Short thesis for the degree of Doctor of Philosophy (Phd)

Investigations of dermatomyositis-specific autoantibodies, disease activity and vitamin D receptor gene polymorphisms in idiopathic inflammatory myopathies

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GYULA PETRÁNYI DOCTORAL SCHOOL OF ALLERGY AND CLINICAL IMMUNOLOGY

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INTRODUCTION

1. Myositis and the significance of myositis-specific antibodies

Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of rare systemic autoimmune diseases collectively called myositis, which causes progressive muscle weakness. Their incidence in Hungary is 0.95/100000/year and their prevalence is 1-6/100000. They are multifactorial diseases; their etiology and pathogenesis are not exatly known. Genetic and environmental factors can contribute to the pathogenesis of IIM. The most commonly used criteria for the classification of IIMs are those that were proposed by Bohan and Peter in 1975.

Autoantibodies are of great importance for the diagnosis of many systemic autoimmune rheumatic diseases, including IIMs. Whether they have a significant role in pathogenesis of myositis, is not known. Antibodies are useful in the diagnosis of IIMs and in the precise definition of disease subsets. With the help of the antibodies, we can make different disease phenotypes that could be important in diagnosis, therapy and prognosis. They can be divided into two parts. The first is the group of myositis-associated autoantibodies (MAAs), the other category is the group of the myositis-specific autoantibodies (MSAs). MAAs can be found in overlapping cases. MSAs can be detected only in myositis and are directed against specific proteins that can be found in the cytoplasm or in the nucleus of the cells. Approximately 38% of IIM patients have MSAs in their sera, and, to our knowledge, the co-existence of two MSAs is considered extremely rare. The presence of the MSA, in many cases, can be detected in the sera some month before clinical manifestation of the symptoms; the serum level may correlate with disease activity. If the MSA disappears, it could be the sign of remission, so they can serve as prognostic markers. The most relevant MSAs are the followings: anti-aminoacyl-transfer RNA-synthetase (anti-

ARS) autoantibodies, anti-SRP, anti-HMGCR, anti-Mup44 and the dermatomyositis (DM)-specific MSAs: anti-Mi-2, and the novel MSAs targeting intracellular proteins, namely anti-TIF1γ, anti-NXP2, anti-SAE and anti-MDA5. The growing number of reviews shows the importance of these antibodies. It has to be underlined that the clinical symptoms associated to the antibodies are not always the same because of the various populations investigated. Most of the studies show that the anti-Mi-2 is associated with DM, and it is associated with an acute disease with good prognosis. The two most important of the newly recognized ones are anti-TIF1γ and anti-NXP2. According to the newest data from the literature their frequency in adult IIM patients are 13-21% and 1.6-17%, in juvenile IIM patients 23-29% and 23-35%, respectively. Their common characteristic is that the symptoms among adults and children are not the same.In adults they are associated with classical skin lesions and the higher frequency of malignancy. Some reviews say that they are good markers for cancer-associated myositis (CAM) in adult patients. In children special skin lesions (skin ulcers and calcinosis) play an important role. Anti-SAE is a rare antibody (<5% in adults and <1% in juvenile patients). At the beginning of the disease only skin lesions are present, muscle weakness and myalgia appear during the progression. By anti-MDA5 positivity the muscle symptoms of the patients are minimal and the serum levels of muscle enzyms are also normal. In Europe typical symptoms are the skin lesions, interstitial lung disease (ILD) and bad prognosis; the frequency is not exatly known.

2. The evaluation and monitoring of disease activity – Disease Activity Core Set Measures

The following of clinical symptoms during disease progress has a very important role when evaluating the disease activity. The organisation called IMACS was created by internationally known researchers and physicians. They established a method called Disease Activity Core Set Measures for evaluating the activity of myositis. Disease activity assesses

the manifestations of myositis which are thought to be reversible that result directly from the inflammatory process. IMACS recommends these core set measures be included in all myositis therapeutic trials and clinical studies that assess disease activity in patients with myositis.

3. Vitamin D receptor gene polymorphisms

Vitamin D is essential for normal development and maintenance of bones. It enhances the intestinal absorption of calcium and phosphorus, and it also has other autocrine and paracrine effects. Vitamin D is considered as an important regulator of the immune system. Most biological activities of vitamin D are mediated by the vitamin D receptor (VDR), which is located in the nucleus. The VDR gene is located onchromosome 12. In recent years many polymorphisms have been identified in the VDR gene, but their effects on VDR activity are still poorly understood. The VDR gene has more than 100 restriction endonuclease recognition sites, but only 4 of them are known polymorphisms: FokI, BsmI, ApaI, and TaqI. The effects of vitamin D and the VDR gene polymorphisms are in connection with each other. The latest findings suggest that allelic variations of VDR gene may partially represent a genetic component associated with incidence, clinical symptoms, or severity of autoimmune diseases or to the susceptibility of them. To the best of our knowledge, there is no information in the literature about the VDR gene polymorphisms and their association with myositis disease.

AIMS

1. Investigations of autoantibodies

1. The aim of my work was to make the clinicopathological and clinicoserological classification of IIM patients treated at the Department of Clinical Immunology, University of Debrecen.

2. The clinical characterization of patients with DM-specific autoantibodies. It is the first work to investigate the anti-TIF1γ, anti-NXP2, anti-SAE and anti-MDA5 antibodies in Hungarian patients.

3. The aim was also to compare the clinical features of patients with DM-specific autoantibodies with the characteristics of patients without MSAs.

4. I also introduce six of our patients with the rare picture of the co-existence of twoMSAs,

2. Investigations of disease activity

1. In this follow-up study I aimed to monitoring the activity of the disease in 219 myositis patients. First I compared the disease activity data in DM és PM patients, because there isn't any previous study that investigated this field.

2. I also investigated the patients whose disease was active during follow-up time. By these patients the correlations between the components of Disease Activity Core Set Mesaures (Manual Muscle Testing - MMT80, MMT150, Health Assessment questionnaire - HAQ, muscle enzymes) were studied.

3. Investigations of vitamin D receptor gene polymorphisms

1. In this work I aimed to reveal the possible connection between myositis and the VDR gene polymorphisms. First the genotyping of VDR-BsmI, VDR-ApaI, VDR-TaqI és VDR-FokI polymorphisms was carried out in IIM patients. Then I followed the study with the same investigations in healty controls. I compared the results to decide wether an association exists between the VDR gene polymorphisms and the susceptibility to myositis. I also investigated whether an association exists between VDR gene polymorphisms and some important markers of myositis (phenotype of disease, age at disease onset, gender, presence of MAAs/MSAs, presence/absence of osteoporosis).

2. With the help of the known genotypes I aimed to make a haplotype analysis, to determine, wether an association exists between haplotypes and myositis. At the end of the project pairwise linkage disequilibrium(LD) between the VDR gene polymorphisms was also made.

METHODS

1. Investigations of autoantibodies

Detection of MSAs

The peripheral blood samples were taken from IIM patients treated at the Department of Clinical Immunology, University of Debrecen.The sera of 330 patients with myositis were stored at -20°C. Anti-Jo-1, anti-PL-7, anti-Pl-12, anti-Mi-2 and anti-SRP antibodies were detected by membrane-fixed immuno-blot (Orgentec Diagnostika). The recently discovered MSAs (anti-TIF1γ, anti-NXP2, anti-SAE and anti-MDA5) were detected in Bath, UK by radiolabelled protein immunoprecipitation.

Muscle biopsies, EMG investigations

Muscle biopsies were made from the musculus deltoideus or musculus quadriceps femoris (Institute of Surgery, University of Debrecen). They were performed on that side where the patient had myalgia or muscle weakness. Samples were stored at -70°C. Immunohistochemical stainings and their evaluation were done at the Department of Neuropathology, Department of Pathology, University of Debrecen. EMG investigations were performed at the EMG Laboratorium, Neurological Department, University of Debrecen.

Detection of extramuscular manifestations

Cardiac involvement (pericarditis, myocarditis) was diagnosed with ECG and echocardiography. Every patient came through HRCT and diffusing capacity of the lungs for carbon monoxide (DLCO) examination in order to detect the presence of pulmonar involvement (alveolitis, fibrosis). High resolution CT (HRCT) and echocardiography were repeated regularly, in order to detect the possible progression. Present dysphagia in anamnesis

meaned a gastrointestinal involvement. Skeletal involvement (arthritis, arthralgia) were investigated with physical examination and X-ray.

Statistical analysis

To compare groups with cathegorical data Pearson Chi-square (χ^2) test was used. To compare groups with small number of cases Fisher's exact test was used. To compare continuous data (CK, LDH, GOT, GPT)Mann-Whitney test was used. Statisitical analysis was made using SPSS 17.0 statistical software. The P value less than 0.05 was regarded as statistically significant.

2. Investigations of disease activity

Data collection

During this follow-up study between 31.07.2012 and 01.02.2015 I investigated 219 myositis patients who were treated at the Autoimmune Outpatient Clinic of the Department of Clinical Immunology, University of Debrecen. At every single visit I evaluated the condition of the patient. Health Assessment Questionnaire was also used.

Evaluating the activity of myositis

The following factors of Disease Activity Core Set Measures were evaluated in this study: physician global activity, patient/parent global activity, MMT, HAQ, serum levels of muscle enzymes, myositis disease activity assessment tool (constitutional disease activity, cutaneous disease activity, skeletal disease activity, gastrointestinal disease activity, pulmonary disease activity, cardiovascular disease activity, other disease activity, extramuscular global activity, muscle disease activity, global disease activity).

Statistical analysis

To compare groups with cathegorical data Pearson Chi-square (χ^2) test was used. To compare groups with small number of cases Fisher's exact test was used. To compare non-categorical data (CK, LDH, GOT, GPT) Mann-Whitney test was used. During normality test investigations of continuous data Kolmogorov-Smirnov test was used; we found non-normal distribution so later we used the non-parametrical Spearmen's correlation. If the correlation coefficient (CC) was between 0.2-0.5; or between 0.5-0.7; or above 0.7, there was a weak, modarate or strong relationship, respectively. Statisitical analysis was made using SPSS 17.0 statistical software. The P value less than 0.05 was regarded as statistically significant.

3. Investigations of vitamin D receptor gene polymorphisms

Patients and controlls

89 patients with IIM were enrolled in the present study, recruited from the Autoimmune Outpatient Clinic of the Department of Clinical Immunology, University of Debrecen. A total of 93 healthy individuals taking no immunosuppressive or immunomodulating medications served as the control group.

Genomic DNA extraction

Genomic DNA for genotyping was extracted from peripheral blood, which was collected in EDTA vacutainers. Before extraction blood was stored at -20°C. Genomic DNA was extracted according to the manufacturer's recommendation using a QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany). DNA was quantitated by UV absorption at 260 nm and 280 nm (spectrophotometer: GeneQuant pro RNA/DNA Calculator) and stored at −20°C until analysed.

Genotyping of of the VDR gene polymorphisms

Genotyping of the BsmI polymorphism was carried out in PCR-amplified DNA by allelic discrimination using TaqMan from Applied Biosystems (Foster City, CA, USA). PCR primers and TaqMan probes specific for the BsmI polymorphism were purchased from Applied Biosystems. Real-time PCR was performed using Corbett Rotor-Gene RG-3000 equipment. The genotypes of the VDR gene FokI, ApaI, and TaqI polymorphisms were determined according to the digestion pattern generated for the amplified DNA fragment using the restriction enzymes FokI, ApaI, andTaqI. FokI genotypes were defined by capital letters in the absence of the restriction site (allele-F) and lowercase letters where the restriction site was present (allele-f). From the 265 bp long PCR product will become two fragments (196 bp and 69 bp long) with FokI enzymatic digestion. For both ApaI and TaqI, genotypes were defined by capital letters in the absence of the restriction site (A and T, resp.) and lowercase letters where the restriction site was present (a and t, resp.). From the 740 bp long PCR product will become two fragments (530 bp and 210 bp long) with ApaI enzymatic digestion. TaqI has two binding sites, one of them generates two fragments (495 bp and 245 bp long), with the help of the other site there will be two smaller fragments (290 bp and 205 bp long) from the first, 495 bp long fragment.

Statistical analysis

Genotype frequencies were calculated by direct counting. Allele frequencies were calculated from genotype frequencies based upon Hardy-Weinberg equilibrium. For comparisons of mean values between patients and controls, statistical analysis was performed using the independent samples *t*-test. Differences in genotypic and allelic distribution of VDR polymorphisms between patients and controls were determined by Pearson Chi-square (χ^2) test using SPSS 20.0 statistical software. The *P* value less than 0.05 was regarded as

statistically significant. Haplotype analysis was performed by CHAPLIN 1.2 software. Pairwise LD between the VDR gene polymorphisms was computed, and LD plots were constructed using Haploview software, version 4.2.

RESULTS

1. Investigations of autoantibodies

Classification of the patients

The classical clinicopathological classification of the 330 patients with IIM gave the following result: 52.81% polymyositis (PM), 22.73% DM, 11.51% overlap myositis (OM), 6.67% CAM, 4.55% juvenile dermatomyositis (JDM), 0.91% juvenile polymyositis (JPM), 0.91% necrotizing autoimmune myopathy (NAM), 0.61% inclusion body myositis (IBM), 0.30% clinically amyopathic dermatomyositis (CADM). 121 patients were MSA positive (36.67%). According to the clinicoserological classification we had 51 anti-Jo-1 positive, 4 anti-PL-7 positive, 1 anti-PL-12 positive, 25 anti-Mi-2 positive and 14 anti-SRP positive patients. The MSA detecting in Bath gave the following results: 12 anti-TIF1γ positive, 4 anti-NXP2 positive, 4 anti-SAE positive patients. There wasn't any patient with anti-MDA5 positivity. Six of our patients showed double MSA positivity.

Characterization of our patients with DM-specific antibodies

Among our anti-Mi-2 positive patients there were 14 patients with PM and 14 patients with DM. Among the newly recognized DM-specific autoantibody positive 20 patients there were 17 DM patients. It has to be underlined that all patients had muscle weakness, 47.92% of them had myalgia. As extramuscular manifestations we could detect the followings: arthritis 18.75%, arthralgia 52.08%, dysphagia 43.75%, lung involvement 18.75%, heart involvement 6.25%. The disease course was monophasic in 29.17% of the patients, chronic in 14.58% of the patients and polyphasic in 56.25% of the patients.

Comparison the clinical features of patients with DM-specific autoantibodies with the characteristics of patients without MSAs

We had 48 patients with DM-specific autoantibody. I created another group of patients without MSA positivity (number of patients: 48, age at disease onset: 38.17 years, female:male ratio 3:1). I investigated 30 patients with PM and 18 patients with DM in this group. The two groups were compared with each other in various ways. The initial MMT score of the patients with DM-specific autoantibody positivity was significantly lower than the MMT score of MSA negative patients (41.54±13.87 vs. 53.19±12.54, p<0,001). The comparison of the initial CK muscle enzyme levels also supported this result (5402.92±10213.48 vs. 2086.06±4415.47, p<0,001). The frequency of most of the classical skin lesions (face erythema: P=0.041, heliotrop rash: p=0.038, Gottron-papule: p=0.04, periorbital oedema: p=0.049, scarf sign: p=0.018) was significantly higher in patients with DM-specific autoantibodies. As extramuscular manifestation, definitive pulmonary fibrosis was significantly more frequent (p=0.031) in patients with DM-specific autoantibodies.

Our patients with double MSA positivity

It has to be underlined that we could detect six patients with double MSA positivity. This is considered to be extremely rare in the literature. These 6 patients are 1.82% of the whole myositis population, and 4.96% of the MSA positive patients. We had two patients with anti-Jo-1 and anti-SRP positivity; the other four patients had anti-Jo-1 and anti-Mi-2; anti-Mi-2 and anti-PL-12; anti-Mi-2 and anti-SRP; anti-SRP and anti-PL-7 positivity. The clinical picture of these patients was very varied.

2. Investigations of disease activity

In the analyzed period (between 31.07.2012 and 01.02.2015) 219 patients with myositis were followed. The collection and evaluation of data from 219 patients happened in a total of 1101 outpatient visits. Only those patients were enrolled, who attended at least three

times during follow-up. Age at disease onset was 44.93 years, the female: male ratio was 2.98:1. According to the clinicopathological classification we had the foloowing gropus: 107 PM patients (48.86%), 61 DM patients (27.85%), 7 JM patients (3.2%), 8 NAM patients (3.65%), 3 IBM patients (1.37%) and 33 OM patients (15.07%).

Disease activity scores of our patients with PM and DM

First I compared the disease activity data in our 107 PM and 61 DM patients, as measured with the help of the Disease Activity Core Set Measures. The patients with PM had statistically significant higher scores in every single case. This also refers to the physician and patient global activity scores. The only exception was the cardiovascular disease activity; here was no difference between the two groups. There was another exception: the activity of skin lesions, which was higher in DM patients.

Correlations of IMACS scores in our active patients

The definition of an active patient was the following: clinical activity signs of myositis (muscle weakness, myalgia, skin lesions) and higher CK enzyme levels at least two times in follow-up time. 44 patients matched these criteria. The measured MMT scores gave unequivocal negative correlations with HAQ scores and CK levels. This can be confirmed with the following statistical data of correlations:MMT80 vs. CK $R=-0.387$ and $p<0.001$; MMT80 vs. LDH $R=-0.353$ and $p<0.001$; MMT80 vs. GOT $R=-0.277$ and $p=0.001$; MMT80 vs. GPT $R=-0.247$ and $p=0.005$; MMT150 vs. CK $R=-0.382$ and $p<0.001$; MMT150 vs. LDH $R=-0.336$ and $p<0.001$; MMT150 vs. GOT $R=-0.300$ and $p<0.001$; MMT150 vs. GPT $R=-0.263$ and $p=0.001$; MMT80 vs. HAQ $R=-0.536$ and $p<0.001$; MMT150 vs. HAQ $R=-0.558$ and $p<0.001$. The physician and patient VAS scores gave strong negative correlations with MMT scores (physician VAS vs. MMT80 $R=-0.714$ and $p<0.001$; patient VAS vs. MMT80 $R=-0.730$ and $p<0.001$). The physician and patient VAS scores gave positive correlations with

HAQ scores and CK levels (physician VAS vs. HAQ R=0.691 and p<0.001; patient VAS vs. HAQ R=0.629 and p<0.001; physician VAS vs. CK R=0.622 and p<0.001; patient VAS vs. CK R=0.615 and p<0.001).

3. Investigations of vitamin D receptor gene polymorphisms

There were 69 female and 20 male in the patient group, the female:male ratio was 3.45:1. The mean age was 52.6, ranging from 9 to 81 years. The distribution of IIM patients was as follows: PM, n = 46; DM, n = 15; JPM/JDM, n = 9; OM, n = 17 (myositis and RA in 10 patients; myositis and systemic sclerosis (SSc) in 5 patients; myositis and mixed connective tissue disease (MCTD) in 2 patients); NAM, n = 1; IBM, n = 1. A total of 93 healthy individuals (mean age 41.2 years, range 14 to 70 years, 52 females and 41 males, female:male ratio 1.27:1) taking no immunosuppressive or immunomodulating medications served as the control group.

Result of genotyping

No significant difference was found for allele frequencies when data were compared between patients with IIM and control individuals. We identified a difference in the frequency of Fand falleles between the IIM population and the control population, but this was not significant. In addition, no significant difference was observedwhen the IIM caseswere grouped into PM,DM, and overlap cases. No significant difference was found in the genotype frequencies when the VDR gene genotypes of patients with IIM and healthy individuals were compared. The same result was confirmed between PM, DM, and overlap cases.

Thepatients were classified into groups according to their gender, age at disease onset, laboratory profiles (myositis-specific and myositis-associated autoantibody positivity), and the presence of osteoporosis. The most important clinical manifestations that are of great importance to the prognosis of IIM were also investigated.These are the following

extramuscularmanifestations: the presence of cardiac involvement (myocarditis), esophagus involvement (dysphagia), other skeletal involvements (arthralgia, arthritis), and lung involvement (ILD). There was no association of VDR gene polymorphisms with clinical manifestations in IIM patients. We detected important associations when investigating gender. The statistical *t*-test showed significant differences as follows: the distribution of BB, Bb, and bbgenotypes was found to be significantly different ($p<0.001$) in males compared to females among myositis patients. We also found a similar distribution for VDR-TaqIpolymorphism. The distribution of TT, Tt, and ttgenotypes was significantly different ($p=0.037$) in male myositis patients than in female myositis patients. We also found statistically significant differences as follows. Comparing the male myositis patients with the entire control group, we established that the distribution of BB, Bb and bbgenotypes was significantly different ($p=0.0323$) in the male IIM patients. The distribution of the BB-Bb-bbgenotypes in the male patient group versus the men in the control group also showed a significant difference ($p=0.0176$). Significant difference was detectable when comparing the frequency of FF, Ff, and ffgenotypes between myositis-associated autoantibody positive patients and the control population ($p=0.0033$). When examining the distribution of AA, Aa, and aa genotypes in female myositis patients and control female patients, the p value was 0.0398.

Haplotype frequencies of VDR gene polymorphisms in IIM patients and linkage disequilibrium

There was no significant difference in the calculated three-marker haplotype frequenciesbetween the control and the patient groups. The baT and the BAt haplotypes were the most frequent in both the patient group (35.22% and 30.28%, resp.) and the control population (48.35% and 31.46%, resp.). bAT haplotype was not as frequent as the above mentioned haplotypes but was also frequent in both groups (19.33% in patients vs 10.16%in

healthy population). The combined frequency of the five remaining haplotypes was less than 10%. The Bat was not present in the patient group, and in the control group, the estimated frequency of BaT was 0%. The most frequent four-marker haplotype in IIM patients was the fbAt haplotype (19.07%); the estimated prevalence of this haplotype in controls was only 1.41%. The second andthird most frequent ones in patients were FBaT and fbAT(18.18% and 16.25%, resp.), while the estimated prevalence in the control group was only 0% and 1.34%, respectively. We should highlight here that, according to our findings, the three most frequent four-marker haplotypes in healthy individuals were as follows: FbaT (29.78%), fbaT (24.50%), and FBAt (22.59%). The estimated frequency of these in patients was 0%, 3.61%, and 0%, respectively.

LD analysis revealed a very strong LD (r^2>0.8) betweenApaI and TaqI polymorphisms, a strong LD (r^2between 0.67and 0.7) between BsmI and ApaI or BsmI and TaqI polymorphisms, and a very weak LD (r^2<0.3) between FokI and otherpolymorphisms in the control group. In patients, very strongLD was found between BsmI and ApaI polymorphisms; moderate LD was found between TaqI-ApaI and TaqI-BsmI polymorphisms. No LD was observed between FokI andother polymorphisms in patients.

DISCUSSION

1. Investigations of autoantibodies

Looking at the detailed investigated antibodies the following conclusions can be reviewed.

Most of the international studies say that anti-Mi-2 positivity is associated primarily with DM. There are some other reviews saying that anti-Mi-2 positive patients do not form a particular group in the myositis-population. As can be seen from the results, my data can confirm these details; anti-Mi-2 was not DM-specific in our patients. As seen in textbooks and in the literature, internal organ manifestations were very rare in this population. The only exception was the dysphagia, which was present in 39.29% of the patients. Many works emphasizes an important data: the mortality due myositis is very low in anti-Mi-2 positive patients. In our patients the mortality was high, six anti-Mi-2 positive patients died. But only one death was linked directly to the myositis.

It has to be underlined that anti-TIF1γ was DM-specific in our patients with myositis. Only one PM patient was in this subgroup. In the whole myositis-population there were less anti-TIF1γ positive patients than seen in the literature. In DM patients the frequency of the antibody was 8% which is lower than seen in the literature. We had 22 CAM patients, two of them was anti-TIF1γ positive. This is also less than other data. Typical clinical manifestations were the muscle weakness and skin lesions – this was detectable in adult and juvenile patients. Extramuscular manifestations were rare. International reviews showed us that this antibody - as well as anti-NXP-2 - is a very important antibody in juvenile myositis patients. I also can stregthen this data. According to these findings the previous data from textbooks should be reevaluated – at least in juvenile cases. It would be worthy to screen adult and juvenile patients for this new antibody.

According to my results the frequency of anti-NXP2 in the whole myositis-population, in PM patients and in DM patients were lower than seen in the literature. There was no juvenile case. It has to be underlined that in 18.18% of all CAM patients anti-TIF1γ or anti-NXP2 positivity could be detected. It would be worthy to screen anti-TIF1γ and anti-NXP2 positive patients for malignancy.

The onset of disease is very acute and serious with typical skin lesions; after that typical proximal muscle weakness can be detected; than the patient has a good prognosis and good response to therapy. This is the typical case of an anti-SAE positive myositis patient in the literature and we also could strengthen this description. It has to be underlined that the frequency of dysphagia was 100% in these patients.

Comparison the clinical features of patients with DM-specific autoantibodies with the characteristics of patients without MSAs

The MMT scores of the patients with antibody-positivity were significantly lower and their muscle enzyme levels were significantly higher. That means a more serious disease onset than in MSA negative patients. It is known that patients with anti-Mi-2 and anti-SAE have a good response to therapy – after this acute onset with severe skin lesions. According to my data we can conclude followings. After therapy there was no significant difference in muscle strength between the two groups. The initial muscle pain occured more often in the control group (almost 70%), this also gave a significant difference between the two groups. There were significant differences in most of the skin symptoms: the frequency of them was lower in the control group. It is known that the frequency of arthritis and arthralgia is lower in anti-Mi-2 positivity, than in anti-Jo-1 positivity and anti-Mi-2 negativity. Investigating all patients with DM-specific antibody a significant difference could be detected; artgralgia and arthritis were more frequent in MSA negative patients.

Initial and last measured MMT scores in PM patients gave a similar tendency as in the full comparison. Investigating only the DM patients the change between initial and last measured MMT scores was not high enough. It means that there was no significant difference compared to the control group. Nonetheless both groups – keeping mind the muscle power – responded well to the therapy.

Double MSA positivity

To date, literature suggests the rarity of the co-existence of two MSAs. Blood tests identified six patients with the rare co-existence of MSAs. Some authors alleged that the coexistence of two MSAs leads to more severe symptoms, interacting in a complex fashion, thus expanding the clinical spectrum of IIM. Investigating six patients with two MSAs in our Hungarian IIM population I conclude the followings. It cannot be stated that these associations all have a more serious course or a more severe phenotype. Only one patient died of an extreme severe form of myositis, the patient with anti-Mi-2 and anti-PL-12 positivity. I can state that only two patients matched the characteristic features of both antibodies; two patients fitted one antibody's anticipated symptoms; two patients had simple clinical manifestations represented only with weakness and myalgia. The most frequent antibody was the anti-SRP but only one patient had typical, rapid progress. Anti-Mi-2 was associated with severe skin lesions only one time. With the exception of anti-PL-7, all anti-ASA were associated with ILD; they were always „dominant". Based on these results the presence of more than one MSA is rather associated with a "mixture" of phenotypes. In clinical practice the physician has to evaluate such findings together with the clinical features.

2. Investigations of disease activity

In my work I used the disease activity scores validated by IMACS. First I compared the disease activity data in DM és PM patients, because there isn't any previous study that

investigated this field. First there was no distinction between the active patients and patients in remission. The physician and patient VAS scores were higher in PM than in DM. It means that patients with PM had more active disease and the patients evaluated their disease similar to the doctor. This can be underlined with the results of the MYOSITIS DISEASE ACTIVITY ASSESSMENT VISUAL ANALOGUE SCALES. There were statistically significant difference in case of constitutional disease activity, muscle disease activity and extramuscular disease activity. Investigating 168 Hungarian patients it can be concluded that the patients with PM had more severe muscle sypmtoms and extramuscular manifestations.

Investigating the correlations between the components of Disease Activity Core Set Mesaures (MMT80, MMT150, HAQ, CK, LDH, GOT, GPT) gave many unequivocal connections in active myositis patients. Muscle strength score (MMT80 and MMT150) gave negative correlations with the enzyme levels and HAQ scores. HAQ scores gave strong negative correlations with muscle strength scores in the whole population as well (MMT80 vs. HAQ CC: -0.690, $p<0.001$; MMT150 HAQ CC: -0.717, $p<0.001$). The result was similar by the physician and patient VAS scores as well: there was strong negative correlation with MMT80 scores and moderate positive correlation with HAQ and CK scores. Similar studies with similar comparisons were made by other workgroups; the results were similar to my data.

3. Investigations of vitamin D receptor gene polymorphisms

The latest findings suggest that allelic variations of VDR gene may partially represent a genetic component associated with incidence, clinical symptoms, or severity of autoimmune diseases or to the susceptibility of them. Many authors investigated the organ-specific and polysystemic autoimmune diseases. The two most investigated diseases are SLE and RA. The Central European literature about the possible association of VDR gene polymorphisms and

autoimmune diseases is very limited. There is no information in the literature about VDR gene polymorphisms and their connection with myositis.

Result of genotyping

Our most important findings were connected to theVDR-BsmI polymorphism,VDR-TaqI polymorphism, and male gender. Based on our data, we can state that the distribution ofBB, Bb, andbb genotypes represents an interesting finding becauseBB andbb genotypes were presented at a very low percentage in the male patient population. The distribution ofTT,Tt, andtt genotypes was significantly different in males than in females in the patient group. These findings may reveal that IIM can be associated with theBb genotype of theVDR-BsmI polymorphism and theTt genotype of theVDR-TaqI polymorphism in males in our cohort. According to the statistical analysis Bb and Tt genotypes can considered to be risk factors in Hungarian males.

Some authors found that RA is associated with theFokI polymorphism of the VDR gene. In my case-control study, 17 patients were included with OM, and 10 of these had RA. Comparing the distribution ofFF,Ff, andff genotypes of the VDR gene identified a significant difference between MSA positive patients and the control population (p=0.0033). TheFf genotype was found to be the most frequent genotype in myositis-associated antibody positive cases (66.67% out of the positive patients). Also, 42.86% of the MAA patients were RF-positive. According to the international results this confirms the diverse distinct distribution of the genotypes in our MAA positive patients.

Haplotype frequencies of VDR gene polymorphisms in IIM patients and linkage disequilibrium

Investigating the three-marker haplotypes it was detected that thebaT, BAt and bAT haplotypes were the most frequent three-marker haplotypes in both the patient and control groups. These three-marker haplotypes were identified as the most frequent haplotypes by other authors as well.

Data demonstrated that the frequencies of the sixteen possible haplotypes showed differences between the patient and the control groups when the four-marker haplotype prevalence was assessed. Several differences that were not statistically significant might accumulate and recombine in individuals resulting in significant differences in the level of four-marker haplotypes. This is the reason why certain haplotypes are represented in patients but are not found in control individuals.

Previous studies resulted that BsmI, ApaIand TaqI stay in strong linkage disequilibrium. I could detect the same result in my work. Many workgroups think that the combination of LD and one or more functional polymorphisms of VDR gene could explain the connection between VDR genes and some diseases (e.g. asthma bronchiale, diabetes mellitus type 1, some type of cancers or multiple sclerosis).

The pathomechanism of IIM is still unclear, but the genetic predisposition and environmental conditions play an important role. According to these findings, the genotype variations of the VDR gene polymorphisms may be one of the factors in the development of IIM which is a polygenic and multifactorial autoimmune disease.

SUMMARY

Idiopathic inflammatory myopathies are chronic systemic autoimmune diseases characterised by symmetrical, proximal muscle weakness.

In the first investigation I made the clinicopathological and the clinicoserological classification of 330 patients with myositis treated at the Department of Clinical Immunology, University of Debrecen. Then I described the frequency and the clinical features of the patients presented with dermatomyositis-antibody positivity (anti-Mi-2, anti-TIF1γ, anti-NXP2, anti-SAE). This was made at first in a Hungarian myositis population. With the exception of anti-Mi-2 and anti-SAE the frequency of these autoantibodies was lower in the investigated Hungarian population than in other international studies. I drew attention to the acute and severe onset of the patients presented with dermatomyositis-specific autoantibodies in contrast to the patients without any myositis-specific antibodies. It was the first Hungarian study that identified patients with double myositis-specific autoantibody positivity. The detection and analysis of these myositis-specific autoantibodies help us to differentiate between myositis-symptoms and they bring us closer to a better therapy.

In the second study I investigated 219 Hungarian patients with myositis according to the IMACS criteria. In this follow-up study I could state that the activity of the followed Hungarian patients with polymyositis was significantly higher than those with dermatomyositis. With the analysis of myositis-patients with active disease I could prove a significant correlation between MMT scores, HAQ scores, levels of muscle enzymes in serum, and the values of the visual analog scales.

The effects of vitamin D and the polymorphisms of the VDR gene are connected. The latest findings suggest that allelic variations of VDR gene may partially represent a genetic component associated with incidence, clinical symptoms, or severity of autoimmune diseases

or to the susceptibility of them. We assessed the VDR gene BsmI, ApaI, TaqI, and FokI polymorphisms in 89 patients with myositis and 93 healthy controls to assess whether a relationship exists between polymorphisms/haplotypes in the VDR gene and any features of PM/DM. We found that a relationship may exist between the BsmI genotype and myositis in males and TaqI genotype and myositis in males as well. Our data demonstrated that the frequencies of the sixteen possible haplotypes showed differences between the patient and the control groups when the four-marker haplotype prevalence was assessed. According to our findings, the genotype variations of the VDR gene polymorphisms may be one of the factors in the development of myositis.

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List of publications related to the dissertation

1. **Bodoki, L.**, Nagy-Vincze, M., Griger, Z., Dankó, K.: Dermatomyositisspezifische Antikörper.
   Z. Rheumatol. 74, 363-369, 2015.
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