SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Clinical and genetic characteristics of anti-Jo-1 positive antisynthetase and scleroderma-myositis overlap syndromes

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 06th of July, 2023 at 13:00

INTRODUCTION

1. Idiopathic inflammatory myopathies:

Idiopathic inflammatory myopathies (IIMs) are rare, heterogenous autoimmune diseases. The incidence of this disease in Hungary is 0,95/100000/year, the prevalence is 1- 6/100000. IIMs are systemic autoimmune connective tissue diseases characterized by chronic muscle inflammation resulting in progressive symmetrical muscle weakness with elevated serum levels of muscle enzymes, electromyographic abnormalities and characteristic mononuclear inflammatory infiltrates in muscle biopsy specimens. Besides the muscle, other organs can be also affected, including the joints, skin, heart, gastrointestinal tract and lungs, and they can even result in the predominant manifestations, supporting that IIM are systemic inflammatory disorders. Inflammation of skeletal muscles and internal organs underpin IIM, leading to irreversible damage and even death.

The clinical diagnosis of IIMs is proposed by EULAR/ACR classification criteria in 2017. IIMs are classified into 4 major subtypes, polymyositis (PM), dermatomyositis (DM), inclusion body myositis (IBM) and necrotizing autoimmune myopathy (NAM).

Autoimmune processes observed in inflammatory myopathies are not fully understood, but it seems that genetic and environmental factors (viral infections, UV light) are likely to interact to confer risk for developing chronic inflammatory diseases such as polymyositis (PM) and dermatomyositis (DM). It is known that the pathogenesis of idiopathic inflammatory myopathies involves strong interactions between dendritic cells, activated Th1 and Th17 cells, B cells, muscle cells, genes and environment. Autoimmune origin is supported by derailed cellular and humoral immune processes. Ethnic differences and the HLA-associations suggest that genetic factors may play a part in the pathomechanism.

Autoantibodies are of great importance for the diagnosis of many systemic autoimmune rheumatic diseases, including IIMs. Myositis-associated autoantibodies (MAAs) are those that

appear in myositis overlap syndromes and in other connective tissue diseases, which correlate with certain clinical and/or pathophysiological conditions of myositis. The myositis-specific autoantibodies (MSAs) are useful markers for clinical diagnosis, classification, and for predicting the prognosis of the IIM. Approximately 38% of IIM patients have MSAs in their sera. The most frequent MSA in the serum of patients with myositis is anti-Jo-1.

Management of myositis is challenging owing to the rarity and heterogeneous nature of this disease. Complexity also arises from variable presentation and disease courses as well as its multiorgan and systemic characteristics.

2. Antisynthetase syndrome:

Presence of anti-Jo-1 defines a distinct clinical phenotype, antisynthetase syndrome (ASS), which is characterized by poor prognosis, and multiple organ involvement, such as myositis, interstitial lung disease (ILD), arthritis, Raynaud's phenomenon, mechanic's hand, skin rashes, and fever.

Considering the etiology of patients with anti-Jo-1 antibody the followings are of great importance. Some special genes may play a role in the development of ASS antibodies. Human leukocyte antigen genes on chromosome 6, particularly HLA-DRB1∗03:01 and the linked allele DQA1∗05:01, have the strongest associations with the presence of anti- Jo-1 antibody in Caucasian patients. HLA-DQA1∗05:01 and HLA-DQA1∗04:01 are associated with this antibody in African-Americans and Hispanics. Smoking appears to be associated with an increased risk of having anti-Jo-1 in HLA-DRB1∗03-positive IIM cases.

3. Overlap Myositis:

The prevalence of overlap myositis in inflammatory myopathies varies from 22 to 49%. Overlap myositis is constituted by myositis occurring in the setting of systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disorder (MCTD), rheumatoid arthritis (RA), and Sjögren's syndrome (SS). One of the most frequent overlap

myositis is represented by myositis and SSc, comprising 15-42,6% of all the overlap syndrome IIMs. On the contrary, muscle disease, or myopathy, in scleroderma has been thought to be a relative bystander in comparison to other organ disease manifestations. Due to a lack of classification criteria of muscle disease in scleroderma, the prevalence of muscle disease in scleroderma varies widely. There is no consensus on whether an inflammatory myopathy in SSc should be considered as disease symptom, or as scleroderma-myositis overlap, but it is becoming more apparent that SSc patients with concomitant muscle disease have poorer outcomes including disability and death. Hence, the clinic-serological identification and early recognition of an IIM-SSc overlap situation is useful to clarify prognosis and facilitate management.

It is well known that genetics could have a crucial role in the pathomechanism of the diseases and is associated with the presence of certain autoantibodies and clinical phenotype. Patients with systemic sclerosis and myositis have characteristic genetic features, such as the presence of HLA-DRB1∗11:04, DQA1∗05:01, and DQB1∗03:01 in SSc and HLA DRB1∗03:01 and DQA1∗05:01 in myositis. Furthermore, clinical characteristics of patients with ASSD and IIM-SSc overlap patients might be similar. The presence of mechanic's hand, interstitial lung disease, Raynaud's phenomenon, and myositis is frequently found in both clinical syndromes. There is also recent literature data that supports the common pathogenic origin of PM/Scl OM and ASSD. However, the genetic features of IIM-SSc overlap patients are not known; thus, comparing clinical profile and genetic features of patients with ASSD and IIM-SSC OM might help us better understand the pathomechanism of this disease.

AIMS

In our study, we tried to create 2 homogeneous Hungarian patient groups within the patients with heterogenous idiopathic inflammatory myopathies.

I. The aims of the study with the **anti-Jo-1 positive antisynthetase syndrome** group were:

- to determine the demographic, clinical, serological, laboratory, and genetic features
- to find any significant correlation between having the HLA- DRB1∗0301 allele and the presence of distinct organ involvement
- to assess relevant markers, or clinical features at the onset of the disease, which can predict the progression of myositis, or the response to the therapy.

II. The aims of the study with the **myositis-scleroderma overlap** group were:

- to determine the demographic, clinical, serological, laboratory, and genetic features
- to define and compare the HLA haplotype of the overlap patients with anti-Jo-1-positive ASSD patients and healthy controls
- to assess relevant markers or clinical features at the onset of the disease, which can predict the progression of myositis or occurrence of major organ involvement.

METHODS

1. Patients

Firstly, data of 49 **anti-Jo-1 positive myositis patients** were analyzed retrospectively. All patients are followed by the Department of Clinical Immunology at the University of Debrecen, Hungary, and medical files of the patients were reviewed. The median follow-up was 10,1 ± 6,51 years. This study meets and is in compliance with all ethical standards of medicine. Informed consent was obtained from all the subjects. This study is in compliance with the Declaration of Helsinki. Diagnosis was made in each case according to the Bohan and Peter criteria and all patients had a definitive or probable diagnosis of idiopathic inflammatory myopathy. None of these patients had other connective tissue disorders or myopathy; secondary Sjögren's syndrome was excluded. ILD involvement was investigated initially by pulmonary function test and high-resolution computed tomography (HRCT). Prognosis was assessed by determination of mortality during the follow-up. In addition, higher maintenance doses of steroids and a need of a higher number of immuno- suppressants (including cyclophosphamide) have been used as surrogated markers of bad prognosis. The occurrence of vasculitic skin lesions (small vessel vasculitis or capillaritis) was assessed clinically based on the presence of purpura and/or skin ulcers with or without histology.

Secondly, all **myositis-scleroderma overlap patients** were selected from the Hungarian patient database used at the University of Debrecen. A total of 414 patients were first selected, having at least one of the systemic sclerosis and one of the myositis International Classification of Diseases (ICD)-10 diagnosis codes. All identified patients were treated at the Department of Clinical Immunology or at the Department of Rheumatology and had at least two outpatient clinic appearances between January 2001 and January 2019. Secondly, these patients were sorted by those who fulfilled the diagnostic criteria for scleroderma (according to 1980 American Rheumatology Association (ARA) or 2013 American College of

Rheumatology/ European League Against Rheumatism (ACR/EULAR) and had a definitive or probable diagnosis for IIM (muscle weakness, high muscle enzyme levels, plus positive electromyography (EMG) and/or positive muscle biopsy in polymyositis or skin symptoms in dermatomyositis, according to Bohan and Peter/or probable/definitive IIM according to the EULAR/ACR myositis criteria). Patients without characteristic dermatomyositis skin symptoms were only classified as having muscle involvement if EMG/or muscle biopsy was performed. Based on these criteria, 39 cases of scleroderma-myositis overlap could be identified. Interstitial lung disease was defined as presented by radiographic findings (high-resolution computed tomography (HRCT)) and pulmonary function tests. Dysphagia was diagnosed by barium radiography of the esophagus. Intestinal involvement except dysphagia was determined based on the presence of either malabsorption, chronic diarrhea/small intestinal bacterial overgrowth, or motility disturbance. Cardiac involvement, indicated by relaxation abnormalities, conduction disturbances, or right ventricular hypertrophy, was assessed by electrocardiogram (ECG), two-dimensional, and Doppler echocardiography. Cardiac involvement was encoded in case of pericarditis, myocarditis, conduction disturbances, myocardial ischemia on ECG, and recurrent arrhythmia. Pulmonary artery systolic pressure (PASP) was calculated with the measurement of the tricuspid regurgitation peak velocity in those patients that had some degree of tricuspid regurgitation. Mean values of measurements from 3 consecutive beats were calculated. The diagnosis of PAH was set up, when estimated PASP was ≥30 mmHg.

2. Immunserology

Immunological analyses included tests for the following autoantibodies. Antinuclear antibodies (ANA), anticentromere antibody (ACA), antihistone antibodies, and anticytoplasmic antibodies were determined by indirect immunofluorescence on HEp-2 cells (Viro-Immun Labor-Diagnostika GmbH, Oberursel, Deutschland); ANA positivity was assessed at 1: 40

dilutions. Anti-Scl70, anti- Sm, and anti-Sm/RNP were determined in all patients by enzyme-linked immunosorbent assay (ELISA) (Hycor Biomedical Inc., Garden Grove, CA, USA). Anti-Jo-1, anti-Mi- 2, anti-Pm-Scl, and anti-Ku antibodies were detected by membrane-fixed immunoblot (ORGENTEC Diagnostika GmbH, Mainz, Deutschland). Anti-Sjögren's-syndrome- related antigen A (SSA) and anti-Sjögren's-syndrome-related antigen B (SSB) were determined by ELISA (Hycor Biomedical Inc., Garden Grove, CA, USA), as well as anti-U1RNP (ORGENTEC Diagnostika GmbH, Mainz, Deutsch- land). Titers of the antibodies against extractable nuclear antigen (ENA) complex, anti-SS-A (Ro) and anti-Jo-1 antibodies, were measured (HYCOR Biomedical Inc., CA, USA) using this latter method. It was assessed at the beginning of the disease, and the titer of anti-Jo-1 was followed during disease progress. IgM rheumatoid factor (RF) was assessed by nephelometry (DIALAB GmbH, Neudorf, Austria). The normal value for IgM RF was <50U/ml. ELISA was used for the measurement of the following autoantibodies: anticyclic citrullinated peptide (CCP) (Euro Diagnostica AB, Lundvagen, Sweden), anti-double-stranded deoxyribonucleic acid (dsDNA), anti-Beta-2-glycoprotein I (B2GPI), anticardiolipin, and antiphospholipid antibodies (ORGENTEC Diagnostica GmbH, Mainz, Deutschland). These commercially available methods were used following the manufacturer's protocol. Antiendothelial cell antibodies (AECA) were determined by a home-made ELISA at the Department of Laboratory Medicine, University of Debrecen.

3. Genotyping

High molecular weight DNA for genotyping was extracted from peripheral blood, which was collected in EDTA Vacutainers. Genomic DNA was extracted according to the manufacturer's recommendation using a QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Germany). DNA was quantified by ultraviolet absorption at 260 and 280 nm and stored at -20∘C until analyzed. Human leukocyte antigen (HLA)-DRB1, -DQA1, and -DQB1 genotyping

was performed with sequence-specific primers (Olerup SSP, GenoVision, Oslo, Norway). All samples were processed according to the manufacturer's instructions based on polymerase chain reactions (PCR). HLA genotypes were determined on the basis of the PCR product pattern obtained using 2% agarose gel electrophoresis.

4. Statistical Analysis

Statistical analysis was made using the SPSS 27 statistical software. For contingency tables, Fisher's exact test or the chi-square ($\chi 2$) test was applied. The values of continuous variables in different groups were com- pared with either Welch's d-test or the nonparametric Mann–Whitney test, depending on the result of the normality test (Shapiro-Wilk). During statistical analysis, the p value less than 0.05 was regarded as statistically significant; for the pairwise comparison of more than two groups, Bonferroni's or Hochberg's correction was applied.

RESULTS

1. Anti-Jo-1 positive antisynthetase syndrome

Demographic Data and Genetic Investigation:

The data of 49 myositis patients with anti-Jo-1 antibodies were evaluated. There were 7 male and 42 female patients. Age at disease onset was between 18 and 70 years, average age was 43,4±13,28 years. Median follow-up time ± SD (years) was 10,1±6,51 years.

HLA-DRB1, -DQA1, and -DQB1 genotypes of 29 patients with anti-Jo-1 positivity were determined using commercial sequence-specific oligonucleotide kit. HLA-DR3 (HLA-DRB1∗03) alleles were present in 20 (68,96%) anti-Jo- 1 positive patients. HLA-DQA1∗051-DQB1∗0201 haplotype was represented in 17 (58,62%) patients. The correlation of HLA-DRB1∗03 positivity and different parameters of myositis (organ involvement, laboratory parameters, serological status) was also investigated. We found that HLA-DRB1∗03 positivity was associated with lower initial CK level in patients with anti-Jo-1 positivity; however no other clinical parameters were influenced by the presence or absence of HLA-DRB1∗03 genotype. In addition, serological or therapeutic features of the patients were also not affected by the HLA-DRB1∗03 genotype. Five out of the 49 patients died during the 10,1 years median follow-up time. None of the investigated parameters were significantly associated with the mortality.

Clinical and Laboratory Findings:

Considering the symptoms of the classical ASS, the frequency of the features was as follows: myositis 100%; ILD 73%; arthritis 88%; dysphagia 12%; Raynaud's phenomenon (RP) 65%; fever 43%; and mechanic's hand 33%. Other skin symptoms were much rarer than mechanic's hand.

Multiple laboratory parameters were followed during disease progress. The average creatine kinase (CK) level at diagnosis was 3003,25 U/L and lactate dehydrogenase (LDH) level 922,33 U/L, whereas the average C- reactive protein (CRP) was 22,49 mg/L and erythrocyte sedimentation rate (ESR) 24,24 mm/h. The anti-Jo-1 titer at diagnosis showed significant correlation with both the initial CK ($p=0,03$; $R=0,328$) and CRP levels ($p=0,016$; $R=0,374$). In addition, both CK levels ($p<0,001$) and CRP levels ($p<0,001$) showed significant positive correlation with the anti-Jo-1 titer collected at the same time during disease course.

The presence and clinical significance of other autoantibodies were also investigated. The most frequently found antibody in the sera of anti-Jo-1 positive patients was anti- SSA (17/49; 35 %). We compared the clinical and laboratory findings of the anti-SSA positive group with anti-SSA negative patients. We found that the age at disease onset and the frequency of interstitial lung disease in patients with anti-SSA were significantly lower (36,12±11,08 years vs. 47,22±12,87 years; $p=0,004$ and 53% vs. 81%; $p=0,039$). In contrast, the minimal stable dose of steroid was significantly higher in the SSA positive group (9,53±12,56 mg vs. 3,47±3,95 mg; $p=0,031$). It should be emphasized that skin erosions were present only in patients having anti-SSA antibodies (18% vs. 0%; $p=0,037$).

Therapy:

Almost all available pharmacologic therapies were used during the disease course of the investigated patients and all of the patients were instructed to do exercises regularly. Concerning medications, 10 patients (20,41%) received only methylprednisolone therapy, whereas two patients (4,08%) refused the steroid treatment. 26 (53,06%) patients got steroid and immunosuppressive drug therapy, more than half of them at least two different drugs. The frequency of the used immunosuppressant was the following: methotrexate (MTX: 21 patients), cyclophosphamide (Cyc: 20 patients), azathioprine (AZA: 17 patients), cyclosporine (CSA: 13 patients), hydroxychloroquine (HQ: 7 patients), and sulfasalazine (6 patients). During the

disease course nine (18.37%) patients received intravenous immunoglobulin (IVIG) treatment, one patient rituximab alone, and one patient IVIG with rituximab.

We compared the clinical symptoms and laboratory parameters found at the diagnosis in those patients who received Cyc with the group that did not during the disease course. In the "cyclophosphamide group" regarding the clinical parameters, the frequency of interstitial lung disease (90% versus 60%, p=0,024), fever (60% versus 31%, p=0,044), Raynaud's phenomenon (85% versus 51%, p=0,016), and vasculitis-like skin lesion (25% versus 3,4%, p=0.035) was significantly higher at the disease onset. Focusing on laboratory parameters, the CRP level was also significantly higher in this subgroup (31,18±21,77 mg/l versus 16,921±20,85 mg/l, p=0,042) at diagnosis.

In addition, we compared the clinical symptoms and laboratory parameters at diagnosis of those patients who received only one immunosuppressant with those who were treated with more. The second group had more frequent fever (68% versus 22%, p=0,001), vasculitis-like skin lesions (27% versus 0%, p=0,005), and higher CRP level at disease onset (30,461±21,51 mg/l versus 16,25±20.9 mg/l, p=0,039). Rheumatoid factor (RF) was detectable more frequently in the second group (59% versus 18%, p=0,003).

Finally, based on the minimum stable dose of methylprednisolone treatment, the patients were categorized into the following two groups: (i) by 65% (n=32) of all patients, less than 8 mg; (ii) 35 % (n=15) of the patients more than 8 mg to control disease activity. We could detect that CRP (17,84±18,32 mg/l versus 36,34±25,39 mg/l; p=0,014); ESR (19,81±10,4 mm/h versus 33,87±22,11 mm/h; p=0,032); and the presence of fever (34% versus 67%; p=0,038) at diagnosis were significantly higher in the group receiving more than 8 mg methylprednisolone during disease course.

2. Myositis scleroderma overlap

Demographic Data, Organ Involvements:

Data of 39 myositis-SSc overlap patients were evaluated. There were 9 male and 30 female patients. The mean age at diagnosis was 42,0±14 (13-76) years, and 56,41% of the overlap patients (n=22) had limited cutaneous (lcSSc), 43,59% (n=17) diffuse cutaneous systemic sclerosis (dcSSc), 92,31% (n=36) PM, and 7,69% (n=3) DM. The two diseases occurred simultaneously in 23 patients (58,97%), while 4 cases (10,26%) in myositis and 12 cases (30,77%) in scleroderma were initially diagnosed, before the onset of the other disease. When not occurring simultaneously, the first diagnose was followed by the second disease in 2,9 (1-6) years.

Raynaud's phenomenon (RP) was present in 38 patients (97,44%) at disease onset. The frequency of the most important internal organ manifestations during disease progression were the following: interstitial lung disease (ILD) 28 patients (71,79%), dysphagia 26 patients (66,67%), intestinal involvement (except dysphagia) 11 patients (28,21%), cardiac involvement 16 patients (41,03%), pulmonary arterial hypertension (PAH) 12 patients (30,77%), and renal involvement 5 patients (12,82%). Fever at diagnosis was present in 7 (17,95%) cases. Regarding skin symptoms, mechanic's hand could be detected in 5 cases (12,82%), sub-cutaneous calcinosis in 8 cases (20,51%), and livedo reticularis in two (5,13%) cases. The average creatine kinase (CK) at diagnosis was 1542,37 ± 1975,33 U/l, and the average lactate dehydrogenase (LDH) was 725,67 ± 360,58 U/l.

Comparison of these results with historical data of our 49 anti-Jo-1-positive patients showed similar age at diagnosis, male/female ratio, average CK, or LDH levels at diagnosis, but ESR levels at diagnosis was significantly higher in overlap group (40,26 vs. 24,24 mm/h; $p < 0,01$). Twenty-eight binary variables (symptoms and laboratory parameters) were compared between the 2 groups with Fisher's exact test, applying Hochberg's correction for multiple comparisons. The frequency of the 3 parameters was significantly different. The presence of

Raynaud's phenomenon ($p < 0,001$, odds ratio (OR) 20,00; 95% confidence interval (CI): 2,54 to 166) and dysphagia ($p < 0,0001$, OR: 15,6; 95% CI: 5,21 to 45,5) was more, whereas livedo reticularis ($p < 0,01$, OR 0,11; 95% CI: 0,02 to 0,52) was less frequently detected in the overlap group than in the anti-Jo-1-positive patients.

Examining the immune-serological parameters, we observed very heterogeneous results. All of our overlap patients were antinuclear antibodies (ANA) positive, but none of the autoantibodies could be identified as a marker for this overlap. There was no anti-Ku or anti-U1-RNP-positive patient.

Genetic investigation:

HLA-DRB1 and DQA1 genotypes of 17 patients with myositis-SSc overlap and 69 healthy individuals were determined using commercial sequence-specific oligonucleotide kits. The most frequent HLA-DRB1 (HLA−DRB1∗03) alleles were present in 14 (82,35%) overlap patients and 19 (27,54%) in controls. Additionally, HLA−DQA1* 05:01 alleles were detected in 17 (88,24%) patients and 19 (30,16%) in controls. A comparison of these results showed that the presence of DRB1∗03 was significantly more frequent in the overlap than in the healthy control group (Fisher's exact test: $p<0,0001$; OR: 12,3; 95% CI: 3,2 to 47,6). Similar results were seen with the frequency of DQA1∗05:01 (Fisher's exact test: $p<0,0001$, OR: 17,4; 95% CI: 3,6 to 83,5).

We then compared this data with genetic results of our anti-Jo-1 ASSD patients but found no significant difference between the frequency of neither the DQA1*05:01 nor the DRB1∗03 genotype in the overlap and anti-Jo-1 group (Fisher's exact test: $p>0,1$). The presence of HLA−DRB1∗03−HLA−DQA1∗05:01 haplotype in overlap patients with different parameters of the disease phenotype (organ involvement, laboratory parameters, and serological status) were also investigated; however, significant correlations could not be detected.

Comparison of Patients with and without Pulmonary Arterial Hypertension (PAH):

PAH, which represents a significant factor in SSc disease progression was detected in 30,77% (12/39 patients) of our overlap population. Looking for predictive factors of PAH, we compared the clinical and laboratory findings of the PAH positive group with PAH-negative patients. We found that the presence of ILD (75% vs. 77,78%), arthritis (83,33% vs. 81,48%), Raynaud phenomenon (100% vs. 96,3%), and dysphagia (75% vs. 62,96%) was quite similar in the PAH-positive and PAH-negative group. In contrast, fever at diagnosis (41,67% vs. 7,41%, p=0,0046), cardiac involvement (83,33% vs. 22,22%, p=0,0008), subcutaneous calcinosis (41,66 vs. 11,11, p=0,01146), and claw hand deformity (25% vs. 11,11%, p=0,00016) were significantly associated with the presence of PAH in our overlap cohort.

Treatment:

Treatment trials have focused specifically on overlap syndromes themselves. It has to be underlined that 100% of patients received corticosteroid treatment and only 31% managed to leave this completely.

DISCUSSION

We can summarize our recent work that the phenotype of the disease is not different in HLA-DRB1∗0301 positive or negative patients who has the anti-Jo-1 antibody, but the initial CK level was significantly higher in the HLA-DRB1∗0301 negative patients. Distinct laboratory parameters measured at disease onset (high CRP, high ESR, anti-SSA, RF) and the presence of certain clinical symptoms (fever, vasculitis-like skin lesions) refer to a more difficult disease course, requiring higher steroid maintenance dose and multiple immunosuppressant treatments during the follow-up of patients with ASS. Anti-Jo-1 titer and the CK and CRP levels were positively correlated at disease onset and during disease course.

Based on our genetic data it seems that having anti-Jo-1 antibodies will determine the phenotypes of the patients more than the presence or absence of certain haplotypes. The comparison of the HLA-DRB1∗03 positive and negative patients with anti-Jo-1 antibody showed that none of the clinical symptoms, organ involvements, minimal stable steroid dose, or inflammatory parameters at disease onset differed in the two groups. Thus, it seems that HLA-DRB1∗03 positivity is one of the major factors which can play a role in the development of anti-Jo-1 antibody, but the organ involvement, i.e., the main phenotype of the disease, is not different in patients who has the antibody and the disease symptoms. Interestingly, the initiating CK level was higher in the HLA-DRB1∗03 negative group, which may indicate a more severe myositis; however, disease severity, including myositis, is affected by certain other parameters (muscle force, gastrointestinal involvement, severity and extent of extramuscular involvements, etc.). Therefore, further work and investigation of a larger patient population are required including the assessment of disease activity core set measures to determine the impact of this phenomenon.

Another aim of our study was to find potential markers, or clinical features at the onset of the disease, which can predict the response to the therapy. This could be notably important,

since before the validation of assessment and improvement criteria, there were no controlled drug studies for the treatment because of the low incidence and the heterogeneity of myositis subtypes. Therefore, recommendations are mainly based on clinical observations. The early administration of immunosuppressants (MTX, AZA, Cyc, CSA) is considered first-line adjuncts to glucocorticoid therapy, or as steroid sparing agents, although there is limited evidence for its use. With no clearly superior agent, the choice of immunosuppressive agent remains dependent on patient factors and clinician preference. Cyclophosphamide can increase the vital capacity and the diffusion capacity and decrease the extent of alveolitis; in addition, it also improves the muscle strength and function. Cyclosporine is also effective and substantially safe in patients with anti-Jo-1 ASS with corticosteroid-refractory ILD. According to Marie et al. in anti-Jo-1 patients with severe oesophageal manifestations, combined high-dose steroids and IVIGs might be proposed as the first-line therapy. The Rituximab in Myositis (RIM) trial showed that there were no significant differences in the 2 treatment arms for the primary and secondary end points, but 83% of adult and juvenile myositis patients with refractory disease met the DOI. Moreover, subanalysis revealed that antisynthetase patients responded better and other limited but encouraging results showed that rituximab may stop the progression of ASS-associated ILD.

Our other novel finding was that in those patients who needed at least two different immunosuppressants, the occurrence of fever was significantly higher at disease onset and their CRP levels were also significantly higher. Similarly, the presence of fever and high CRP at diagnosis was found more often in those patients, who were treated later with cyclophosphamide. The presence of RF and the vasculitis-like skin lesions were also more frequent in these patients. The mortality results of our cohort did not show any significant associations, which could be accountable for the low number of patients enrolled. Nevertheless, our results indicate that higher steroid doses were used in those patients, whose ESR and CRP

levels were high at disease onset or in whom fever was present. This suggests that these factors could be used as possible prognostic markers at disease onset. In these cases, the physician could predict a more difficult disease course with higher steroid demand and a need of a higher number of immunosuppressants, which might be surrogate markers of poor prognosis and treatment failure. Therefore, further longitudinal, ideally prospective studies are required to assess the exact predictive value of the abovementioned parameters on the prognosis and treatment response using the newly developed therapeutic response criteria.

The comparison of our data with results of other workgroups is challenging, since ASS is a complex disease, and the clinical picture might evolve during the follow-up and there are no well-established classification criteria. Some of the groups were selecting ASS patients with a dominant muscle disease, but in other cohorts the selection was made based on the positivity of anti- Jo-1 test and the presence of at least one clinical finding between arthritis, myositis, and ILD. That could be the reason of marked differences in the presence of myositis within the different ASS population. In our cohort only those patients who fulfilled the probable or definite Bohan and Peter criteria, were included, and that is why the presence of myositis was 100 %, and anti-Jo-1 positive patients without myositis were excluded. Therefore, the comparison of the phenotypes of the patients is defying. However, it seems that the majority of our demographic (average age at disease onset) and some of the clinical data of our patients were similar to those found in the literature. The causes of the differences may be the distinct genetic and environmental factors or the selection criteria of the cohorts discussed above. Therefore, the development of classification criteria for ASS based on differential impact for various clinical, pathological, and serological variables is needed, and this was proposed recently by others as well.

Intensive research is under way to show that the titer of anti-Jo-1 autoantibody is also part of the immune process and appears not just as a marker. Some publications have

highlighted that anti-Jo-1 autoantibodies may play a role in disease propagation and pathogenesis. The authors suggested that autoantigenic aminoacyl-tRNA synthetases are overexpressed in damaged muscle cells and their proinflammatory properties promote the immune response, which leads to the development of myositis. The pathogenic role of anti- Jo-1 antibody is supported by evidence that anti-Jo-1 levels showed modest correlation with CK, myositis, and joint disease activity. We found in our population that there was a significant correlation between the initial CK and CRP levels and the anti-Jo-1 titer at diagnosis. Strong associations of anti-Jo-1 titer and CK/CRP levels during disease progress were also detected. These data argue for the assumption that the presence of ant-Jo-1 titer is not only a diagnostic marker. Therefore, we propose that measurement of the anti-Jo-1 titer might be an advantageous additional component of standard disease activity core set measures during disease course in the antisynthetase patients.

Anti-SSA antibody is often found in the sera of anti- Jo-1 positive myositis patients. The cooccurrence of anti-SSA/Ro may have an effect on ASS prognosis: it was associated with more severe ILD, which was not confirmed in a larger cohort. Marie et al. failed to show a significant difference regarding progression of ILD between patients with and without anti-Ro52 antibody and concluded the association of more severe myositis, joint impairment, and increased risk of cancer with the coexistence of anti- Ro52 antibody in anti-Jo-1 positive patients. We could notice that the anti-SSA positive patients were younger, the presence of skin erosions was more extensive, and ILD was less frequent compared to the SSA negative group. In our population the steroid demand was higher in the SSA positive group, which could be a surrogate marker of a more progressive disease; however, the prevalence of ILD was lower in this group. The cause of this phenomenon needs further clarification; thus, we did not investigate the severity of lung involvement. Nevertheless, it seems that having anti-SSA antibody may define a distinct subgroup within the ASS patients and further studies are required

to better characterize the phenotype of ASS associated with anti- SSA and to improve treatment for these patients.

In second part of our work, the clinical, laboratory and genetic features of SSc-myositis overlap syndrome were assessed in a Hungarian cohort of 39 patients. We confirmed that the genetic characteristics, namely, the HLA-DRB1 and DQA1 genotypes of the overlap patients, are clearly different from healthy individuals, but not significantly different from genetics of patients with anti-Jo-1 ASSD. Myositis-SSc overlap patients have a distinct and unique clinical phenotype, which seems similar in many ways with the phenotype of anti-Jo-1 ASSD patients. Besides the sclerodactyly, the main differences are the more frequent presence of dysphagia, Raynaud's phenomenon, and less frequent presence of livedo reticularis. Distinct clinical parameters measured at disease onset (fever at diagnosis, subcutaneous calcinosis, cardiac involvement and claw hand deformity) were associated with the presence of PAH in the overlap patients.

To date there are not many studies reported in the literature of scleroderma-myositis overlap. Most of them are limited to a small number of overlap cases. The comparison of our data with the results of other workgroups is challenging, since despite the definitions of overlap syndromes being obvious, sometimes it is not clear, whether myositis is represented as muscle involvement of SSc or as a different disease. In view of the clinical picture, we found that the ratios of lcSSc and dcSSc were quite similar, whereas PM over DM dominance was detected regarding the IIM phenotype in the overlap patients. Besides sharing clinical features of both diseases, these patients may have characteristic autoantibody positivity. In the context of SSc-myositis overlap, autoantibodies against Pm-Scl protein complex are the most relevant. In our Hungarian population, none of the autoantibodies could be identified as a dominant marker for this overlap syndrome. Interestingly, 12 cases of anti-DNA positivity were detected, but only 3 cases of lupus could be diagnosed. Other overlapping systemic autoimmune disorders, such as

rheumatoid arthritis or Sjögren's syndrome, were not detected. There was no anti-Ku or anti-U1-RNP-positive patient in this cohort, which is a fact of interest because these are myositis-associated antibodies which can be found in overlap cases.

Anti-Jo-1-positive ASSD is a complex disease without well-established classification criteria, and the clinical picture of these patients could be similar to IIM-SSc overlap patients. Presumably, when myositis-specific antibodies (MSA) testing is not available, the majority of patients can be categorized into both disease groups. With our recent work, we could confirm that based on the comparable presence of HLA−DRB1∗03 and HLA−DQA1∗05:01 alleles, the two entities share similar genetic features too, which is evidently different from genetic results of healthy individuals. However, it should be emphasized that despite the unique anti-Jo-1 autoantibody positivity of our ASSD cohort, both ASSD and overlap diseases show intense heterogeneity regarding the organ involvement and the clinical picture. Nevertheless, it seems that dysphagia and RP are more characteristic, but livedo reticularis is less typical to IIM-SSc overlap than to anti-Jo-1-positive ASSD patients. The similarities of the two cohorts could be, at least partly, explained by the fact that all of our anti-Jo-1-positive patients had myositis, which could be different in other ASSD cohorts, since some groups are selecting ASSD patients based on the positivity of anti-Jo-1 test and the presence of at least one clinical finding between arthritis, myositis, and ILD. Therefore, development of new classification criteria of ASSD based on differential weights for various clinical, pathological, and serological variables might help to select appropriate patients to this disease and compare more unified cohorts.

The most frequently reported genetic associations with SSc are HLA−DRB1∗11, HLA−DQB1∗03 among European and North American Caucasians, and HLADRB1∗03:01 and DQA1∗05:01 in myositis. It is also reported that distinct HLA associations have been described in IIM in different populations, clinical subgroups, and with specific clinical features; however, the strongest HLA associations are found when stratifying by autoantibody status. In the setting

of Caucasian myositis patients, anti-PM/Scl autoantibody was strongly associated with HLA−DQB1∗02:01, anti-Jo-1 with HLA−B∗08:01, and HLA−DRB1∗03:01 alleles. Our overlap population did not have a unique autoantibody profile, and the presence of autoantibodies showed high variance. However, the genetic results showed quite similar data than those found in patients with anti- Jo-1 ASSD, which indicates a different link between genetic susceptibility, autoantibodies, and disease phenotype.

PAH is a significant factor of morbidity and mortality of patients with IIM and/or SSc. The occurrence ratio of PAH was 30,77% in our SSc-myositis cohort, which seems higher than those found in patients with SSc (7-12%) and in myositis (5-17). This interesting finding could demonstrate that the presence of myositis might be associated with cardiac involvement in SSc. PAH is often associated with extensive ILD as a secondary feature and does not differ clinically from idiopathic PAH. However, in our cohort, the presence of ILD was similar in the PAH-positive and PAH-negative group, supporting an alternative development of the pulmonary hypertension, but it should be noted that the extension and severity of ILD and fibrosis was not compared between the two groups in this study. Nevertheless, the results that the presence of fever at diagnosis, subcutaneous calcinosis, and claw hand deformity was associated with PAH in our cohort might facilitate more frequent assessments of PAH diagnostic procedures in selected cases, which could lead to early diagnosis and adequate treatment. We have found that the mortality results of the two groups did not show any significant associations, which could be accountable for the low number of patients enrolled and the short follow-up.

The possible limitations of this study should be acknowledged. This work was a single center study from a national myositis unit in Hungary, and the number of participants in the study was relatively low. The lower number of patients with genetic examinations could be a cause for selection bias, and determination of PAH was performed via noninvasive methods, which has lower specificity than Swan-Ganz catheterization procedure.

SUMMARY, NEW SCIENTIFIC ACHIEVEMENTS

In the present work, we determined the demographic, clinical, serological, laboratory, and genetic features of Hungarian anti-Jo-1 positive myositis and SSc-myositis overlap syndrome patients. We tried to assess relevant markers or clinical features at the onset of the disease, which can predict the progression of myositis, or the response to the therapy.

It can be concluded that:

1. The organ involvement of **anti-Jo-1 positive patients** with myositis was not affected, however initial CK level was influenced by the HLA-DRB1∗03 genotype.

2. There is a positive correlation between anti-Jo-1 titer and the CK and CRP levels at disease onset and during disease course.

3. Distinct laboratory results measured at the diagnosis (higher CRP, -ESR level; -RF positivity) and the presence of certain clinical symptoms (fever, vasculitic skin lesions) may indicate a higher steroid demand and more difficult disease course within anti-Jo-1 positive antisynthetase patients.

4. The occurrence of PAH seems higher in **SSc-myositis overlap syndrome** than in other subgroups and is associated with certain clinical features, such as fever at diagnosis, claw hand deformity, and subcutaneous calcinosis, drawing attention to more frequent diagnostic screening.

5. SSc-IIM overlap patients show notable similarities with anti-Jo-1- positive ASSD patients regarding HLA alleles and major clinical characteristics with certain distinct exceptions (Raynaud syndrome, dysphagia, intestinal involvement, livedo reticularis).

We believe that our findings may have potential support during the care of ASS and SSc-Myositis overlap syndrome patients, but further investigations are required to assess the exact impact of these factors on prognosis and treatment response.

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List of publications related to the dissertation

1. **Szabó, K.**, Bodoki, L., Nagy-Vincze, M., Béldi, T., Vincze, A., Zilahi, E., Varga, J., Szűcs, G., Dankó, K., Griger, Z.: Clinical, Serological, and Genetic Characteristics of a Hungarian Myositis-Scleroderma Overlap Cohort.
Biomed Res. Int. 2022, 1-9, 2022.
DOI: http://dx.doi.org/10.1155/2022/6251232
IF: 3.246 (2021)

2. **Szabó, K.**, Bodoki, L., Nagy-Vincze, M., Vincze, A., Zilahi, E., Szodoray, P., Dankó, K., Griger, Z.: Effect of Genetic and Laboratory Findings on Clinical Course of Antisynthetase Syndrome in a Hungarian Cohort.
Biomed Res. Int. 2018, 1-9, 2018.
DOI: http://dx.doi.org/10.1155/2018/6416378
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List of other publications

3. Nagy-Vincze, M., Béldi, T., **Szabó, K.**, Vincze, A., Miltényi-Szabó, B., Varga, Z., Varga, J., Griger, Z.: Incidence, features and outcome of disease relapse after Covid-19 vaccination in patients with idiopathic inflammatory myopathies.
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DOI: http://dx.doi.org/10.1002/mus.27811
IF: 3.852 (2021)

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