beginning. As part of therapy, α-interferon and GM-CSF were both used. A potential side effect of interferon is the flare-up of the autoimmune disease, but fortunately, we did not experience that.

Serological markers in Sjögren's syndrome: importance of anti-Ro and anti-La antibodies in determining disease subtype

Some findings in SS and SLE suggest that the subtypes and distribution of the anti-Ro/SSA autoantibodies can be in connection with the disease type (if it is coexisting with another autoimmune disease or not) and activity. Anti-Ro52-kD – anti-Ro60-kD/SSA antibody profiles differed between patients with primary SS and patients with SS/SLE overlapping disease (27).

According to our results, of the subtypes of anti-Ro/SSA autoantibodies, the occurrence of anti-Ro52-kD/SSA positivity looks to be a more frequent finding in Sjögren's syndrome than

the anti-Ro60-kD positivity. Consequently, the use of ELISA kits measuring only anti-Ro60-kD/SSA in the diagnostic process of Sjögren's syndrome can lead to establish anti-Ro/SSA negativity and misdiagnosing the disease. Regardless of presence or absence of anti-La/SSB positivity, the occurrence of anti-Ro60-kD/SSA positivity looks to be more frequent, when SLE complicates SS, compared to primary SS cases. On the other hand, occurrence of anti-Ro-52-kD/SSA and anti-Ro60-kD/SSA does not look to be influenced by the lack or presence of extraglandular symptoms or anti-La/SSB positivity in primary Sjögren's syndrome. These findings must be confirmed in larger scale studies.

Considering our and others' results in Sjögren's syndrome, a possible relation exists between the positivity for and subtype spectrum of the serum anti-Ro/SSA autoantibodies and the subtype (primary or secondary) of the disease, however the relevant data are contradictionary. Even, according to the few accessible literature data it looks to be more probable that the fine specificity, rather than the serum levels and distribution of these autoantibodies is in stronger connection with the disease type and its activity in primary and secondary Sjögren's syndrome (28, 29, 30).

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CUMULATIVE IMPACT FACTOR: 56,04

10. Curriculum Vitae

Name: **ISTVÁN GÁL**

Nationality: Hungarian

Birthplace and date: Debrecen, Hungary, january 8th 1974

Present address: Derék str. 213. 4/13.

Debrecen, H-4031

Present occupation: medical doctor

2nd Dept. of Internal Medicine

Kenézy Gyula Hospital

Bartók B. str.. 2-23.

Debrecen, H-4028

Tel.: 06-52-511-777

Fax: 06-52-511-755

Assistant Professor

Department of Orthopedic Surgery

Section of Biochemistry and Molecular Biology

Rush-Presbyterian-St. Luke's Medical Center

1653 W.Congress Parkway

Chicago, IL 60612, USA

Phone: 312-942-9691

Fax: 312-942-8828

e-mail: gistvan@hotmail.com

Education: medical doctor, 1998 University of Debrecen, Medical Faculty

Licensure: 57454

Scientific activity:

1998. september-1999 march:

PhD student, 3rd Dept of Internal Medicine, University of Debrecen supervisor: Dr. Margit Zeher

2000. september-2002 march:

research fellow, Department of Orthopedic Surgery, Rush University, Chicago

2002 march-present: assistant professor, Department of Orthopedic Surgery, Rush University, Chicago

Clinical practice:

1996 july: visiting medical student, Dept. of Surgery, Göteborgs Universiteit, Gothenburg, Sweden

1997 august: visiting medical student, Depts. Of Surgery, Pediatrics and Nephrology, Vrije Universiteit, Akademisch Ziekenhuis, Amsterdam, The Netherlands

1998 september-2000 september:

resident, 3rd Dept. of Internal Medicine, University of Debrecen

2003 september-present:

resident, 2nd Dept. of Internal Medicine, Kenézy Gyula Hospital, Debrecen

Educational activity:

1993 september-1998 june:

teaching biophysics in Hungarian and in English, Dept. of Biophysics, University of Debrecen

1998 september-2000 september:

teaching internal medicine in Hungarian and in English, 3rd Dept. of Internal Medicine, University of Debrecen

Awards:

1998: Weszprémy-award

1998: TEVA-Biogal Pharmaceutical Company, award for research activity

Memberships:

1998-present: Hungarian Medical Chamber

1998-present: Hungarian Association of Immunologists

1998-present: Hungarian Association of Cardiologists

march 2004-present: Hungarian Association of Gastroenterologists