THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

INVESTIGATION OF IDIOPATHIC INFLAMMATORY MYOPATHIES, RELEVANCE OF NEW CLINICOSEROLOGICAL RESULTS.

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INTRODUCTION

Idiopathic inflammatory myopathy

Idiopathic inflammatory myopathy are heterogenous diseases with unknown origin, characteristic features are the muscle weakness, inflammation due to the autoimmune inflammatory processes, which mostly affects the proximal muscles with or without skin involvement and biopsy proven mononuclear inflammatory infiltration. The idiopathic inflammatory myopathies ((IIM)s are a heterogeneous group of disorders characterized by limb girdle muscle weakness and inflammation with or without cutaneous inflammatory disease and biopsy proven mononuclear inflammatory infiltration. IIM include polymyositis (PM), inclusion body myositis (IBM), dermatomyositis (DM) and juvenile DM, each of which is considered a distinct clinicopathologic entity. Wagner described the first case of polymyositis in 1863 and Unverricht defined dermatomyositis in 1887, but named it in 1891. Inclusion body myositis was reported first by Chou in 1967, but Yunis and Samaha named it in 1971. During the last decades early diagnosis and properly started agressive immunosuppressive treatment protocols resulted in greatly improved survival of these patients. The distribution of DM follows a bimodal pattern with peaks around ages 5-15 and 45-65. On contrary PM is only frequent between ages 45-65. There is a 2:1 female predominance of the disease.

The etiology is multifactorial with gene polimorphism, environmental and hormonal factors all play a role. The pathomechanism of DM is based on inflammatory cells mostly of T-helper-2 type (Th2) CD4+ T lymphocytes and macropahges, dendritic cells predominantly witha perivascular and perimyseal pattern. The earliest pathogenic event is the activation of the complement cascade, deposition of complement and MAC complex formation leading to microangiopathy through damage of endothelial cells. In PM MHC I. expression miofibrills ar attacked by CD8+ T lymphocytes, macrophages, dendritic cells located in the endomyseal compartment, utilising perforin dependent destructive mechanisms. In IBM two parallel processes takes part, as one degenerative process is accompanied by T-cell mediated inflammation. The role of several cytokines in IBM is not clearly explored. Immunohistochemical analysis of muscle biopsies revealed IL-1 expression on mononuclear and endothelial cells. TNF-α positive macrophages and lymphocytes can be detected endomyseally and perivascularly. Transforming growth factor beta (TGF-β) is also detected in these biopsy samples, suggesting a dual role as anti infalmmatory and fibrogenetic activity.

During the last decade early diagnosis and proper agressive immunosuppressive treatment protocols greatly improved survival for these patients. Immunological and molecular biologic studies provided us with valuable information regarding patomechanism, and led the way to develop more specific biological therapies for IIM patients. It is getting clear nowadays that for these patients, besides corticosteroids and other immunosuppressive drugs there is a need for individual and more specific therapy to get even better control of the disease. This eventually leads to even better disease control and better quality of life on the long term setting.

Therapeutic protocols used nowadays are based on non-controlled studies. There is only very limited data provided from controlled studies, because it is very difficult to have standardized outcome and disease activity parameteres. However recently the International Myositis Assessment and Clinical Studies Group (IMACS) published these data, making future studies possible.

Clinical trials in IIM face the problem of classification of this patients into proper subgroups. There are 3 different diagnosctic classification criteria presently (Bohan&Peter 1975, Tanimoto 1995, Hoogendijk 2004). The most widely accepted and used criteria is the Bohan&Peter. However even this classification criteria has its own disadvantages as it does not involve muscle necroenzyme levels and it is not defining IBM. Also it is not taking into consideration myositis specific antibodies (MSA) and myositis associated antibodies (MAA), underdiagnosing OM cases as PM. According to muscle biopsy criteria some muscle dystrophies are also classified as myositis. IBM is misdiagnosed as PM according to this system, as IBM was not described when this criteria were published. With all these drawbacks this is the system used nowadays.

The classification system based on clinical symptoms (Bohan&Peter) was refined by the used of MSA autoantibodies, redefining subgroups with more homogenous disease course and possible treatment outcomes. With the general use of MSA testing more OM cases are diagnosed, as described by Troyanov et al. Leading to better prognostic evaluation and better, more specific therapy leading to better treatment outcomes.

Juvenile myositis cases are also diagnosed according to the Bohan&Peter criteria, so the criteria were revised by pediatric rheumatologists (Juvenile Dermatomyositis Network).

The European Neuromuscular Center (ENMC) modified the criteria for muscle biopsy evaluation proposed by Hoogendijk in 2003 and 2 new subgroups were created:

- non-specific myositis (mon specific perimyseal/perivascular infiltrate is present in the sample without any other DM/PM specific finding)

- immune-mediated necrotising myopathy (no inflammatory cells are present)

Pregnancy and myositis

There is very limited data available on pregnancy outcome in IIM. Fetal lymphocytes may traffic over to maternal circulation and may persist for years. This is called microchimerism. This is proposed to have a role in autoimmune diseaseas as well as chronic graft versus host disease both requiring specific class II HLA antigens (HLADRB1). According to the immunological pathways involved different autoimmune disease may activate or disappear as a response of hormonal changes like found in pregnancy. It is well known that pregnancy induces a Th2 shift in the mother, resulting Th1 suppression. This leads to recovery of symptoms in Th1 mediated autoimmune diseases like rheumatoid arthritis (RA). However relapse may occur in the postpartum period. Patients suffering from systemic lupus erythematosus (SLE), which is a predominantly Th2 mediated disease, usually flair up during pregnancy. Diffuse active scleroderma (SSc) also inversely affects pregnancies. There is not so clear association in IIM, as DM is predominantly Th2 and PM is Th1 like disease.

Pregnancy provoked myositis is very rare, due to the fact that disease starts after childbearing years. Only 14% of female IIM patients' cases start during 15-30 years of age. Occurence of the disease leads to first trimester abortion, intrauterin retardation of the fetus and stillbirth or premature birth. In pregnancy induced myositis during the first trimester, fetal mortality is 62%. In juvenile chronic cases there is a 40% chance of relapse and the fetal mortality is negligible. In adult IIM cases, maintaning remission with corticosteroids and immunosuppressive agents bears with a 16% chance of relapse during pregnancy and the fetal prognosis is slightly worse than in juvenile patients.

Interstitial lung disease in inlammatory myopathies

Mills and Matthews described the first DM associated intertitial lung disease (ILD) in 1956. Since the first description the association between ILD and myositis is well known, leading the mortality causes in myositis. There is no perspective trial yet to define the incidence of ILD in myositis. Earlier cross-sectional studies, based on clinical, radiological, functional and pathological criteria described ILD frequency between 5 and 46%. This great variability was due to the lack of universal diagnostic criteria of myositis and lung disease

(high-resolution CT (HRCT), bronchoalveolar lavage (BAL) and also due to different disease stages. According to clinical appearance there are 3 forms of ILD (acut onset /Hamman-Rich like/, slow progressive and asymptomatic).

In general DM associated ILD bears worse prognosis with less response to corticosteroids. Agressive, fatal ILD has been reported in an amyopathic DM patient. It is not clearly explored whether pulmonary involvement in myositis has the same patomechanism as idiopathic pulmonary fibrosis, neither immunological mechanisms in the background has been fully explored. The presence of MSA has a distinctive role in the initiation and patogenesis of ILD, and also in skin and muscle tissues as well. The most relevant predictive factor for the presence of ILD in myositis is the presence of different aminoacyl-tRNA synthase directed autoantibodies in the sera. There is strong associtation between the presence of Jo-1 autoantibody and ILD, as 70% of Jo-1 positive patients have ILD. Co-existence of anti-Jo-1 and anti-Ro-52 resulted in increased TNFα/IL10 ratio in the sera. This may be explained by genetic factors mediating more severe disease course. The presence of microangiopathy detected by nailbed capillary microscopy also predict pulmonary involvement. Patients with ILD require more agressive therpay and their response rate is worse compared to others. This necessiates the early identification and proper agressive treatment of these cases.

Myositis overlap syndromes

The patomechanism of myositis is more or less described today, but clinicians face more diverse symptoms in a great number of cases. In case the symptoms fulfill diagnostic criteria of other autoimmune diseases (RA, SLE, SSc, SS-Sjögrens) overlap syndrome is diagnosed, resulting in usually more agressive disease course, and internal organ involvement according to the disease type present. Between 11% to 40% percent of IIM cases may actually be overlap myositis with a female predominance. SSc patients has SSc-OM in 5-17% of cases, ususally appearing in a diffuse form. In SLE about 4-16% of cases are SLE-OM and in RA about 3-5% of cases are RA-OM. During the last few years several authors highlighted that the most important predictor of the co-existence of several autoimmune disease is serology. The presence of disease specific autoantibodies may predict overlap myositis, requiring more attention and more agressive treatment. I describe overlap cases with and without clinical symptoms, highlighting the importance of serological testing in all IIM patients.

OBJECTIVES

I was examining clinical characteristics of IIM patients, using extensive serological and immunological testing to reveal immunological abnormalities predicting disease course and response to therapy.

1. I collected data on all pregnancies occuring during myositis. I highlight differencies between pregancies in active and inactive disease stages, with focus on therpay and fetal outcomes as well. I examined pregnancy provoked myositis cases, their outcome and therapy.
2. Anti-SS-A positive and negative antisynthetase syndrome patients' pulmonary involvement were compared. The difference in HRCT score is reported. I examined disease course and response to therpay in this two patient groups. I examined if the presence of anti-SS-A autoantibody in antisynthetase syndrome patients predicts a worse prognostic group with fibrotizing lung involvement.
3. Other connective tissue disease associated IIM patients (OM cases) were investigated regarding their clinical symptoms, disease course, outcome, treatments required. MSA and MAA antibodies weere analyzed and compared to IIM cases to specify presence of this rare autoantibodies and ot correlated their presence with overlap symptoms. I examined what is the impact of MSA presence in patients' sera in the Bohan&Peter classification.
4. I report a case of DM patient with a long disease course. Eventually the long lasting disease and immunosuppressive treatment resulted in sarcoidosis, than B-cell follicular non-Hodgkin lymphoma.

PATIENTS AND METHODS

Pregnancy outcome in idiopathic inflammatory myopathy

Between 1988 and 2007 there were 374 IIM patients followed in our department. Their data was analyzed. We found 173 female patients (134 PM and 39 DM) where pregnancies were examined. Mean age was 41.91 (10.3-77.3) at disease start. Patients gender, age, diagnosis date, disease form, clinical and serological characteristics were collected. Normal delivery was reported when healthy baby of more than 2500 gramm was delivered aftre the 37th week. Premature birth was reported between weeks 28-37. Abortion was reporetd before week 28.

Interstitial lung disease in idiopathic inflammatory myopathy patients

From a group of 315 IIM patients we could identify 27 (8.6%) anti-synthetase syndrome myositis cases (25 female and 2 male). There were 17 PM, 5 DM, 3 SSc-OM and 2 RA-OM cases. Mean age at diagnosis was 39.96 years (17.9-67.3). Mean disease duration was 46.6 (4-198) months. We documented presence of autoimmune diseases, specific symptoms, internal organ involvement. Detailed laboratory and imaging studies were done to evaluate these patients. To establish diagnosis we were maintaining the non-invasive approach, and was focusing on the patients symptoms.

Myositis overlap syndromes

Since 1988 374 IIM cases are followed in our department. There are 279 females and 95 males (212 PM, 63 DM, 18 juvenile DM, 39 OM, 42 cancer associated myositis-CAM) IIM was always diagnosed according to the Bohan&Peter diagnostic criteria. Polysystemic autoimmune diseases were diagnosed according to the relevant diagnostic criteria. RA and SLE according to ACR criteria, SSc according to LeRoy criteria and SS according to the European Community Study Group (ECSG) criteria. Mean age at diagnosis was 44.72 years (PM), 51.53 years (DM), 9.21 years (JDM), 42.9 years (OM), 54.05 yeras (CAM). To compare primary myositis to overlap cases the 39 OM cases (37 females 2 males) were compared to 130 primary myositis cases (95 females, 35 males). We used only 130 patients as

complete MSA and MAA panel results were available on these patients only at the time of publication. Mean age at diagnosis was 43.45 years (17.9-71.7) in primary myositis and 42.9 years (23.3-62) in overlap myositis. Mean duration of follow up was 67.5 months (0.8-372). Data was analysed retrospectively using patients charts and electronic records.

Peripheral blood samples, detection of autoantibodies

Serum samples were obtained at the time of diagnosis and stored at -70 ^{o}C until further use. Anti-dsDNA, -topoisomerase I (-Scl-70), and the anti-histidyl-tRNA synthetase (anti-Jo-1) MSA were detected by ELISA (HYCOR, Biomedical Inc., CA, USA). For sera positive for anti-Jo-1 determined by ELISA, reactivity was also confirmed by immunoblot assay (Euroline-WB assay, Euroimmun Laboratory, Luebeck, Germany). Anti-threonyl- (PL-7) and anti-alanyl-tRNA synthetase (PL-12) and anti-SRP antibodies were also detected by the immunoblot method (Euroline-WB assay, Euroimmun Laboratory, Luebeck, Germany). Among MAAs, extractable nuclear antigens (anti-Ro/SS-A, anti-La/SS-B) were detected by ELISA (HYCOR Biomedical Inc., CA, USA). The cut-off value was 10 U/ml for anti-Jo-1, -SS-A, -SS-B, -Scl-70 as recommended by the manufacturer. Anti-Ku, -U1snRNP and –PM/Scl antibodies were detected by immunoblot method (Euroline-WB assay, Euroimmun Laboratory, Luebeck, Germany). All tests were performed according to manufacturer's instructions. Line immunoassay (LIA) was performed by the Euroline-WB assay (Euroimmun AG, Luebeck, Germany). All sera were retested and autoantibody positivity was confirmed when LIA was available. The specificity of the reactivities was validated by using known positive and negative controls. All antibodies were assessed according to the manufacturer's instructions. IgM rheumatoid factor was measured with quantitative nephelometry (Cobas Mira Plus, Roche, Basel, Switzerland and Dialab GmBH, Austria) Anti-CCP autoantibodies were measured by second generation ELISA (Inova). Presence of anti-cardiolipin antibodies were measured by ELISA (Orgentec Diagnostica GmBH)

Immunfluorescence

Antinuclear (ANA) and anti-centromere autoantibodies were detected by indirect immunfluorescence on Hep-2 cells. Sera was considered positive if positivity was found below 1:40 dilution. Samples were examined under fluorescent microscope. Positivity patterns were also recorded.

Muscle biopsy

Biopsies were taken from the deltoid muscle under local anaesthesia. Samples were frozen at -70oC until immunohistochemical studies were done at the Histopathology Laboratory at the Dept. of Neurology. Perivascular and endomyseal inflammatory cells, sarcolemma and perifascicular fiber MHC I expression, MAC presence in capillary walls were detected. The presence of necrotic muscle fibers were also investigated. The presence of CD3, CD4, CD8 T lymphocytes were examined with specific monoclonal antibodies.

EMG

EMG was performed at the EMG Laboratory of the Dept. of Neurology.

Muscle strength measurement

Muscle strength of the patinets was measured using the UK Medical Research Council System scale (0-5) and MMT (manual muscle strength testing) methods: shoulder abduction, knee bending and extension, strength of fingers, hip bending and abduction, knee extension, foot dorsalflexion and neck flexion. Zero was the lowest value and 5 is the highest, thus the maximum result is 85 representing muscle strength.

Plethysmography and HRCT

HRCT was performed with slices of 1 mm interval and scored semiquantitatively according to Kazerooni by an independent radiologist who was unaware of any clinical or physiological findings. Alveolar or interstitial score ≥2 was used to define lung involvement. Pulmonary function tests were performed using a computer based device (Piston, Hungary, PDT-111). Results were expressed as percentage of predictive values based on a patient's sex, age, height and weight.

Statistical analysis

Statistical analysis was performed using SPSS 15.0 software. Differences were compared between groups with Mann-Whitney test. Chi square and Fisher's exact test was used to compare frequencies of tested variables among groups. When possible, associations were quantified with the odds ratio (OR) with 95% confidence interval (95% CI). To assess the relationship of autoantibodies and clinical manifestations, two variable logistic regression test was used. Correlation analyses were performed to assess the correlation structure of the variables (Spearman's rank correlation). Spearman's rank correlation coefficient was used to assess the relationship between variables. A separate logistic regression model was obtained for MSAs with a binary indicator of its presence or absence as the dependent variable, and with clinical characteristics as the candidate independent variables. The categorical variables entered in the regression models were: arthritis, interstitial lung disease, fever, Raynaud's phenomenon, dysphagia, calcinosis, mechanic hand. Statistical significance was noted, when the probability (p) value was less than 0.05.

RESULTS

Pregnancy in idiopathic inflammatory myopathy

We identified 173 female IIM patients (134 PM, 39 DM) and recorded 186 pregnancies, however only 9 (11.5%) patients had pregnancy after disease onset (4 PM, 5 DM). There was no OM detected. Mean age at diagnosis for the 9 pateints was 26.7 years (10-42). There was an average of 28 months (10-56) from disease onset to first pregnancy. We recorded 14 pregnancies in the 9 patients and 8 healthy child was born (5 girls 3 boys). There was 6 normal deliveries and 2 premature births. There were no twins. There were 6 unsuccesful pregnancies, 3 spontaneous abortion in the active disease state and 2 arteficial abortions and 1 intrauterin death in the 3rd trimester. We diagnosed 2 cases with anti-synthetase syndrome based on Jo-1 positivity and clinical symptoms. Five cases had a polycyclic disease course (3PM, 2 DM), 4 monocyclic (1 PM, 3 DM). There was a DM patient who developed the IIM int he 28th week of pregnancy. Using slowly tapered high dose methylprednisolon (1 mg/kg) resulted in delivery of a 2200 gramm healthy girl. After delivery second-line immunosuppressive treatment (cyclosporin A /CsA/ and azathyoprin /AzA/) was started and the patients achieved a remission in 3 months. In inactive myositis cases mean delivery time was 38.1 weeks (37-40), whereas in active cases it was 36.7 weeeks (35-38). Mean wight of newborns were 2193 gramm (1680-2700) in active cases and 3167 gramm (2800-3800) in inactive cases. Six cases had a benign disease course (2 PM, 4 DM), they responded to first line high dose corticosteroid therapy and did not require second line immunosuppression. There were 2 cases (1 PM? 1 DM) refractery to corticosteroids and requireing second line immunosuppressive agents (cyclophosphamide /CPH/, CsA, Aza). Remission was achieved within 12.6 month at an average. There were 2 monocyclis course DM patients who achieved long lasting treatment free remission, other 3 cases –including the pregnancy provoked one- could be controlled with low dose (0.1 mg/kg) steroids. See data on table 1.

Table 1: Outcome of pregnancies in myositis patients

Patient number	Age (yr)	G	Diagnosis	Disease activity	Fetal outcome	Treatment during pregnancy
1	20	1999	PM	active	prematurely born, healthy	methylprednisolon
	22	2001	PM	inactive	abortion 1st trimester*	methylprednisolon
2.	32	2001	PM	inactive	healthy	methylprednisolon
3.	24	1991	DM	inactive	healthy	methylprednisolon
	35	2002	DM	inactive	abortion (week 7)*	methylprednisolon
4.	31	1997	DM	inactive	healthy	methylprednisolon
5.	25	1991	PM	active	abortion	methylprednisolon
	26	1992	PM	active	abortion	methylprednisolon
	27	1993	PM	active	healthy	methylprednisolon
6.	35	1994	DM	inactive	healthy	no treatment
7.	37	1993	DM	active in 28th week of pregnancy	prematurely born, healthy	methylprednisolon
8.	35	2005	DM	inactive	healthy	no treatment
9.	24	2004	PM	active	abortion	methylprednisolon
		2005	PM	active	abortion	methylprednisolon

*at the patients request

Interstitial lung disease in idiopathic inflammatory myopathy patients

From 315 IIM patients 27 (8.6%) antisynthetase syndrome patients (2:25, male:female) with a mean age of 39.96 years (range 17.9-67.3) at the time of diagnosis were identified. There were 17 PM cases, 5 DM cases, 5 OM (3 SSc, 2 RA). ANA was positive in 44.4%_of the patients and SS-A positivity was also found in 44.4%. Mean age at diagnosis was 37.3 (+/- 10.2) years was in the SS-A positive and 42.5 (+/- 14.7) years in the SS-A negative group. Mean disease duration (from first symptoms until present) was 46.6 (range 4-198) months. No significant differences were found in age, sex, disease duration between the two groups.

Prevalence of interstitial lung disease was found to be 21% in all 315 myositis cases, but 70.3% among the Jo-1 positive cases . There was no clinically significant difference in the characteristic clinical symptoms between the two groups.

ILD was revealed at diagnosis in 52.6% of the antisynthetase cases and 30% of patients were asymptomatic at diagnosis. Normal chest radiograph has been found in 20% of patients (n=2), where HRCT confirmed interstitial lung disease. Interstitial lung disease appeared later in 47.4% of cases approximately 3.12 years (range 0.08-6 years) after the myositis diagnosis. 73.3% of SS-A negative patients developed ILD compared to 66.7% of SS-A positive patients. 54.4% of SS-A negative AS patients with ILD were asymptomatic at diagnosis, but ground-glass opacity could be revealed in four of these cases by HRCT examination.

Mean alveolar score was 1.27 (±SD:1.34), mean interstitial score was 2.27 (±SD:0.91) in the SS-A negative group. However in the SS-A positive group there were no patients with HRCT alveolar score greater than 2, whereas the mean interstitial score was 2.75 (±SD:2.05) ($p<0.05$). HRCT pattern of interstitial lung disease in the SS-A negative patients was found to be less coarse with more extent ground-glass opacity as can be seen in non specific interstitial pneumonia. HRCT pattern of ILD was found to be similar as can be seen in usual interstitial pneumonia in the SS-A positive cases.

FEV1% before therapy was found to be 69.3% (±SD: 5.78) in the SS-A negative subgroup, and 80.1% (±SD: 3.77) after therapy ($p<0.05$). FEV1% was found to be 78.46% (±SD: 5.94) in the SS-A positive subgroup and 78.87% (±SD: 3.86) after therapy. FVC was found to be 2.36 l (±SD: 0.61) in the SS-A negative subgroup before therapy and 2.83 l (±SD: 0.72) after therapy. FVC was found to be 2.3 l (±SD: 0.6) in the SS-A positive subgroup before therapy and 2.38 l (±SD: 0.72) after therapy (Figure 1,2).

FEV1% and FVC increased significantly after therapy in the SS-A negative subgroup ($p<0.05$) compared to the SS-A positive subgroup. Overall 72.7% of SS-A negative patients who were treated improved or remained stable on treatment compared with only 12.5% of SS-A positive patients. Two of the SS-A positive antisynthetase syndrome patients received also intravenous immunoglobulin therapy in addition to the previous immunosuppressive agents due to refractory, progressive ILD. Three (25%) of the SS-A positive antisynthetase patients died due to pulmonary or secondary cardiac complications, their ILD seemed to be refractory to immunosuppressive therapy, partially due to diagnostic delays, and progressive irreversible fibrosis.

Figure 1.: Changes in FVC after immunosuppressive treatment

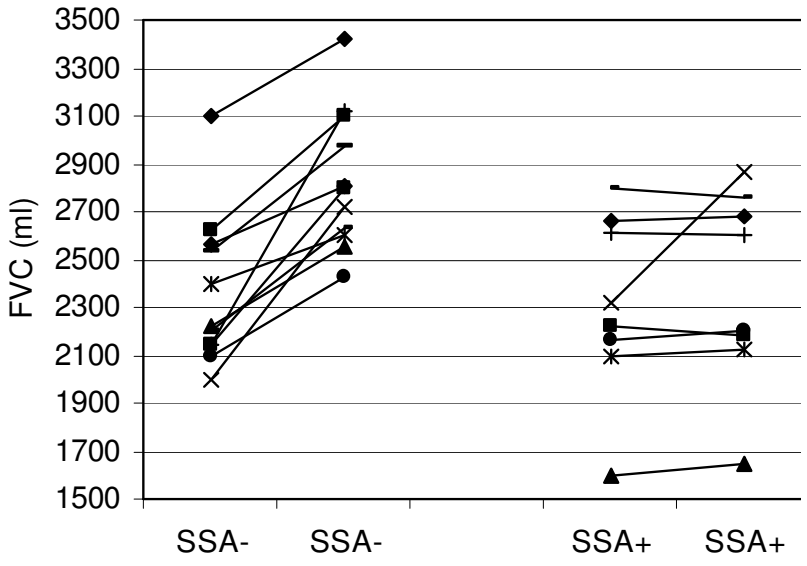
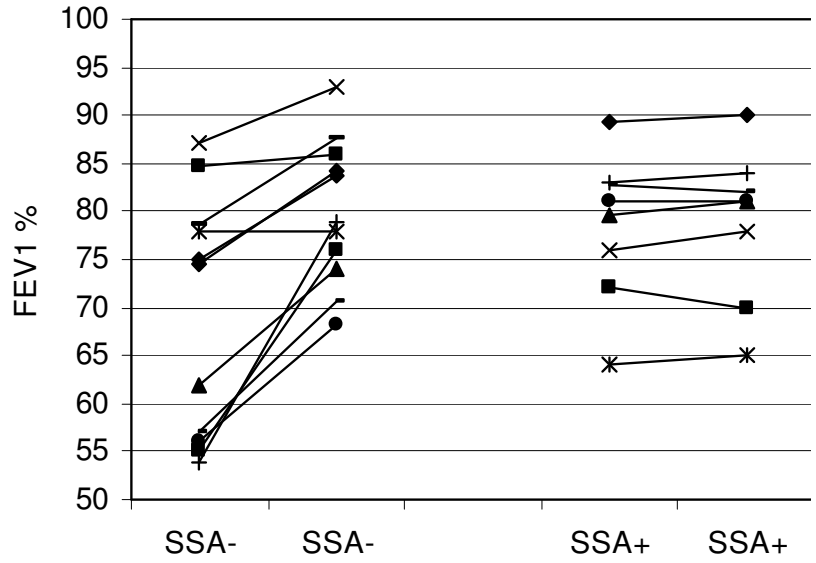


Figure 2.: Changes in FEV1% after immunosuppressive treatment



Each dot represents a measurement value.

Myositis overlap syndromes

Prevalence of OM was 10.4% (n=39) was in our patients. All OM patients were Caucasians and a significant female predominance was seen in both the overlap (female to male ratio 18.5:1) and primary myositis (female to male ratio 2.7:1) group ($p<0.05$). The mean age at diagnosis was 42.9 years (range 23.3 to 62 years) in the overlap myositis patients, and 43.5 years (range 17.9 to 71.7 years) in the primary myositis group. The mean duration of follow-up from myositis diagnosis was 67.5 (range 0.8 to 372) months. Eleven patients were lost to complete follow up during the period. No malignancies were detected during the follow up of the patients. Among the 39 overlap myositis patients, 13 (7.7%) had SSc (scleromyositis), 12 (7,1%) had RA, 9 (5.3%) had SS and 5 (2.9%) had SLE. PM was the most common IIM associated with other CTD (87.2%, n=34) and SSc was the most frequently associated autoimmune disease (33.3%, n=13).

ANA was present in 61.5% (n=24) of the overlap myositis patients which was significantly higher percentage than in primary myositis patients (25.4% n=33, p<0.05) (Table 2.). MSAs or MAAs were present in 67 myositis patients (39.6%). Anti-synthetase autoantibodies were present in 20.1% of myositis patients (n=35). Among MSAs, anti-Jo-1 was the most common, however the frequency of anti-Jo-1 positivity was similar in both overlap and in primary myositis patients (17.9% vs 18.5%, respectively) (Table 2). The presence of anti Jo-1 antibody was predominantly associated with PM (n=26, 83.8%) in context with anti-synthetase syndrome.

Table 2. Myositis specific (MSA) and myositis associated (MSA) antibodies in the 169 IIM patients

	PM/DM *(n=130)*	***OM*** *(n=39)*	***SSc*** *(n=13)*	***RA*** *(n=12)*	***SLE*** *(n=5)*	***SS*** *(n=9)*
ANA (1:40)	33 (25,4%)	24 (61,5%)*	9	7	4	4
dsDNA	13 (10%)	7 (17,9%)	1	1	5	0
ENA	33 (25,4%)	14 (35,9%)	5	3	4	2
MSA	38 (29,2%)	10 (25,6%)				
Jo-1	24 (18,5%)	7 (17,9%)	2	4	1	0
Pl-7	2 (1,5%)	1 (2,6%)	0	0	0	1
Pl-12	1	0	0	0	0	0
Mi-2	10 (7,7%)	1 (2,6%)	0	1	0	0
SRP	1 (0,1%)	1 (2,6%)	0	0	0	1
MAA	29 (22,3%)	25 (58,9%)*				
SS-A	11 (8,5%)	11 (28,2%)*	5	1	4	1
SS-B	7 (5,4%)	6 (15,4%)	3	0	2	1
U1snRNP	3 (2,3%)	4 (10,3%)*	1	1	2	0
PM-Scl	3 (2,3%)	2 (5,1 %)	1	1	0	0
Ku	5 (3,8%)	1 (2,6%)	0	0	1	0

*p<0,05

Surprisingly, we found MAAs including anti-Ku, -Pm/Scl, - SS-A, -SS-B, -U1snRNP, -Scl-70 or -centromere in 8.5% of primary myositis patients (n=11). Thus, upon these autoantibody specificities, 8.5% of primary IIM cases may be reclassified as overlap myositis and therefore the percentage of overlap myositis would increase from 23% to 29.6%. Both anti-SS-A (28.2% vs. 8.5%, $p<0.05$) and anti-U1snRNP (10.3% vs. 2.3%, $p<0.05$) antibodies were significantly more common in the overlap in comparison to the primary myositis patients.

Patterns of autoantibody combinations were also investigated. The most frequent antibody combinations were anti-Jo-1/SS-A (n=11, 35.3% of IIM patients). Coexpression of anti-Jo-1 and anti-SS-A antibodies showed weak but significant correlation (Spearman's R=0.316; p=0.001). We detected a triple combination of anti-Jo-1/SS-A/centromere in one SSc-PM overlap patient. Coexistence of anti-Mi-2 and anti-Ku antibodies was found in one patient with primary IIM.

Table 3.: Clinical symptoms, disease course and response to therapy in the 169 IIM patients

	PM/DM		*OM*		*Pl-7 (n=3) 1.7%*	*Mi-2 (n=11) 6.5%*	*Pm-scl (n=5) 2.9%*	*Ku (n=6) 3.5%*	*U1snRNP (n=7) 4.1%*
	Jo-1 positive (n=24)	*Jo-1 negative (n=106)*	*Jo-1 positive (n=7)*	*Jo-1 negative (n=32)*					
Skin symptoms	4 (16,6%)	42 (39,6%)	1 (14,3%)	4 (12,5%)	0	3	2	1	3
Calcinosis	2 (8,3%)	4 (3,7%)	2 (28,6%)	4 (12,5%)	0	1	0	0	0
Dysphagia	5 (20,8%)	27 (25,5%)	2 (28,6%)	7 (21,8%)	1	0	1	1	1
Fever	8 (33,3%)*	13 (12,3%)	3 42,8%)*	2 (6,3%)	0	2	1	1	0
Arthritis	13 (54,2%)*	16 (15,1%)	5 (71,4%)	15 (46,9%)	0	2	3	1	4
Mechanic hand	6 (25%)*	1 (1%)	2 (28,6%)	1 (3,1%)	0	0	2	0	0
Interstitial lung disease	14 (58,3%)*	15 (14,1%)	4 (57,1%)	11 (33,3%)	0	1	3	0	3
Raynaud	14 (58,3%)	44 (41,5%)	3 (42,8%)	16 (50%)	3	3	5	3	2
Monocyclic	6 (25%)*	52 (49,1%)	3 (42,8%)	8 (25%)	2	5	2	3	1
Polycyclic	16 (66,7%)*	39 (36,8%)	4 (57,2%)	20 (60,1%)	1	6	3	3	6
Chronic	2 (8,3%)	15 (14,1%)	-	4 (12,5%)	-	-	-	-	-
Steroid monotherapy	8 (33,3%)	52 (49%)	3 (42,8%)	13 (40,6%)	3	6	2	2	1
Second-line immunosuppressive treatment	16 (66,7%)	54 (50,9%)	4 (57,1%)	20 (62,5%)	-	5	3	4	6

‡skin symptoms, *p<0.05

The association of characteristic autoantibodies with clinical symptoms, disease course and response to therapy is presented in Table 3. Asterix indicates significant differences between antibody positive and negative cases. Two-variable logistic regression model confirmed the association of Jo-1 antibody with interstitial lung disease, fever, arthritis, mechanic hand. This can not be confirmed in the case of Raynaud's symptom (p=0.25) We could not detect overlap signs in MAA positive IIM patients.

Regarding relationship of MSA positivity and clinical manifestations, among the three (1.7%) anti-PL-7 positive patients, two had PM and one had SS-myositis overlap syndrome. In these cases the course of myositis was benign and responsive to corticosteroid monotherapy.

Anti Mi-2 were present in 6.5 % of all patients. It was associated with both DM (n=3) and PM (n=8) and 45.5% of these patients were refractory to corticosteroid monotherapy. One polymyositis / SSc and one dermatomyositis patient's sera were found to be positive for SRP.

Anti-PM/Scl antibody was present in three (2.3%) primary myositis patients (2 DM and 1 PM), as well as in one scleromyositis patient, altogether 2.9% of cases. This autoantibody positivity was accompanied by clinical features resembling antisynthetase syndrome as the course of myositis was mono- (40%) or polycyclic (60%) and the disease was refractory to corticosteroid monotherapy in 60% of the patients.

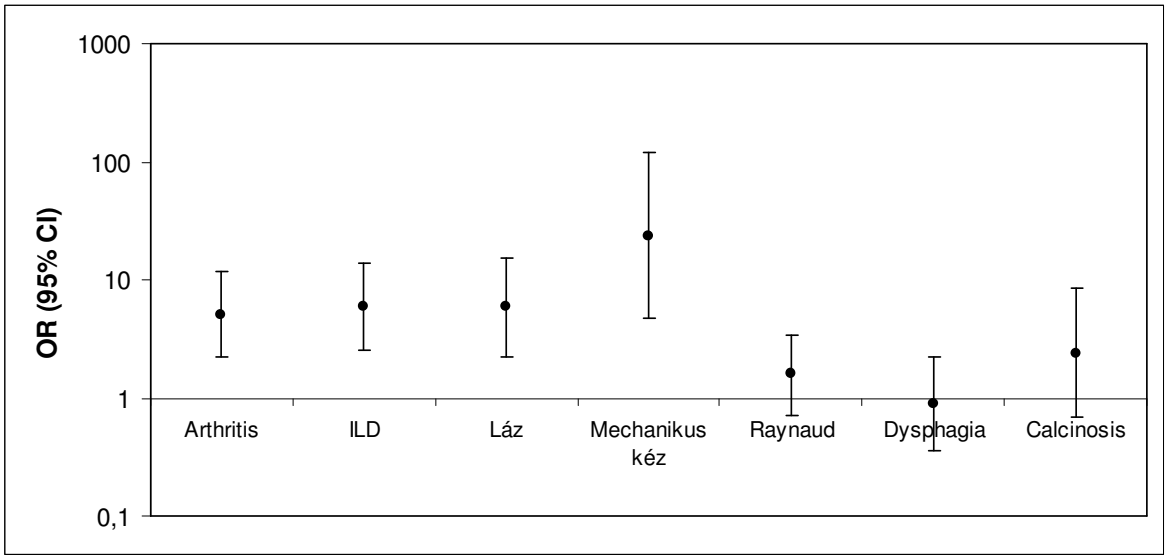
. The frequency of anti-Ku was 3.5% (n=6) as it was present in 4 PM, one DM and one SLE-myositis overlap patients. Altogether 33% of these patients were refractory to corticosteroid monotherapy. We could not detect anti-centromer or anti-scl-70 antibody in the anti-Ku or anti-PM/Scl positive patients' sera.

Three patients (4.1%) with anti-U1snRNP antibody positivity were originally classified as having primary myositis (3 DM). Other four anti-U1snRNP positive patients had overlap with SSc, RA or SLE. Altogether 85.7 % of these patients had refractory disease.

Regarding disease course and treatment responsiveness, two-third of anti-Jo-1 positive myositis patients (20/31; 64.5 %) had refractory myositis compared to those without anti-Jo-1 antibody (74/138; 53.9 %). Patients with anti-Jo-1 antibody had a slightly increased need for second line treatment in all myositis patients (OR: 1.57, CI: 0.7-3.5) or in the primary myositis group (OR: 1.95, CI: 0.8-4.9) but not in the overlap myositis group (OR: 0.8, CI: 0.1-4.2). Polycyclic disease course was more frequent in patients with anti-Jo-1 positivity compared to anti-Jo-1 negative patients. This difference was significant among primary IIM patients (p=0.023). This clinical course required frequent check-ups and prolonged treatment with corticosteroids and additional second line agents.

The association of characteristic autoantibodies with clinical symptoms is presented in Table 3. The increased risk is also indicated as odds ratio (OR). The strongest correlation was with anti-Jo-1 positivity, however anti-U1-snRNP positivity also predicted more frequent association with other CTD, as well as the presence of arthritis.

Table 3.: Associtaion of anti Jo-1 with clinical symptoms



B-cell extranodal follicular lymphoma associated with dermatomyositis

A case of a 63 year old lady is presented, who had been diagnosed with DM at the age of 36. After the myositis diagnosis she developed type I. sarcoidosis and later B cell follicular NHL. I presented this case to highlight the association of autoimmune diseases with lymphoid malignancy due to immune dysregulation and immunosuppressive treatment. The apperarance of B cell activation antigens, sustained polyclonal B cell activation, lack of apoptosis will eventually immortalize cells. Co-existence of DM and NHL is rare, only 15-20 cases are reported. Our case was extremely resistant to NHL chemoterapy highlighting a possible complex immunological disturbance in the background.

DISCUSSION

Pregnancy outcome in idiopathic inflammatory myopathy

Our 3 cases are valuable addition to the data of pregnancy in myositis. The first case reports a gravidity initiated DM, where she delivered a healthy girl on week 37 during steroid monotherapy. Our PM patients with policyclic disease course had 2 abortions and on corticosteroid therapy she delivered a healthy child. The anti-synthetase syndrome patient was in an active state, but with monthly intravenous immunoglobulin IVIG therapy she delivered a healthy but premature child.

According to our data we could not conclude any association with the immunopathogenesis of the disease. We proved, that myositis seldom starts during pregnancy and in the few cases reported and in our case as well, high dose corticosteroid is enough, no second line treatment is needed before delivery. In case pregnancy occurs during active disease state, the monthly IVIG therapy is the option to reduce fetal and maternal mortality.

Before our publication there were only a few case reports and one retrospective analysis published. One case was presented where anti-synthetase syndrome started in the 2nd trimester of the disease with anti threonyl-tRNA synthetase antibody. This was refractory to corticosteroid, azathyoprin therapy resulting in fetal loss. Toyo and colleagues reported another case where triple pregnancy initiated Jo-1 positive DM and fetal loss occured on week 11. There is data on myositis ossificans and amyotrophic dermatomyositis starting during pregnancy. The data support, that in inactive cases there is a minimal risk for fetal loss, however in active cases there is a clearly elevated risk that had to be closely monitored and managed together with gynecologyst, rheumatologist and internist specialists.

Interstitial lung disease in idiopathic inflammatory myopathy patients

From 315 IIM patients the occurence of lung involvement was examined, and was found to be similar to published data (21%). It is a challenge in myositis therapy to identify patients requiring early agressive therapy. Occurence of ILD showed a female predominance in our population in contrary to a swedish data where male predominance was found. We identified anti-Jo-1 anti synthetase syndrome in 27 cases, and 70% (n=19) of these patients had ILD which corresponds with published data. I highlighted the importance of early diagnosis of

ILD with the use of HRCT. A great portion (30%) of cases had no symptom at diagnosis. We were looking for predictive factors, differentiating ILD cases into agressive and non-agressive groups. In our study the anti-Jo-1 and anti-SS-A positive patients has HRCT pattern similar to interstitial pneumonia, requiring agressive treatment than in anti-SS-A negative cases. In these later patients the HRCT pattern was more like non-specific interstitial pneumonia, and the disease required less agressive immunosuppressive treatment, just for myositis in the majority of cases. Both radiological picture and clinical course was different in the two subgroups. This data is also confirmed by the study published by Corte et. al. According to the fact that the presence of ILD in IIM is an unfavourable prognostic factor it is important to perform HRCT and pulmonary function tests at the diagnosis of myositis as well as checking for antisynthetase antibodies. The role of subclinical ILD is not yet clearly identified, long term follow up prospective studies are needed.

Myositis overlap syndromes

A comparison report is presented between 130 primary and 39 overlap myositis patinets followed since 1988. Serological, clinical caharteristics as well as disease course and response to therapy is reported. The occurence of OM in our patient group was 23.1% (10.4% from all patients), which corresponds to literature data where Bohan&Peter criteria were used. However it is much lower compared to those reports where the new clinicoserological classification is used. From the IIM subgroups PM associated most frequently with overlap disease, most frequently with SSc (33.3%). This is lower than published (42.6%) in the literature.

From MSA the presence of Jo-1 associates with PM subgroup, and strong association was found with ILD, non erosive polyarthritis, fever. No association was found with Raynaud's symptoms. Presence of Jo-1 antibody characterized a medium prognostic group with corticosteroid refracter disease policyclic disease course just as published in the literature.The presence of anti-SS-A antibody associated with Jo-1 in 11 cases (35.5%), and a week corrleation could be confirmed with analysis suggesting common immunogenetic background. ILD was significantly more frequent in OM irrespectively of anti-Jo-1 positivity.We confirmed similar anti-Mi-2 frequency as described earlier, but we could not confirm its association with DM only as described by Koenig et al. earlier.

The frequency of anti-PM/Scl antibody was 2.3% in primary myositis and 7.7% is SSc-OM, which was significantly lower than published previously (6-8%; 24%) Anti-PM/Scl positivity resulted in similar symptoms to anti-synthetase syndrome suggesting a strong immunogenetic correlation between Jo-1 and PM/Scl. Anti PM/Scl antibody positive cases have a monocyclic disease course and responded to low dose corticosteroid therapy. The presence of anti-Ku has been confirmed in several autoimmune diseases, however we did not find any difference in frequency between primary myositis and OM. Anti-Jo-1 antibody was found in all subgroups. It was present in 20% of SLE-OM, 15.4% SSc-OM cases. Within OM subgroups 33.3% of RA-OM cases for anti-Jo-1 positive, however due to low patient number no consequences could be drawn. The distribution of MSA was not different in primary myositis and OM case in our study. This contradicts to the literature, where in anti-synthetase syndrome cases (SSc-OM) its frequency is less than 1%.

We could detect MSA antibodies in 8.5% of primary myositis patients diagnosed according to Bohan&Peter criteria. However their presence clearly shifts them into the new clinicoserological classification's overlap cases.

The disease course, response to therpay of OM cases clearly depends on the presence of different MSA and MAA antibodies. Early detection of these antibodies is important to identify and properly treat overlap cases before clinical symptoms appear.

NEW FINDINGS, SUMMARY

By investigating clinical and serological parameters in inflammatory myopathies I found the following new results:

1. The occurence of pregnancy induced myositis is rare, we had one case of pregnancy induced DM. I confirmed that in inactive myositis cases, the mean duration of pregnancy was 38 weeks and in active cases it was 36.7 weeks. Mean weight of child was 3167 g in inactive and 2193g in active cases. I confirmed that high dose corticosteroid treatment can controll the active phase of the disease. Monthly IVIG therapy was succesful in one active anti-synthetase syndrome patient.

2. Pulmonary involvement was found in 21% of all cases and in 70% of Jo-1 positive, anti-synthetase syndrome cases. I could detect the presence of SS-A autoantibody in 44.4% of anti-synthetase syndrome patients and confirmed that the co-existence of anti-Jo-1 and anti-SS-A resulted in a more serious interstitial lung disease resembling interstitial pneumonia, requiring more aggressive immunosuppressive treatment than SS-A negative cases ($p<0.05$).

3. The occurence of overlap myositis in our patients according to the Bohan&Peter criteria was 23.1%, but according to the clinicoserological classification it was higher 29.6%.

4. From IIM subgroups PM was the most frequent (87.2%) to associate with another autoimmune disease and the most frequent disease was SSc (33.3%)

5. MAA or MSA occured in 39.6% of myositis cases. MSA was present in 29%, antisysnthetase antibody in 20.1%, most frequently anti-Jo1 in 18.3% of cases.

6. The presence of anti-Jo-1 associated with PM (83.8%) and there was a strong association with ILD, non-erosive polyarthritis and fever as independent predictive factors.

7. Presence of anti Jo-1 autoantibody predicted corticosteroid refractery (64.5%) policyclic disease course identifying a middle prognostic group, compared to Jo-1 negative patients (53.9%; $p=0.023$)

8. The presence of anti Jo-1 could be confirmed in all subgroups, in one SLE-OM, 15.4% of SSc-OM cases and in RA-OM as well. From within OM subgroups it occured most frequently in RA-OM (33.3%).

9. We could confirm a weak correlation between anti Jo-1 and anti-SS-A ($r=0.316$, $p=0.001$) suggesting immunogenetic link between them.

10. The occurence of Mi-2 was 6.5% in our patients, but we could not confirm its only presence in DM, as other cases were also positive.

11. The presence of anti-PM/Scl antibody was confirmed in 2.9% of cases, however in 2.3% of primary myositis cases, 7.7% of scleromyositis cases. This was significantly lower than data published earlier. The disease course of anti-PM/Scl positive patients was mono- (n=2) or polycyclic (n=6) in contrary to literature data, where mostly monocyclic, corticosteroid responsive benign cases are reported.

12. The presence of anti-Ku was not found significantly different between primary myositis and OM (3.8% vs. 2.6%).

PUBLICATIONS:

In extenso publications from the thesis:

1 **A. Váncsa**, A. Ponyi, T. Konstantin, K. Dankó, M. Zeher: Pregnancy outcome in idiopathic inflammatory myopathy. Rheumatology International 2007;27: 435-9. **IF: 1,27**

2. **A. Váncsa**, I. Csípő, J. Németh, K. Dévényi, L Gergely, K. Dankó: Characteristics of interstitial lung disease in SS-A/Jo-1 positive inflammatory myopathy patients. Rheumatology International, Volume 29, Issue 9 (2009), p989 **IF: 1,327**

3. **Váncsa A**, Ponyi A, Constantin T, Gergely L, Dankó K: Dermatomyositishez társuló, késői megjelenésű extranodális follicularis lymphoma. LAM 2004;14:139-42.

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Cumulative impact factor: 4,55

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