Diploma Thesis

Clinical Relevance of Myositis-Specific Antibodies

submitted by

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Graz, am 12.01.2019 *Viktoria Tiefenthaler eh*

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Zusammenfassung

Hintergrund: Der Nachweis von Myositis-spezifischen Antikörpern spielt eine wichtige Rolle in der Diagnose von idiopathischen inflammatorischen Myopathien (IIM)(1). Im Rahmen dieser Studie wurde ein besonderes Augenmerk auf die klinische Relevanz von Myositis-spezifischen Antikörpern (MSA) und Myositis-assoziierten Antikörpern (MAA) in der Diagnostik von Myositiden gelegt. Aus diesem Grund wurden unter anderem als eines der primären Ziele positive Vorhersagewerte (PPV) für alle untersuchten Antikörper berechnet.

Methoden: Die Studie gestaltete sich als eine retrospektive Analyse aller Krankenakten von Patienten und Patientinnen mit einem positiven Testergebnis für MSA und/oder MAA im Zeitraum von Oktober 2014 bis Oktober 2017. Die Krankenakten wurden auf prädefinierte klinische Merkmale und Laborparameter untersucht. Weiters wurden alle notwendigen Parameter für die Anwendung der EULAR/ACR Klassifikationskriterien für idiopathische inflammatorische Myopathien erhoben, um deren Anwendbarkeit im klinischen Alltag zu überprüfen. Außerdem wurden die Daten auf das Vorliegen signifikanter Korrelationen überprüft. Abschließend wurden positiv prädikative Werte für das Vorliegen eines Myositis-Subtyps, einer anderen Autoimmunerkrankung als Myositis, einer malignen Erkrankung und für das Vorhandensein einer interstitiellen Lungenerkrankung (ILD) bei positivem Antikörpernachweis berechnet.

Resultate: Die positiven Vorhersagewerte für das Vorhandensein von Myositis bei positivem Antikörpertiter sind in unserer Patientenkohorte relativ gering mit Ausnahme des positiven Vorhersagewertes von anti-Jo1 (42%) und anti-Mi-2α (73%). Die berechneten positiven Vorhersagewerte für das Vorliegen einer malignen Erkrankung sind relativ klein in der untersuchten Patientengruppe. Die höchsten positiven prädikativen Werte für das Vorliegen einer anderen Autoimmunerkrankung als Myositis wurden für anti-TIF1γ (47%), anti-PMScl-100 (52%), anti-PMScl-75 (40%), anti-PL-7 (46%) und anti-Mi-2β (43%) bestimmt. Die Prävalenz für ILD in der untersuchten Patientengruppe liegt bei 21% und ist somit circa die Hälfte der Prävalenz, die in der Literatur angegeben wird(2). Relativ am häufigsten wurde ILD bei Patienten und Patientinnen nachgewiesen, die positiv für anti-PL-7 (25%), anti-Jo1 (21%), anti-PL-12 (15%) oder anti-SRP (12%) sind. Eine

signifikante Korrelation zwischen positiven Antikörpertitern von anti-MDA5 und anti-SAE im Normbereich wurde detektiert.
Das Ergebnis der EULAR/ACR Klassifikationskriterien stimmte in 88% der Patienten und Patientinnen mit den klinisch diagnostizierten Myositiden überein.

Schlussfolgerung: Da eines der primären Ziele die Beurteilung der klinischen Relevanz von Myositis-spezifischen und Myositis-assoziierten Antikörpern anhand der Berechnung der positiven Vorhersagewerte für das Vorliegen einer Myositis war, kann diese Arbeit als Basis für die Interpretation von positiven Autoantikörpertiter verwendet werden.

Schlüsselwörter: Idiopathische Inflammatorische Myopathien, Myositis-spezifische/assoziierte Antikörper, Positiver Vorhersagewert, EULAR/ACR Klassifikationskriterien.

Abstract

Background: The detection of myositis-specific antibodies is known as an important diagnostic method of idiopathic inflammatory myopathies (IIM)(1). By conducting this study, the clinical relevance of myositis-specific and myositis-associated antibodies in the diagnostic pathway of IIMs was investigated. Therefore, one of the primary aims was to determine the positive predictive values for each studied autoantibody.

Methods: The study investigated retrospectively medical records of all patients tested positive for MSA and/or MAA between October 2014 to October 2017. The patients' medical records got analyzed thoroughly and the data was checked for predefined clinical features and laboratory parameters. The parameters necessary for the use of the new EULAR/ACR classification criteria for idiopathic inflammatory myopathies were filtered out of the medical records in order to prove the applicability of those criteria. Furthermore, correlations between positive antibody test results and the presence of an IIM subtype as well as other autoimmune diseases, malignancies or interstitial lung disease have been investigated. Finally, the positive predictive values of each tested autoantibody for suffering from an IIM subtype, for having a positive history of autoimmunity or malignancy and the PPV for having ILD were calculated.

Results: The PPVs of the analyzed autoantibodies within our patient cohort for having myositis are rather low except for the PPV of anti-Jo1 (42%) and anti-Mi-2α (73%). The PPVs for having a malignant disease determined in this study are relatively low. The highest PPVs for having another autoimmune disease than myositis were obtained for anti-TIF1γ (47%), anti-PMScl-100 (52%), anti-PMScl-75 (40%), anti-PL-7 (46%) and anti-Mi-2β (43%). The prevalence of ILD in our patient cohort is not higher than 21% and hence only half of the prevalence reported in literature(2). The highest relative frequencies of ILD were found in patients positive for anti-PL-7 (25%), followed by anti-Jo-1 (21%), anti-PL-12 (15%) and anti-SRP (12%). Furthermore, the data was checked for significant correlations ending up with one significant correlation between anti-MDA5 and anti-SAE within the normal range.

Finally, the EULAR/ACR classification criteria confirmed in 88% (213 patients out of 242) the actual situation of patients concerning the presence or absence of an IIM subtype.

Conclusion: Since one of the primary aims was to evaluate the clinical relevance of MSA and MAA by calculating the positive predictive values for having an IIM subtype, this thesis can serve as a basis for clinicians for interpreting a positive autoantibody test result.

Key words: Idiopathic Inflammatory Myopathies, Myositis-Specific/Associated Antibodies, Positive Predictive Value, EULAR/ACR Classification Criteria.

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Abbreviations

Ab	Antibody
ACR	American College of Rheumatology
AI	Autoimmunity
ALS	Amyotrophic Lateral Sclerosis
ALT	Alanine Transaminase
ARS	Aminoacyl Transfer RNA Synthetase
ASS	Anti-Synthetase Syndrome
AST	Aspartate Transaminase
CADM	Clinically Amyopathic Dermatomyositis
CK	Creatine Kinase
CTD	Connective Tissue Disease
DM	Dermatomyositis
EMG	Electromyography
ER	Endoplasmic Reticulum
EULAR	European League Against Rheumatism
HLA	Human Leukocyte Antigen
HMGCR	3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase
IIM	Idiopathic Inflammatory Myopathy
ILD	Interstitial Lung Disease
IP	Immunoprecipitation
MAA	Myositis-Associated Antibodies
MHC	Major Histocompatibility Complex
MSA	Myositis-Specific Antibodies
NAM	Necrotizing Autoimmune Myopathy
NPV	Negative Predictive Value
PAMP	Pathogen-Associated Molecular Pattern
PM	Polymyositis
PPV	Positive Predictive Value
RA	Rheumatoid Arthritis
SAD	Systemic Autoimmune Disease
SLE	Systemic Lupus Erythematosus
SPSS	Superior Performing Statistical Software

SRP	Signal Recognition Particle
SS	Sjögren-Syndrome
SSc	Systemic Sclerosis
TIF1γ	Transcription Intermediary Factor 1γ
TRIM	Tripartite Motif
tRNA	Transfer Ribonucleic Acid
UCTD	Undifferentiated Connective Tissue Disease

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1 Introduction

1.1 General Overview on Idiopathic Inflammatory Myopathies

1.1.1 Definition and Subtypes

Idiopathic inflammatory myopathies, abbreviated as IIMs and also known as myositis, represent a group of heterogeneous muscle disorders, which are characterized by weakness of the proximal muscles and inflammation of the skeletal muscles(1,3). IIMs are rare and sporadic occurring disorders of the skeletal muscles(4). A yearly incidence of 1 in 100 000 inhabitants is reported in the United States for idiopathic inflammatory myopathies(5). Subtypes belonging to IIMs are for example dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM) in adults and juvenile dermatomyositis is described in children(3). Tan et al.(1) mention further subtypes of IIMs such as myositis associated with other systemic autoimmune diseases (SAD) and myositis associated with malignancy. An example of systemic autoimmune disease with myositis is the anti-synthetase syndrome (ASS). This disease is additionally to proximal muscle weakness characterized by arthritis and interstitial lung disease (ILD).(1)

1.1.2 Clinical Presentation of IIM

Overall, patients suffering from inflammatory muscle diseases show similar clinical symptoms although the symptoms can vary in intensity as well as in the time of occurrence in the course of disease. As mentioned above, patients with inflammatory muscle diseases depict typically weakness of the proximal muscles, like weakness of the quadriceps and of the biceps. Therefore, those patients have problems in rising from a chair, in climbing stairs as well as in hoisting objects. Furthermore, in all subtypes it is possible that pharyngeal muscles and neck extensors get affected which results in dysphagia or in difficulties stabilizing the head. In rare IIM cases, muscles of the respiratory tract can be also involved. Muscle pain and tenderness as well as muscle atrophy can occur.(6)
Inflammatory muscle diseases do not only affect muscles, there can be also extramuscular manifestations in all subtypes of inflammatory myopathies. Systemic clinical features like fever, arthralgia and Raynaud´s phenomenon can

be observed. Additionally, cardiac manifestations as cardiac arrhythmias or ventricular dysfunction are seen in rare cases. Finally, also pulmonary complications can occur.(6) Interstitial lung disease which has a prevalence of 40% in IIM patients is a very common and important complication(2).

1.2 Subtypes of Idiopathic Inflammatory Myopathies

1.2.1 Dermatomyositis

1.2.1.1 Definition

Dermatomyositis is defined as a systemic inflammatory myositis with typical skin affections(7).

1.2.1.2 Epidemiology

Dermatomyositis is more frequent in women than in men (female-to-male ratio 2:1) and the incidence of DM in adults varies from 0.6 to 1.0 per 100 000 inhabitants per year. The juvenile DM has an incidence of 0.2 per 100 000 inhabitants per year.(8)

1.2.1.3 Signs and Symptoms

Patients with dermatomyositis show typical skin changes either in parallel or before muscle weakness starts. The specific skin manifestations include the heliotrope edematous rash, erythematous rash and the so-called Gottron's rash. The heliotrope rash occurs typically around the eye and has a blue-purple color. Furthermore, the erythematous rash can be found on joints, like the knees, elbows, as well as in the face, neck, anterior chest (V-sign), back, shoulders and malleoli. The Gottron's rash is located on the knuckles and can be described as a violet eruption. All those skin changes which can be found in dermatomyositis patients are sensitive to ultraviolet light and can get worse when patients are exposed to sunlight. Another characteristic of dermatomyositis are the so-called "mechanic's hands" which are defined as fingertips with clefts and fissures(6,9). Furthermore, loops formed by dilated capillaries found at the base of the fingernails are typically found in DM patients. In an advanced state, calcifications originating from the subcutis can be observed, but those are more frequently found in children than in adults. In severe cases these calcifications reach the surface of

the skin and thereby causing ulcerations which are then starting points of infections.(6)

As described in section 1.1.2 (Clinical Presentation of IIM) idiopathic inflammatory myopathies and therefore also dermatomyositis are characterized by muscle weakness especially of the proximal muscles which results in difficulties getting up from a chair or combing one's hair. It is also possible that muscle strength is normal and dermatomyositis is limited to skin changes, then the correct description is amyopathic dermatomyositis.(6)

Beside the typical skin changes, usually elevated creatine kinase (CK) levels can be detected. Those levels can reach up to 50 times the upper limit of normal. It is also possible that sometimes creatine kinase levels are within the normal range. In the muscle biopsy, perivascular, perimysial and perifascicular inflammation, necrotic fibers, perifascicular atrophy and a reduced number of capillaries can be detected. The perifascicular atrophy is caused by hypoperfusion which is resulting from microinfarcts. Those microinfarcts are themselves caused by the perivascular inflammatory reaction. Autoantibodies that are associated with dermatomyositis are anti-MDA-5, anti-Mi-2, anti-TIF-1 and anti-NXP-2.(6)

1.2.1.4 Pictures of DM Patients with Typical Skin Manifestations

In the following chapter some typical skin manifestations that can occur in dermatomyositis patients are illustrated by pictures thankfully provided by the Dermatology Department of the University Hospital Graz.

In Figure 1, one of the most typical skin manifestations of dermatomyositis, the heliotrope rash around the eyes, is shown. By analyzing the pictures A to D of Figure 1, the heliotrope rash in its various severities can be seen. In picture A, the heliotrope rash can be described as a very subtle erythema around the eyes, whereas in picture B and C, the heliotrope rash is more intense, while in picture D the most severe periorbital heliotrope rash can be observed. Figure 1 illustrates that dermatomyositis can manifest in the form of a very subtle erythema as well as in the form of a more obvious erythematous and edematous rash.

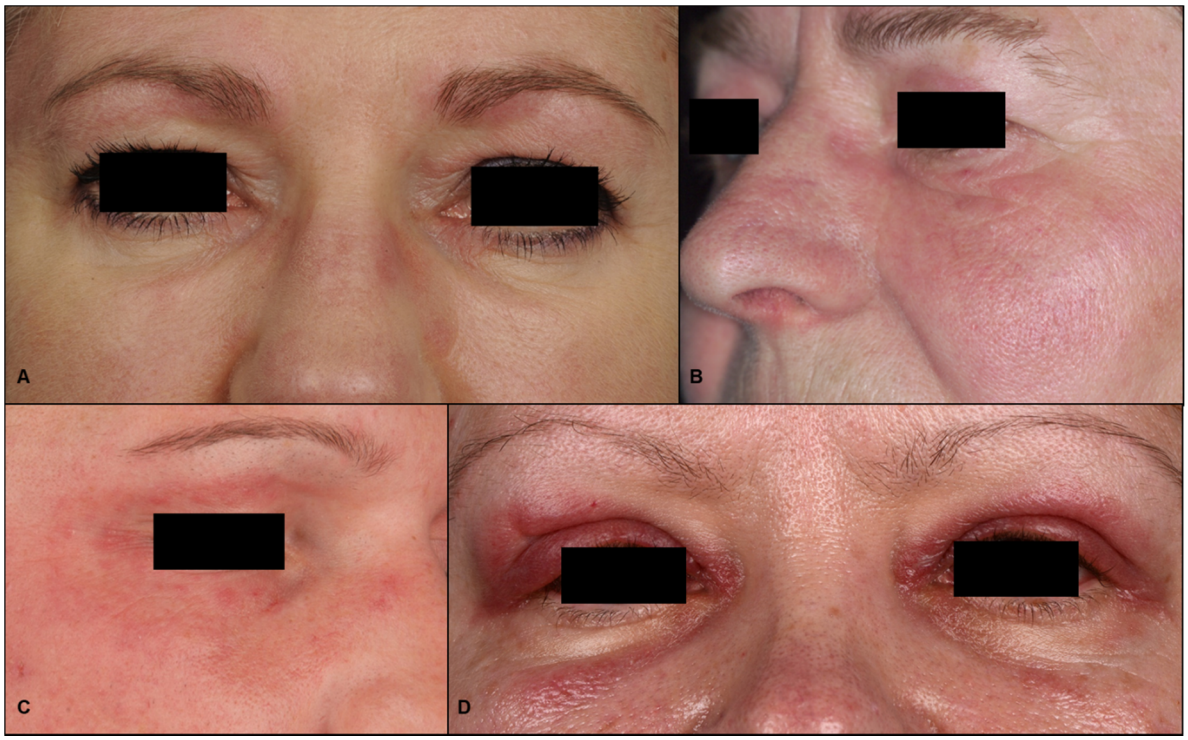

Figure 1: Dermatomyositis patients with heliotrope rash of increasing severity from picture A to D. The pictures are thankfully provided by the Dermatology Department of the University Hospital Graz.

In Figure 2, two examples of dermatomyositis patients with erythematous rash on the anterior chest, which can be also described as V-sign, are depicted. The V-sign again is a skin manifestation typically found in DM patients(6).

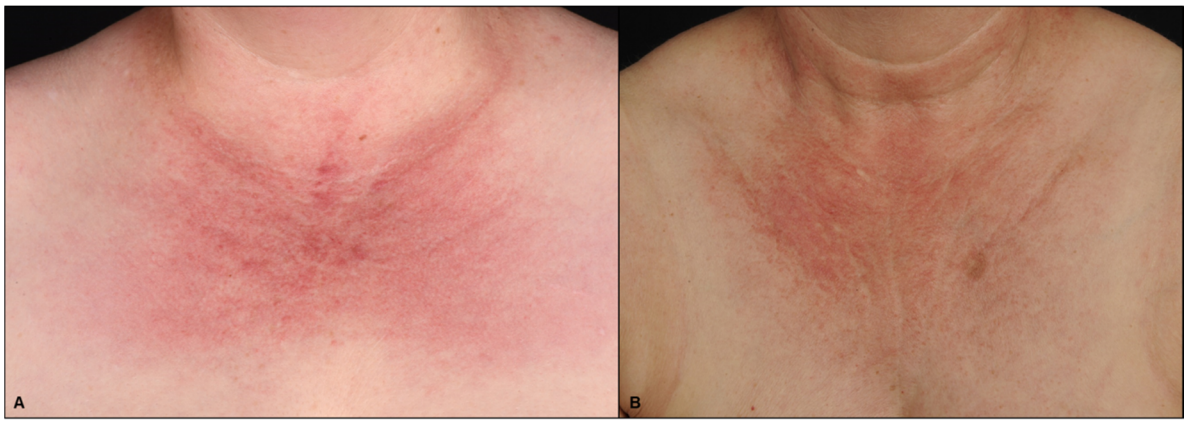

Figure 2: Erythematous rash on the anterior chest of DM patients. The pictures are thankfully provided by the Dermatology Department of the University Hospital Graz.

In Figure 3, another skin manifestation typically found in DM patients, the so-called Gottron's papules are illustrated(6). In picture A only a subtle thickening of the skin over the knuckles can be detected, while in picture B and C the Gottron's papules are more obvious and can be described as red-purple papules located on the dorsum of the hand in the region of the finger joints.

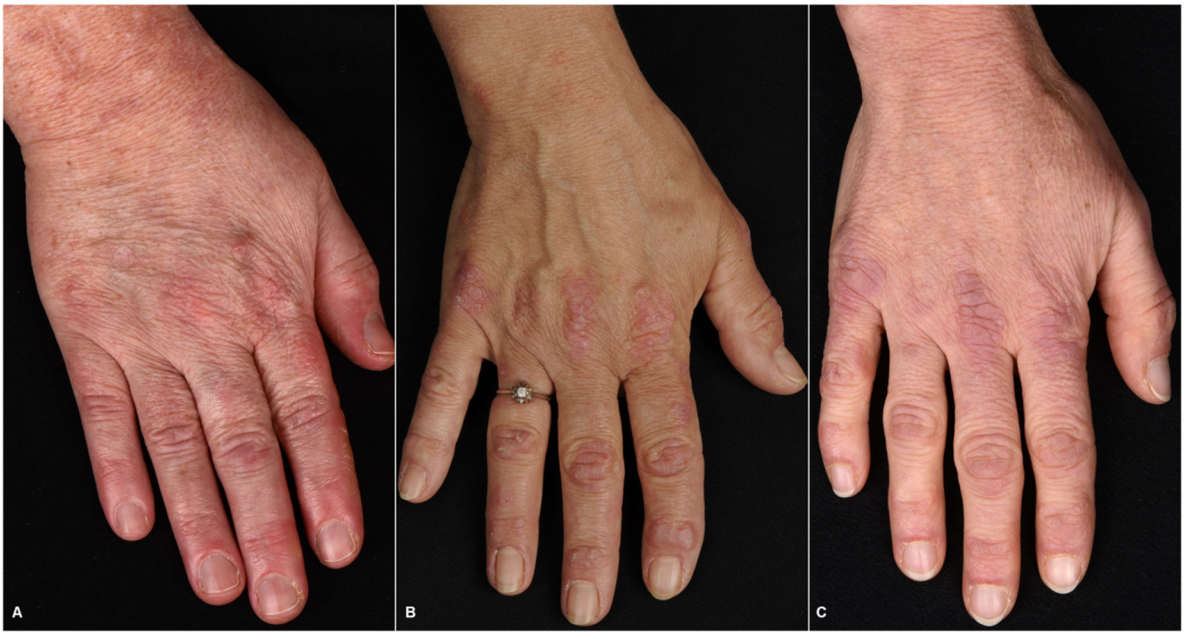

Figure 3: Gottron's papules on the knuckles of dermatomyositis patients in different severity grades. The pictures are thankfully provided by the Dermatology Department of the University Hospital Graz.

By performing direct-light microscopy, in some dermatomyositis patients dilated capillary loops located at the basis of the fingernail can be detected as shown in Figure 4. This is a further typical clinical feature of DM patients.(6)

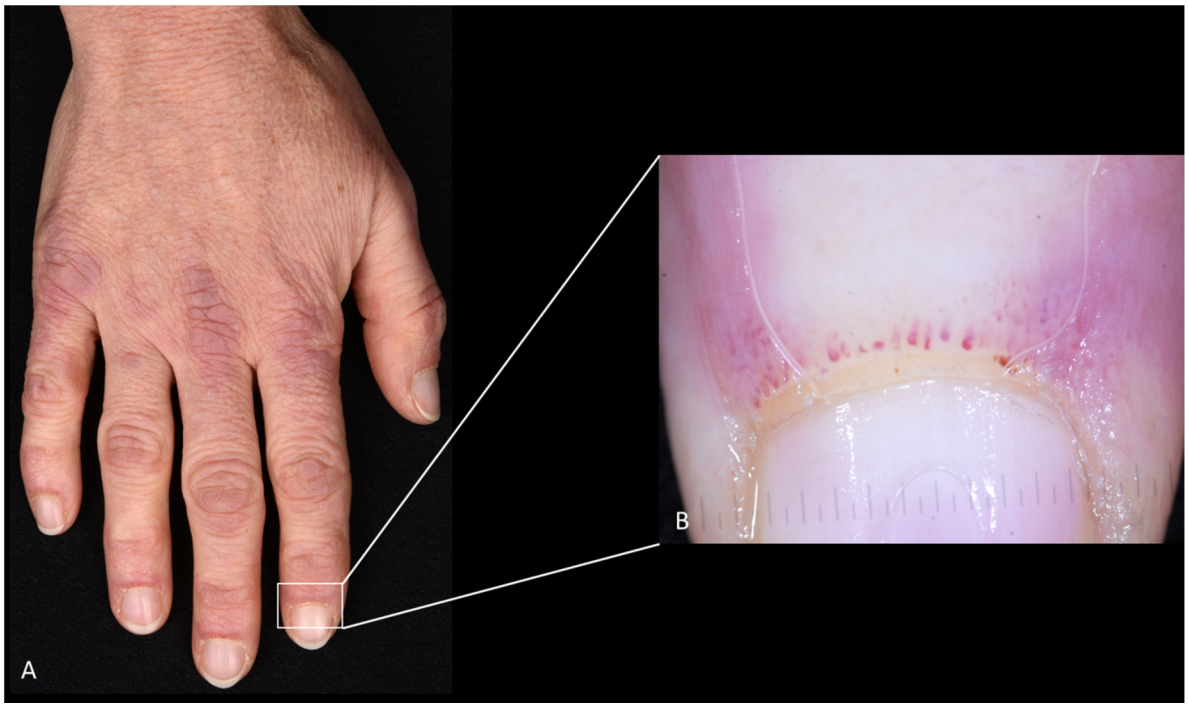

Figure 4: Dilated capillaries located at the basis of the fingernails. The pictures are thankfully provided by the Dermatology Department of the University Hospital Graz.

1.2.2 Polymyositis

1.2.2.1 Definition

Polymyositis is defined as an inflammatory systemic disease of skeletal muscles resulting in myalgia, tenderness and weakness of especially the proximal muscles.(7)

1.2.2.2 Epidemiology

Polymyositis is a rare muscle disease with an incidence of 5-10 per 1 000 000 inhabitants per year.(10)

1.2.2.3 Signs and Symptoms

The clinical presentation of polymyositis is nearly the same as of dermatomyositis except the skin changes(7). The muscle affections are described in section 1.1.2 (Clinical Presentation of IIM).

Polymyositis is an exclusion diagnosis which is described as subacute proximal myopathy in adults without any rash, with a negative family history of neuromuscular diseases and no myotoxic drug exposure like for example to statins, steroids, chloroquin and hydroxychloroquin, pencillamine, zidovudine etc. Furthermore, affections of the muscles of the face as well as of the extraocular

muscles and endocrine disorders should be excluded beforehand diagnosing PM.(6)
Especially in early active disease, the creatine kinase levels usually are high and can reach levels up to 50 times higher than the normal upper limit. It is possible that the CK levels lower again and linger at up to 10 times the normal upper limit. Invasion of healthy, non-necrotic muscle fibers, expressing the MHC-class-1 antigen, with $CD8^+$ T-cells is typically found in muscle biopsies of PM patients. Inflammation is typically located in multiple endomysial foci.(6)
In idiopathic inflammatory myopathies, MHC-class-1 gets upregulated due to increased secretion of interferons and other cytokines. Thereby muscle fibers get targeted and are recognized by cytotoxic $CD8^+$ T-cells initiating a myodegenerative process. Moreover, Tieu et al.(11) describe that MHC-class-1 molecules do not only play an important role in the inflammatory reaction of IIMs, but rather represent pathogenic molecules themselves. In an MHC-class-1 transgenic mouse model it was shown that the mice suffer first from weakness followed by lymphocytic infiltration. The upregulation of MHC-class-1 antigens can be found in early as well as in advanced stages and represents an important diagnostic marker for IIM.(11)

1.2.3 Necrotizing Autoimmune Myositis

1.2.3.1 Definition

Necrotizing autoimmune myopathy (NAM) represents a rare subtype of idiopathic inflammatory myopathies which is associated with some risk factors like statin medication, malignant diseases or connective tissue disorders. For example, people with scleroderma can also show NAM. When people under statin therapy develop muscle weakness and the myopathy even gets worse after stopping statin medication, this would be an indicator for NAM. However, if the myopathy improves within a time period of four to six weeks after terminating statin therapy, the myopathy was most likely due to toxic effects of the medication rather due to inflammatory reaction. It is also described in literature that necrotizing autoimmune myopathy can occur after viral infections.(6) However, in more than one half of patients suffering from necrotizing autoimmune myopathy the trigger is not known(5).

1.2.3.2 Epidemiology

Necrotizing autoimmune myopathy can basically occur at any age, however for the main part adults are affected(6). According to Khan et al.(5), NAM represents about 16% of all IIM cases in the United States.

1.2.3.3 Signs and Symptoms

The necrotizing autoimmune myositis can start acutely which means that within weeks or even days the maximum of clinical symptoms is reached. On the other hand, necrotizing autoimmune myositis can begin in a subacute manner and thereby resulting in more severe muscle weakness.(6) Typically, patients with necrotizing autoimmune myositis show severe proximal muscle weakness, weakness of the lower extremities and extreme fatigue. Pharyngeal and respiratory muscles are affected only in very rare cases.(5)
The creatine kinase levels in patients with necrotizing autoimmune myopathy are typically extremely high and reach levels even higher than 50 times the upper limit of normal in early stages of the disease. In muscle biopsies, lots of necrotic muscle fibers can be detected which are either surrounded or invaded by macrophages. In contrast to polymyositis, there are only few lymphocytes infiltrating the muscles. Moreover, like in patients suffering from PM, MHC-class-1 expression can be found too, however these MHC-class-1 positive cells in necrotizing autoimmune myositis are mostly located beside the necrotic fibers and not within the muscle fibers as it applies for polymyositis. Furthermore, complement deposits can be found on capillaries. Additionally, necrotizing autoimmune myositis is associated with specific antibodies against SRP (signal recognition particle) and HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase), the pharmacological target of statins. Antibodies against HMGCR are detected in 22% of patients suffering from necrotizing autoimmune myositis independent of medication with statins. The presence of anti-HMGCR also correlates with the CK levels and strength.(6)

1.2.4 Inclusion Body Myositis

1.2.4.1 Definition

Inclusion body myositis (IBM) is defined as another subtype of idiopathic inflammatory myopathies.(6,12) The reason for developing IBM is not known so

far, but an interplay between environmental and genetic factors as well as aging is postulated(12).

1.2.4.2 Epidemiology

The sporadic inclusion body myositis is the most frequent inflammatory myopathy among people at the age of 50 years or older(6). Since physicians have a greater awareness of the disease now, IBM is diagnosed more frequently than in former days(13). The prevalence of inclusion body myositis varies between groups of different ethnicity and also between different populations(12). In the Netherlands for example, the prevalence is estimated to 4.9 cases per 1 000 000 inhabitants. By adjusting to the age of 50, the prevalence rises up to 16 cases per million people.(6,12)

1.2.4.3 Signs and Symptoms

Inclusion body myositis typically shows an insidious beginning and takes a long period of time, usually over years, to develop. The muscle weakness can be symmetrically or even asymmetrically which means that for example one arm is more severe affected than the other one.(6) In patients with IBM selective patterns of muscle weakness can be observed especially affecting the forearm flexors and the quadriceps muscles. Consecutively, those people are prone to fall more easily.(12) Moreover, muscles like the finger flexors and foot extensors show reduced strength in early stages of the disease. In more than 50% of patients with IBM, dysphagia is described due to involvement of the pharyngeal muscles. The axial muscles can be affected too resulting in a forward bending of the spine.(6)
In inclusion body myositis, two different mechanisms are described. On the one hand, inflammatory reactions take place leading to upregulation of inflammatory mediators and thereby attracting $CD8^+$ T-cells. These cells destroy the healthy muscle fibers expressing MHC-class 1-antigen.(12) The second mechanism observed in IBM is that molecules like β-amyloid aggregate within the muscle fibers. According to Machado et al.(12), it is still questionable whether IBM is an inflammatory myopathy or a degenerative muscle disorder with an accompanying inflammation. Askanas et al.(13) suggest that the accumulation of posttranslational modified proteins in muscle fibers is the reason for the T-cell inflammatory reaction. There is an IBM mouse model that supports their hypothesis that at first protein aggregation takes place followed by an inflammation.(13) This mouse

model is based on the overexpression of mutated gelsolin D187N. In that mouse model, misfolded, congophilic proteins like β-amyloid and gelsolin accumulated within the muscle fibers of aged mice. Moreover, lymphocytic infiltrations were detected perivascular and endomysial.(14) It is suggested that accumulation of mutated abnormal proteins is followed by secondary inflammation(13).
Interestingly, patients with inclusion body myositis cannot be treated satisfactorily by anti-inflammatory therapies as PM patients. Given the hypothesis that inflammation is a secondary event and not the primary cause of IBM, it makes sense that anti-inflammatory therapies do not improve the clinical presentation of IBM patients in a satisfactory manner.(13)
Most histopathological changes in muscle biopsies of PM also apply for IBM. Additionally, autophagic vacuoles, ragged-red or ragged-blue fibers and congophilic amyloid deposits can be detected. An increase in connective tissue is associated with the chronic development of the disease.(6)
The CK level can be elevated up to 10 times the normal upper limit. It is also possible that the CK level is normal or only slightly elevated. The autoantibody anti-cytosolic-5'-nucleotidase-1A (anti-cN1A) is associated with IBM.(6)

1.2.5 Antisynthetase Syndrome

1.2.5.1 Definition

The antisynthetase syndrome is another rare subtype of idiopathic inflammatory myopathies. This IIM subtype is defined by the detection of antisynthetase antibodies and the presence of one or more of the following symptoms like myositis, ILD, Raynaud's phenomenon, mechanic's hands, arthritis and/or fever.(9)

1.2.5.2 Signs and Symptoms

The antisynthetase syndrome goes along with inflammation of the muscles, polyarthritis especially of the small joints of the feet and hands as well as lung affection in terms of ILD. Additionally, fever, so-called "mechanic's hands" and the Raynaud's phenomenon are typical characteristics of the antisynthetase syndrome.(9) "Mechanic's hands" are defined as fingertips with clefts and fissures and can be also detected in DM patients(6,9). The Raynaud's phenomenon is defined as a phenomenon where paroxysmal, painful vasospasms of the hand's

blood vessels occur as part of an underlying primary disease. It is characterized by a white color of the skin in the beginning due to vasospasm of the hand's blood vessels, followed by a cyanotic state and finally, the skin appears red due to reactive vasodilatation.(15)

1.2.6 Overlap Myositis

1.2.6.1 Definition

Overlap syndromes are defined as clinical conditions where either the classification criteria of two or more systemic diseases are fulfilled or the main symptoms of these systemic diseases are present. At least two of the following systemic diseases must be present at the same time that one can diagnose an overlap syndrome: Systemic lupus erythematosus (SLE), systemic sclerosis (SSc), idiopathic myositis (IM), Sjögren-syndrome (SS) or rheumatoid arthritis (RA).(16) Consequently, overlap myositis is a condition where clinical features of both idiopathic inflammatory myositis, like for example dermatomyositis, and of at least one of the diseases described above can be observed.(6)

1.2.6.2 Epidemiology

Concerning prevalence and incidence of overlap myositis no data is available. The reason for missing epidemiological data may be that overlap myositis has not been recognized as a distinct entity of idiopathic inflammatory myositis for a long time.(17) In recent years, overlap myositis started to be detected as a distinct entity of IIM(6).

1.2.6.3 Signs and Symptoms

The symptoms of overlap myositis are very diverse and depend on which diseases are overlapping. It manifests typically with pathologic changes in the perifascicular, interfascicular and perimysial regions of the muscles resulting in muscle weakness.(6)

1.3 Diagnosis of Idiopathic Inflammatory Myositis

In order to be able to diagnose idiopathic inflammatory myopathies, many different clinical features as well as laboratory parameters and histological findings have to be taken into consideration. It is important to pay attention to the patient's clinical

history, progression of the disease, muscle involvement, elevated levels of muscle enzymes (CK levels), results of electromyographic investigations, findings in muscle biopsies and finally whether myositis-associated or myositis-specific autoantibodies can be detected.(6)
Moreover, in the diagnosis of idiopathic inflammatory myositis, several different diagnostic criteria are available like for example the most widely used Bohan and Peter criteria (described in section 1.3.5) or the classification criteria of the European League Against Rheumatism (EULAR) / American College of Rheumatology (ACR) (described in chapter 1.3.6 more in detail).(3)

1.3.1 Electromyography

Electromyography (EMG) plays an important role in the diagnosis of idiopathic inflammatory myositis since it can distinguish between myopathic and neuropathic disorders. For example, both IIMs and ALS (amyotrophic lateral sclerosis) can be presented by muscle weakness and increased levels of CK.(18) Hence, EMG is performed in order to rule out neurogenic causes for the clinical symptoms.(6)

1.3.2 Magnetic Resonance Imaging

Muscle imaging is performed in a next step. Thereby, muscles get checked for abnormalities like abnormal signal intensity, destruction of the common muscle morphology or a decreased muscle mass.(18) On the one hand magnetic resonance imaging (MRI) is performed in order to identify the muscles affected by idiopathic inflammatory myopathies and to determine the activity of the disease. Especially muscle edema that is present in active muscle inflammation and fatty infiltrations indicating a chronic inflammatory process are detected by MRI.(6,18) Secondly, another aim of muscle imaging is to identify the best biopsy sites represented by highly active muscle inflammation.(18)

1.3.3 Muscle Biopsy

The gold standard in the diagnostic pathway of IIMs is the muscle biopsy which is important for the confirmation of idiopathic inflammatory myopathy, differentiation between the IIM subtypes and exclusion of other diseases like muscle dystrophies or metabolic myopathies(6,11). The characteristic histopathological muscular changes are described in section 1.2 for each IIM subtype.

1.3.4 Myositis-Specific and Myositis-Associated Antibodies

Autoantibodies play an important role in the diagnosis of various systemic autoimmune diseases like for example in diagnosing idiopathic inflammatory myositis. There are basically two different groups of antibodies that are essential to know when dealing with IIM. On the one hand there are the so-called myositis-specific antibodies (MSA) and on the other hand there are myositis-associated antibodies (MAA). The main difference between those two groups is whether they can be found exclusively in IIM patients or also in patients suffering from other systemic diseases. Myositis-associated antibodies are defined as autoantibodies that can be detected also in other connective tissue diseases (CTD) like in systemic sclerosis or systemic lupus erythematosus. In contrast, myositis-specific antibodies are reported to be found nearly solely in idiopathic inflammatory myositis.(11,19)

In Table 1, an overview of different types of autoantibodies important for IIM is given. As it can be seen in Table 1, myositis-specific antibodies are for example antisynthetase antibodies (anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ etc.), anti-Mi2, anti-SRP, anti-TIF1γ, anti-SAE, anti-MDA-5 and anti-NXP-2 antibodies. Autoantibodies like anti-Ku, anti-PM-Scl75 and anti-PM-Scl100 are examples of myositis-associated antibodies.(19)

Table 1: Myositis-Specific/-Associated-Antibodies and their Targets.(11,19)

Antibody subtype	Name	Target
Myositis-specific antibodies	anti-Jo-1	Histidyl transfer RNA synthetase
	anti-Mi2	Mi-2 autoantigen
	anti-SRP	Signal recognition particle
	anti-TIF1γ	Transcription intermediary factor-1γ
	anti-SAE	Small ubiquitin-like molecule activating enzyme
	anti-MDA-5	Melanoma differentiation associated gene 5
	anti-NXP-2	Nuclear matrix protein 2
	anti-HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase(11)
	anti-cN1A	Cytoplasmic 5'-nucleotidase 1A(11)
Myositis-associated antibodies	anti-Ku	DNA repair enzyme(20)
	anti-PM-Scl75	Human exosome complex
	anti-PM-Scl100	Human exosome complex

1.3.4.1 Myositis-Specific Antibodies

1.3.4.1.1 Antisynthetase Antibodies

The antisynthetase antibodies represent the most common myositis-specific antibodies and they are reported to be identified in 35 – 40% of IIM patients(11). These autoantibodies are targeted against cytosolic enzymes, called aminoacyl-transfer-RNA-synthetases (ARSs) that are involved in protein synthesis. The function of the tRNA-synthetases is to catalyze the binding between a specific amino acid and the correct tRNA during the process of protein synthesis. Due to the fact that the anti-ARSs can be already detected before disease' outbreak, it is assumed that these antisynthetase antibodies play an important role in pathogenesis.(11)

In Table 2, the eight antibodies against aminoacyl-tRNA-synthetases that are known and described up to now can be seen. This table includes the antisynthetase antibody's name, its target and which diseases are associated with the specific antibody.

Table 2: Antisynthetase antibodies, their targets and their clinical occurrence.(11)

Antisynthetase Ab	tRNA synthetase	Clinical occurrence
Anti-Jo-1	Histidyl	PM, DM + ILD
Anti-PL-7	Threonyl	PM, DM + ILD
Anti-PL-12	Alanyl	ILD > myositis
Anti-EJ	Glycyl	PM > DM + ILD
Anti-OJ	Isoleucyl	ILD + PM/DM
Anti-KS	Asparaginyl	ILD > myositis
Anti-Zo	Phenylalanyl	ILD + PM/DM
Anti-Ha	Tyrosyl	ILD + PM/DM

By analyzing Table 2, it can be seen that the different antisynthetase antibodies are associated with different clinical diagnosis. The most frequent antisynthetase antibody is anti-Jo-1. The presence of this special type of antibody is associated with an increased risk of developing myositis, while most other antisynthetase antibodies are more likely to be present in patients suffering from interstitial lung disease (ILD). Myositis is mostly associated with antibodies like anti-Jo-1, anti-PL-7 and anti-EJ.(11)

The antisynthetases belong to the group of myositis-specific antibodies and are typically associated with a combination of clinical symptoms as interstitial lung disease, myositis, fever, mechanics' hands, inflammatory arthritis and Raynaud's phenomenon.(11)

1.3.4.1.2 Anti-Mi-2 Antibodies

The target of anti-Mi-2 antibodies is the autoantigen Mi-2 which is part of a nucleosome remodeling and histone deacetylase complex.(11) Mi-2 is composed of two subunits – Mi-2α and Mi-2β(21). This complex takes part in gene expression and binding of nucleic acids. Anti-Mi-2 is typically associated with dermatomyositis and its characteristic skin lesions, including Gottron's papules, heliotrope rash, shawl rash, V-sign, cuticular hypertrophy and photosensitivity. Mi-2 antibodies are detected in 10 – 30% of patients suffering from idiopathic inflammatory myositis.(11) Furthermore, if anti-Mi-2 antibodies are found, those patients usually respond well to treatment and have a good disease prognosis. Finally, it is reported that anti-Mi-2 antibodies show a low risk for developing ILD or a malignant disease.(11,22)

The Mi-2-autoantigen has an important role in the regulation of developmental processes. Studies performed in the past demonstrated that tissue repair processes lead to high levels of Mi-2 in skin and muscle in DM. In different words, when muscles or skin are injured, the expression of Mi-2 rises in the repairing cells, which leads to further attack by the immune system. Thereby injury is caused again, further repair is needed and a vicious cycle of damage is induced.(22)

1.3.4.1.3 Anti-MDA5 Antibodies

MDA5 (melanoma differentiation associated gene 5) is an RNA-specific helicase that is found in the cytoplasm and is able to recognize single-stranded RNA viruses. This helicase can be described as a PAMP (pathogen-associated molecular pattern) sensor for viral RNA and hence has an important role in controlling viral infections. MDA5 induces type 1 interferon production and anti-viral gene expression.(22)

Anti-MDA5 antibodies belong to the group of DM-specific antibodies and are mostly associated with clinically amyopathic dermatomyositis (CADM). Therefore, those antibodies were originally termed anti-CADM. In Asian patients suffering

from DM, anti-MDA5 antibodies are found in 20 – 30%. In Caucasians those antibodies are detected less frequently than in Asians.(11)
Patients with anti-MDA5 antibodies typically show absent or only mild muscle involvement, but they have an elevated risk for rapidly progressive interstitial lung disease. Moreover, those patients with anti-MDA5 antibodies show skin manifestations such as skin ulcers and palmar papules typically located at the lateral nailfolds, Gottron´s papules and elbows.(11,22) Also oral ulcers and arthritis are likely to be detected in MDA5 positive patients(22). In the United States, DM patients with anti-MDA5 antibodies are associated with bad disease prognosis and a reduced survival(11).

1.3.4.1.4 Anti-TIF1γ Antibodies

TIF1γ (transcription intermediary factor 1γ) is part of the so-called TIF1 family which represents tripartite motif (TRIM)-containing proteins like TIF1α, TIF1β and TIF1γ. Those proteins are known to play an important role in carcinogenesis and other key pathways. For example TIF1γ inactivates Smad4[1] which in turn inhibits TGFβ signaling and at the same time growth and differentiation of cells get increased. TIF1α inactivates p53 by ubiquitination and activates other genes involved in tumor development. TIF1β leads to an increase in p53 ubiquitination and prevents acetylation of p53 thereby avoiding apoptosis.(22) So all three members of the TIF1 family somehow contribute to cancer development. There are speculations that anti-TIF1 antibodies get produced as an anti-tumor response, so in different words, autoimmunity is representing an anti-cancer process.(22)
Anti-TIF1γ antibodies are reported to be found in about 20 – 30% of DM patients and they are associated with malignancy in adult DM. On the other hand, those antibodies come along with a reduced risk of developing ILD. Skin changes that can be observed in patients with anti-TIF1γ antibodies are photoerythema.(11)

[1] Smad4 is a protein that gets activated by TGFβ signaling and regulates gene expression in the nucleus. Smad4 is a tumor suppressor and inhibits epithelial cell proliferation. If there are mutations in Smad4, various malignancies like pancreatic cancer can be detected.(38)

1.3.4.1.5 Anti-NXP2 Antibodies

NXP2 (nuclear matrix protein 2) is the target of anti-NXP2 antibodies which are detected in about 25% of juvenile DM and they are also found in adult patients suffering from dermatomyositis(11). The nuclear matrix protein 2 is a protein that moves into the nucleus and is proposed to be involved in RNA processing since NXP2 has an RNA-binding site.(21) Anti-NXP2 antibodies are associated with severe muscle weakness, joint contractures, polyarthritis, calcinosis and severe skin changes.(11,22) Moreover, there is also an association to malignancy. In patients with cancer-associated dermatomyositis the autoantibodies TIF1γ and NXP2 are found in more than one half of the patients.(11)

1.3.4.1.6 Anti-SAE Antibodies

Small ubiquitin-like modifier activating enzyme (SAE) is a protein detected in the nucleus which takes part in posttranslational conjugation of proteins. Anti-SAE antibodies belong to dermatomyositis-specific autoantibodies and they are found in approximately 8% of adult DM cases. Although these antibodies indicate a higher risk of dysphagia and severe cutaneous changes, those patients have good disease prognosis.(11)

1.3.4.1.7 Anti-SRP Antibodies

SRP is the abbreviation for signal recognition particle which is involved in the transport of newly translated proteins to the endoplasmic reticulum (ER). The SRP binds to a special signal sequence of the protein, targets it to the ER where the protein is then translocated into the cell organelle.(22) Anti-SRP antibodies are found in about 4 – 6% of IIM patients and those antibodies are associated with necrotizing autoimmune myositis. Furthermore, the presence of those antibodies is associated with a rapid progressive myopathy, relevantly increased CK levels and disabled act of swallowing.(11) Patients suffering from an anti-SRP myopathy do not respond well to therapies and often need more immunosuppressive treatments.(22)

1.3.4.1.8 Anti-HMGCR Antibodies

The target of the anti-HMGCR is the so-called 3-hydroxy-3-methylglutaryl-CoA-reductase (HMGCR), which is also targeted by statin medication. Those anti-HMGCR autoantibodies are associated with necrotizing autoimmune myositis and

their development is highly associated with the intake of statin medication as well as with the HLA subtype DRB1*11. Tieu et al.(11) describe that within patients suffering from myositis, those people carrying HLA-DR11 under statin therapy have the highest risk for developing autoantibodies against HMGCR.(11) Anti-HMGCR autoantibodies can be detected in approximately one fifth (22%) of people suffering from necrotizing autoimmune myopathy. Furthermore, a correlation between the anti-HMGCR and the CK levels as well as the muscle weakness is described in literature.(6)

1.3.4.1.9 Anti-cN1A Antibodies

Anti-cN1A autoantibodies are targeted against cytoplasmic-5'-nucleotidase-1A (cN1A) and belong to the group of myositis-specific autoantibodies. cN1A antibodies are typically present in patients suffering from inclusion body myositis. Moreover, antibodies against cN1A can be also detected in some patients having another autoimmune disease diagnosed, like systemic lupus erythematosus for example.(11) According to Pluk et al.(23), the anti-cN1A antibodies, also called Mup44 autoantibodies, were detected in 33% of patients suffering from IBM while the occurrence of those antibodies in patients with DM or PM was described to be less than 5%(23).

1.3.4.2 Myositis-Associated Antibodies

As already described in section 1.3.4, myositis-associated antibodies (MAAs) are defined as autoantibodies that can be also detected in other connective tissue diseases like systemic sclerosis or systemic lupus erythematosus.

1.3.4.2.1 Anti-PMScl Antibodies

Anti-PMScl antibodies are defined as anti-nucleolar antibodies and are targeted against human exosome complex. These antibodies against the autoantigens PMScl-75 and PMScl-100 are predominantly described in patients suffering from PM, SSc or PM/SSc overlap syndrome. Patients with PM or DM showing anti-PMScl antibodies often have lung and esophagus affected. Moreover, it is reported that those antibodies may coexist with malignant diseases in PM or DM patients and they represent a bad disease prognosis. Hence, patients with PMScl antibodies should undergo regular follow ups.(11)

1.3.4.2.2 Anti-Ku Antibodies

Also anti-Ku antibodies belong to the group of MAAs and can be identified in up to 55% of patients showing an overlap syndrome of PM and SSc. In those patients, involvement of the joints and Raynaud's phenomenon are often described. Finally, they have an increased risk of developing ILD.(11)

1.3.4.3 Detection of Antibodies

Since autoantibodies play an important role in the diagnosis of different systemic autoimmune diseases, it is essential to know the different possibilities how they can be detected and also the differences between the various detection methods. This chapter will focus on the traditional immunoprecipitation (IP) and the line blot (LB), a commercially available kit based on mostly recombinant antigens.(19)

1.3.4.3.1 Immunoprecipitation

Immunoprecipitation was developed based on traditional column affinity chromatography which consists of a column packed with a porous resin with fixed antibodies and allows sample and different solutions to pass through. Instead of a column, immunoprecipitation requires only a small amount of resin in a microcentrifuge tube. The solution to be tested is added to the beads, mixed and incubated. Finally, at the end of each incubation step, the tube gets centrifuged. Thereby, the beads settle to the bottom and the solution can easily be removed. If a specific antibody is contained in the test solution, it can be detected by western blot[2] or other quantitative or semi-quantitative methods.(24)

1.3.4.3.2 Line Blot Assay

There are so-called line blot test kits available (Myositis Profile Euroline) that are developed for the detection of human immunoglobulin G autoantibodies against myositis antigens. Basically, native mammalian Jo-1 and recombinant antigens (Mi-2, Ku, PL-7, PL-12 etc.) are applied to test nitrocellulose strips. The test plasma or serum is applied and if autoantibodies are present in the test

[2] Western blotting is a protein detection technique. First of all, the proteins are separated by gel electrophoresis based on their size or electric charges. After protein separation, the protein bands are transferred to a nitrocellulose membrane where the protein of interest can then be detected by using antibody detection techniques.(39)

serum/plasma they bind to the fixed antigen.(25) In a second incubation step those antibodies react with anti-human antibodies that carry alkaline phosphatase. In the third and last step, a special chromogenic/substrate solution is added. Finally, if there are specific antibodies present in the patient serum, a color change can be detected.(26)

1.3.4.3.3 Comparison of Immunoprecipitation and Line Blot Assay

Since many commercial assays get released without sufficient validation, it is important to compare those new test kits with IP as the gold standard. According to Cavazzana et al.(19), the overall agreement between homemade IP and commercially available LB was 77% (K=0.32 → fair agreement). For anti-MDA5, NXP2, TIF1γ antibodies a good concordance was obtained. A moderate concordance was reached for anti-EJ, anti-Mi-2 and anti-Ku antibodies. Anti-Jo1 showed the worst agreement between IP and LB.(19)
Cavazzana et al.(19) performed the comparison of IP and LB in IIM patients' sera to check for the agreement of these two methods in already known IIM patients. Since the study only included known IIM patients and not a randomized patient group, one cannot say how good these two methods really agree in daily routine. Therefore, it is still of great importance to perform further studies on the accuracy of LB assays so that it will be possible for clinicians to rely on those inexpensive and easily available LB assays in near future(27).
According to Infantino et al.(27), it is absolutely necessary to improve the specificity of the commercially available LB assays in order to reduce the number of false positive test results. Since commercially available assays are increasingly used also by non-specialized people due to their advantages, like less time consuming than IP and easily to conduct, it is of great importance to increase specificity and thereby reducing false positive test results. By reducing false positives, the number of wrong diagnosis, wrong therapies and worried patients will drop.(27)

1.3.5 Bohan and Peter Criteria for the Diagnosis of PM and DM

The Bohan and Peter Criteria for diagnosing polymyositis and dermatomyositis were developed in 1975 and are listed in Table 3. If four criteria are fulfilled, PM or DM exists definitely. PM or DM is probable, if three criteria are fulfilled and if only

two criteria are proven, PM or DM is possible. Polymyositis and dermatomyositis get distinguished whether the typical skin changes of DM are present or not.(28)

Table 3: Bohan and Peter Criteria(28)

1.	Weakness of the proximal muscles (typically symmetrically)
2.	Elevated levels of serum muscle enzymes (CK, aldolase)
3.	Electromyographic abnormalities (myopathic potentials, fibrillations, positive sharp waves, complex repetitive discharges)
4.	Pathologies in muscle biopsies (necrosis, phagocytosis, regeneration, inflammation)
5.	Skin changes typical for DM (Gottron's papules, Gottron's sign, heliotrope rash)

The Bohan and Peter criteria are most widely used, but nevertheless they show some limitations. According to Lundberg et al.(3), the criteria of Bohan and Peter do not define how patients with other myopathies can be excluded properly. For example, it is possible that patients with IBM are classified as PM patients when applying these criteria.(3)

1.3.6 Criteria of the European League Against Rheumatism/American College of Rheumatology

In 2017 new classification criteria for adult and juvenile idiopathic inflammatory myopathies were published by the European League Against Rheumatism and the American College of Rheumatology. They are based on clinical features like muscle weakness, skin manifestations, dysphagia or esophageal dysmotility. Furthermore, laboratory parameters and muscle biopsy findings are included. The two major aims of the project of Lundberg et al.(3) were to firstly, discriminate IIMs and other diseases with muscle involvement and secondly, to allocate the IIM patients to the different subgroups of idiopathic inflammatory myopathies. Therefore, they tried to define a minimum set of parameters that are accessible and available easily to physicians.(3)

Basically, they defined candidate criteria by considering already published myositis criteria and expert opinion, ending up with 93 variables. One group of patients with known IIM and another group with known non-IIM, were analyzed concerning the candidate criteria. The data needed were collected from various different clinical departments from all over the world such as rheumatology, dermatology, neurology and pediatric clinics. Finally, by applying different statistical operations

the EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies could be defined. The new classification criteria which are listed in Table 4 consist of a total number of 16 variables that were found to best differentiate IIM patients and patients without IIM. Each variable has a defined score which represents the variable's importance for differentiating IIM patients from non-IIM patients. The scores for each known variable get aggregated ending up with a total score and a probability of IIM. By using the online web calculator (www.imm.ki.se/biostatistics/calculators/iim), the EULAR/ACR criteria can be applied easily. The outputs of the web calculator are the total score, the probability for IIM and the IIM subgroup.(3)

Table 4: EULAR/ACR classification criteria for adult and juvenile IIMs (based on the table in Lundberg et al.(3)).

Category	Variable	Definition	Score points without muscle biopsy	Score points with muscle biopsy
Age of onset	Age of onset of first symptom related to the disease ≥18 and <40 years	Patients age ≥18 and <40 years	1.3	1.5
	Age of onset of first symptom related to the disease ≥40 years	Patients age ≥40 years	2.1	2.2
Muscle weakness	Objective symmetric weakness of the proximal upper extremities, usually progressive	Progressive weakness of both proximal upper extremities defined by objective strength testing	0.7	0.7
	Objective symmetric weakness of the proximal lower extremities, usually progressive	Progressive weakness of both proximal lower extremities defined by objective strength testing	0.8	0.5
	Neck flexors are relatively weaker than neck extensors	Relative muscle weakness of the neck flexors compared to neck extensors	1.9	1.6

		defined by objective strength testing		
	Proximal leg muscles are relatively weaker than distal muscle legs	Relative muscle weakness of the proximal leg muscles compared to distal leg muscles defined by objective strength testing	0.9	1.2
Skin manifestations	Heliotrope rash	Purple or erythematous periorbital patches, often with periorbital edema	3.1	3.2
	Gottron's papules	Erythematous papules over the extensor surfaces of joints (finger joints, elbows, knees, malleoli, toes)	2.1	2.7
	Gottron's sign	Erythematous macules over the extensor surfaces of joints	3.3	3.7
Other clinical manifestations	Dysphagia or esophageal dysmotility	Difficulties in swallowing or abnormal esophageal motility	0.7	0.6
Laboratory measurements	Presence of Anti-Jo-1	Anti-Jo-1 detected in patients serum	3.9	3.8
	Elevated CK or LDH or AST or ALT	Enzyme level elevated above the upper limit of normal (highest absolute value during the disease course)	1.3	1.4
Muscle Biopsy	Endomysial infiltration of mononuclear cells surrounding, but not invading myofibers	Mononuclear cells surrounding healthy, non-necrotic muscle fibers		1.7
	Perimysial and/or perivascular infiltration of mononuclear cells	Mononuclear cells located in the perimysium and/or surrounding the blood vessels		1.2
	Perifascicular atrophy	Several muscle fibers in the perifascicular		1.9

		region are smaller than muscle fibers located in the center of fascicules		
	Rimmed vacuoles	Blue vacuoles by hematoxylin and eosin staining, Red vacuoles by modified Gomori trichrome stain		3.1

In order to be able to understand the results of the online web calculator, one must know which score is corresponding to “definite IIM”, “probable IIM”, “possible IIM” and “no IIM” which is illustrated in Table 5.

Table 5: Scores and the probabilities for “Definite IIM”, “Probable IIM”, “Possible IIM” and “No IIM”(3).

	Total score without biopsy	Total score with biopsy	Probability of having IIM
No IIM	<5.3	<6.5	<50%
Possible IIM	≥5.3 and <5.5	≥6.5 and <6.7	≥50% and <55%
Probable IIM	≥5.5	≥6.7	≥55%
Definite IIM	≥7.5	≥8.7	≥90%

One of the advantages of the EULAR/ACR classification criteria is their high sensitivity (93%) and specificity (88%) compared to other criteria. The Bohan and Peter criteria have even a higher sensitivity (98%) but on the other side, the specificity is low (55%). Another important advantage is the fact that only a minimum set of clinical features and lab parameters is required.(3)

If patients get classified by the EULAR/ACR criteria having IIM, these patients can further be assigned to subgroups as it is depicted in Figure 5(3).

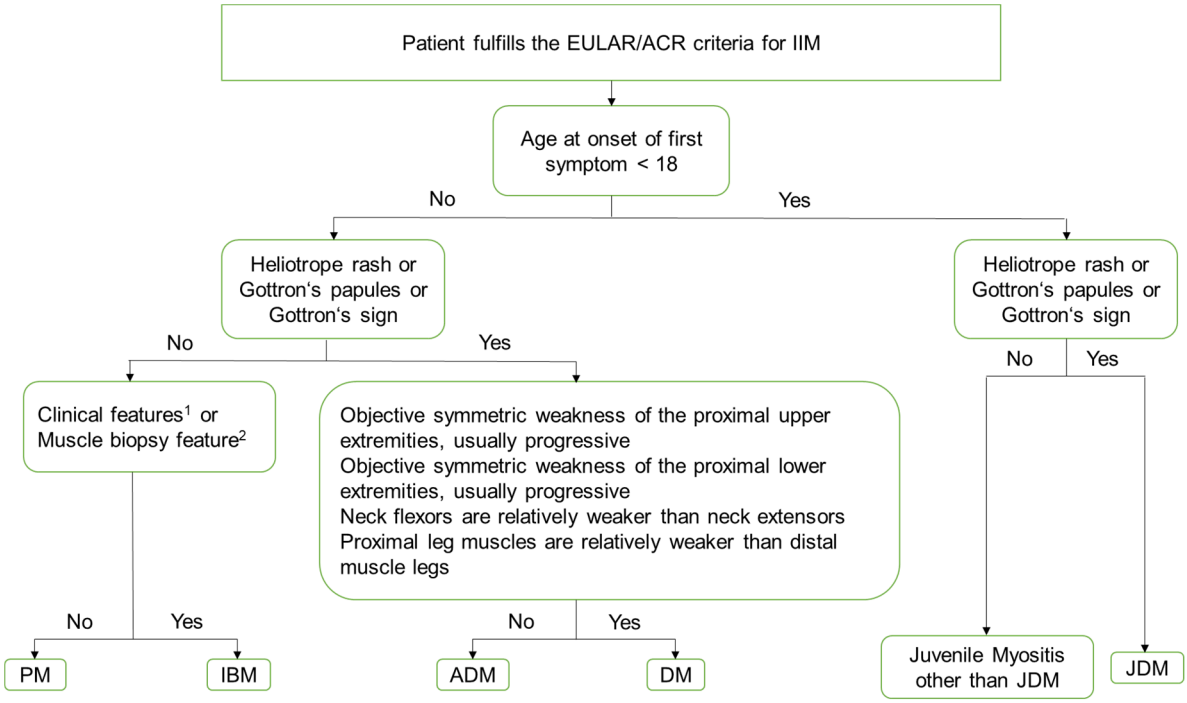

Figure 5: Classification pathway for sub-classifying IIMs. This figure is based on the information provided in Lundberg et al.(3).

[1] Finger flexor weakness and treatment response have not improved. [2]Rimmed vacuoles

Patients under the age of 18 years can be sub-classified to JDM if typical skin changes exist. Secondly, patients with an age greater equal than 18 can be sub-classified into PM, IBM, ADM or DM.(3)

2 Aim of the Thesis

The detection of myositis-specific antibodies is known as an important diagnostic method of idiopathic inflammatory myopathies. There are new commercially available line immunoassays which show a reduced specificity compared to immunoprecipitation known as the gold standard in detecting myositis-specific autoantibodies(1). However according to Ghirardello et al.(25), line blot immune assays represent good alternatives to other more time consuming methods like immunoprecipitation. Since immune precipitation techniques need to be performed in special laboratories with the required equipment and expect highly educated specialists for the interpretation of the obtained results(19,29), line immunoassays, which are commercially available and less costly in terms of time, are used at the University Hospital Graz to screen for myositis-specific and myositis-associated antibodies.

The overall aim of this study has been on defining the clinical relevance of myositis-specific/associated antibodies. Therefore, I investigated the hypothesis whether those commercially available line immunoassays are useful in the daily routine of clinicians for supporting them in the diagnostic pathway of idiopathic inflammatory myopathies. In order to prove the hypothesis, positive predictive values of each tested autoantibody for suffering from an IIM subtype, for having a positive history of autoimmunity or malignancy and finally, the PPVs for having ILD were calculated.

The second aim of the study was to examine the applicability of the new EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies. Therefore, I investigated in how many cases the output of the EULAR/ACR classification criteria confirm the clinical diagnosis myositis found in the patients' medical records.

3 Materials and Methods

3.1 Data Request & Preparation of the Data

In the first step, the main topic and the aim of the study as described in section 2 were defined. As the investigation of the clinical relevance of myositis-specific antibodies, testing the applicability of the new EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathy as well as correlations between positive antibody test results and different clinical features were defined as the primary focus of this study, I obtained a list from the immunological laboratory of the University Hospital Graz including all patients tested positive for myositis-specific and/or myositis-associated antibodies in the time period of October 2014 to October 2017. Since several patients were tested positive for the presence of MSA/MAA twice or even more often, I checked the obtained list for patients tested several times. Moreover, I modified the list in a way that patients occur just once in the file and the redundant data got deleted. Finally, I deleted all patients without sufficient data and I ended up with a sample size of n=242 as shown in Figure 6.

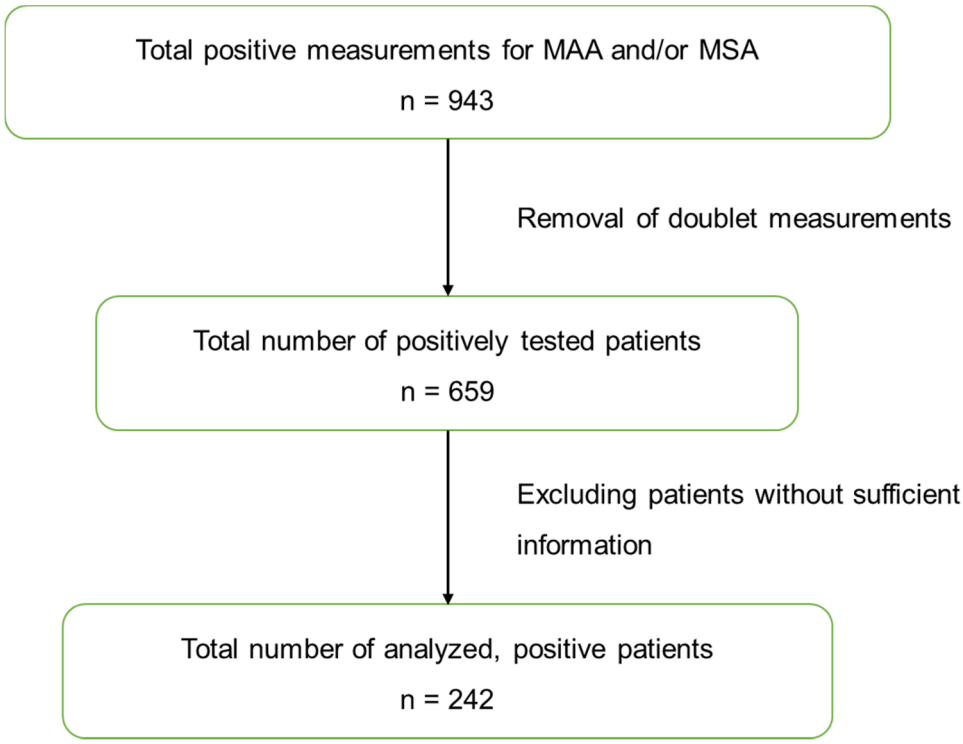

Figure 6: Illustration of how the final sample size of n=242 patients was obtained.

3.2 Definition of the Parameters of Interest

In the next step, clinical features and laboratory parameters of interest were clarified. The medical records got analyzed for the presence of the EULAR/ACR classification criteria - age of onset, muscle weakness, skin manifestations, other clinical manifestations like esophageal dysmotility or dysphagia, laboratory measurements (increased levels of CK or LDH or AST or ALT) and muscle biopsy findings. The criteria are described in more detail in chapter 1.3.6.

At the University Hospital Graz, the evaluation of muscle weakness is performed by applying a manual muscle strength test, the MMT8. Thereby, muscles of the upper extremities like the deltoid muscles, biceps muscles, extensors of the wrist, as well as muscles of the lower extremities like the quadriceps muscles and the dorsal flexors of the ankle are examined on the left and the right side.

Furthermore, the strength of the neck flexors gets investigated by performing the muscle strength test. If the muscle strength is not reduced at all, a maximum of 10 points is recorded. The more reduced the muscle strength is, the less points are achieved by the patients.(30) Hence, the various muscles which are part of the MMT8 get evaluated by using a scale from 0 (corresponding to severe weakness) to 10 (no muscle weakness), the so called Kendall scale which is shown in Table 6 (30,31).

Table 6: Kendall 10 point scale based on the table provided by Pfister et al.(30).

Test procedure	Muscle function	Score	Severity
No Movement	No visible Movement	0	Severe weakness
Test Movement	**Movement in horizontal plane**		
	Moves (partial range of motion)	1	
	Moves (complete range of motion)	2	
	Movement against gravity		
	Moves (partial range of motion)	3	
Test position	Gradual release from test position	4	Moderate weakness
	Holding test position	5	
	Holding test position against slight pressure	6	
	Holding test position against slight to moderate pressure	7	Mild weakness
	Holding test position against moderate pressure	8	
	Holding test position against moderate to strong pressure	9	
	Holding test position against strong pressure	10	No weakness

Since one aim of the study was to apply retrospectively the EULAR/ACR classification criteria for IIMs to all patients tested positive for any MSA and/or MAA, the medical records got analyzed whether muscle strength testing had been performed. If the test was performed, also the scores for the muscles tested were recorded. Furthermore, a muscle strength test score of equal to or less than seven was defined as significantly reduced muscle strength by specialists of the Rheumatology Department of the University Hospital Graz. By taking this into consideration, the muscle strength test scores could be used for the evaluation of the four muscle parameters of the EULAR/ACR classification criteria for each patient as described in Table 7.

Table 7: Muscle weakness parameters of the EULAR/ACR classification criteria for IIMs and the corresponding muscle strength score used in the study.

EULAR/ACR muscle parameters	Muscle strength score
Objective muscle weakness of the upper extremities (symmetrically)	Muscle strength score ≤7 of deltoid muscle and/or biceps muscle on the left and right side
Objective muscle weakness of the lower extremities (symmetrically)	Muscle strength ≤7 of quadriceps muscle on the left and right side
Neck flexors relatively weaker than neck extensors	Not applicable since neck extensors are not recorded in the medical records of the University Hospital Graz
Proximal leg muscles are relatively weaker than distal leg muscles	Muscle strength score of quadriceps muscles is lower than the score of the dorsal flexors

Additionally, the patients' medical records got checked whether other autoimmune diseases, malignancies or interstitial lung disease were described. If further autoimmune diseases or malignancies were detected, they got further classified and the subtypes got recorded.

3.3 Analysis of the Data

After defining all the necessary parameters of interest, a retrospective analysis of the patients' medical records was started. Jalia Mirzayeva (Erasmus student, Charles University of Prague, Faculty of Medicine in Pilsen) supported me in analyzing all the patient's medical records.

I calculated the positive predictive values (PPV) for each antibody for having a diagnosis of myositis, malignancy, another autoimmune disease and for having interstitial lung disease since the study focused on the clinical relevance of MSA and MAA. The PPV is of great importance for clinicians in evaluating a positive antibody test result of an individual as it describes how many of the people that are tested positive by a clinical test do really have the disease. One fact that has to be taken into account is that the PPV depends on the prevalence of the disease within the tested population. If the prevalence of the disease is high within the tested population, the PPV is high too. Therefore, the probability that a positive

tested individual really shows the disease is higher.(32) I calculated the positive predictive values by applying the following formula:

Equation 1: Positive Predictive Value

$$PPV = \frac{True\ positives\ (A)}{True\ positives\ (A) + False\ positives(C)} * 100\%$$

In Table 8, there are two columns which depict the actual situation of the individuals, whether they are ill or healthy. The rows show the positive and negative test results.

Table 8 Illustration of sensitivity, specificity, positive predictive value and negative predictive value based on the figure of the PennState Eberly College of Science(32).

		Truth	
		Disease Number	Healthy Number
Test result	Positive	True positive (A)	False positive (C)
	Negative	False negative (B)	True negative (D)

Further statistical parameters whose formulas can be derived based on the information given in Table 8 are sensitivity (Equation 2), specificity (Equation 3) and the negative predictive value (NPV, Equation 4) which are mentioned for completeness but cannot be calculated with the data collected in this study.

Equation 2: Sensitivity

$$Sensitivity = \frac{True\ positives\ (A)}{True\ positives\ (A) + False\ negatives(B)} * 100\%$$

Equation 3: Specificity

$$Specificity = \frac{True\ negatives\ (D)}{True\ negatives\ (D) + False\ positives(C)} * 100\%$$

Equation 4: Negative Predictive Value

$$NPV = \frac{True\ negatives\ (D)}{True\ negatives\ (D) + False\ negatives\ (B)} * 100\%$$

3.4 Statistical Analysis

By applying the statistical software IBM SPSS (Superior Performing Statistical Software) Statistics 23, the data was tested for significant correlations. The different antibodies, the age of the patients when the blood sampling was done and the actual date of blood sampling were checked for correlations. First of all, these parameters were tested whether they are normally distributed by performing the Shapiro-Wilk Test. Since the data was not normally distributed, the Spearman correlation coefficients were calculated.

4 Results

4.1 General Overview

In Table 9, a general overview is presented. On the one hand, the total number of patients tested positive for the specified antibodies in the time period of October 2014 to October 2017 is given. Since some patients were tested multiple times positive for the same antibody within the specified time period, redundant data got deleted ending up with the total number of positive patients for each antibody given in the second column. The immunological laboratory of the University Hospital Graz mainly performs antibody tests on sera handed in by internal departments like the Rheumatology, Dermatology or the Neurology Department. Furthermore, other KAGes[3] hospitals and independent physicians send patients' sera to the immunological laboratory of the University Hospital Graz in order to test for myositis-specific and myositis-associated antibodies. In the third column of Table 9, the total number of patients tested positive where the necessary data was accessible and therefore used for further analysis is shown (total number of analyzed, positive patients), including patients of the Department of Rheumatology and Immunology Graz and patients of other KAGes hospitals. Finally, for each antibody the absolute numbers of included females and males with a positive test result are illustrated, as well as the relative frequencies.

[3] KAGes is defined as „Steiermärkische Krankenanstaltengesellschaft“ (www.kages.at)

Table 9: General overview of the antibodies tested.

Antibody	Total number of positive patients	Total number of analyzed, positive patients	Number of ♀	Number of ♂	Percentage of ♀	Percentage of ♂
Jo-1	36	19	8	11	42	58
TIF1γ	43	19	15	4	79	21
MDA-5	29	13	4	9	31	69
NXP-2	22	11	6	5	55	45
SAE	11	3	1	2	33	67
PM-Scl100	52	25	11	14	44	56
PM-Scl75	162	65	33	32	51	49
PL-7	49	24	7	17	29	71
EJ	4	1	0	1	0	100
OJ	11	3	2	1	67	33
PL-12	56	27	12	15	44	56
SRP	68	33	14	19	42	58
Mi-2α	22	11	5	6	45	55
Mi-2β	46	21	9	12	43	57
Ku	48	24	14	10	58	42

4.2 Myositis-Specific/Associated-Antibodies and their Positive Predictive Values for having Idiopathic Inflammatory Myopathy

In order to be able to evaluate the clinical relevance of myositis-specific and myositis-associated antibodies, positive predictive values have been calculated as one main part of this study. As it can be seen in Table 10, positive predictive values have been calculated for various parameters, like the PPV for *clinical diagnosis*[4] of myositis, PPV for probable, definite and probable and/or definite myositis according to the EULAR/ACR classification criteria.

Table 10: Illustration of the relative frequency of IIM diagnosis for the specified antibodies and the positive predictive values for myositis.

Antibody	Total number of analyzed positive patients	PPV for clinical diagnosis[4] of myositis [%]	PPV for probable myositis [%]	PPV for definite myositis [%]	PPV for probable and/or definite myositis [%]
Jo-1	19	42	37	21	58
TIF1γ	19	11	5	5	11
MDA-5	13	23	23	0	23
NXP-2	11	27	27	0	27
SAE	3	33	33	0	33
PM-Scl100	25	16	4	4	8
PM-Scl75	65	5	5	0	5
PL-7	24	25	0	0	0
EJ	1	0	0	0	0
OJ	3	0	0	0	0
PL-12	27	4	0	0	0
SRP	33	24	6	3	9
Mi-2α	11	73	18	18	36
Mi-2β	21	24	10	5	14
Ku	24	17	4	0	4

In Figure 7 to Figure 11, the relative frequencies and the total number of patients with and without a documented clinical diagnosis are depicted. Furthermore, the different types of myositis diagnoses found within the patient groups positive for

[4] As *clinical diagnosis* IIM subtypes are summarized including PM, DM, antisynthetase-syndrome, necrotizing PM, undifferentiated. myositis, juvenile DM, overlap myositis and eosinophilic myositis.

the specified antibodies are shown. In Figure 7, it can be seen that the number of patients with a positive clinical diagnosis (eight patients) is not equal to the number of clinical diagnoses found within that patient group (nine documented clinical diagnoses). The explanation for this discrepancy is that there was one patient with two subtypes of IIM diagnosed.

In case of anti-Jo1 (Figure 7) and anti-Mi2α (Figure 8), the results are illustrated in form of pie diagrams since the patient groups positive for those two antibodies show a high relative frequency of having a clinical diagnosis documented. Furthermore, for both antibodies the IIM diagnoses are depicted.

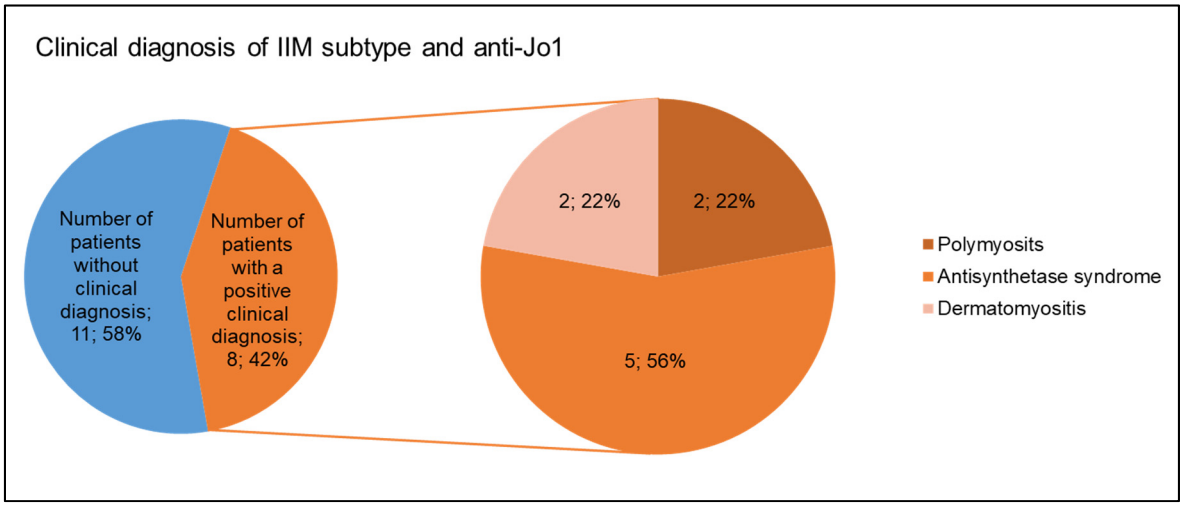

Figure 7: Illustrating the number of patients positive for anti-Jo1 without and with a documented clinical diagnosis. Furthermore, the IIM diagnoses present in this patient group are listed.

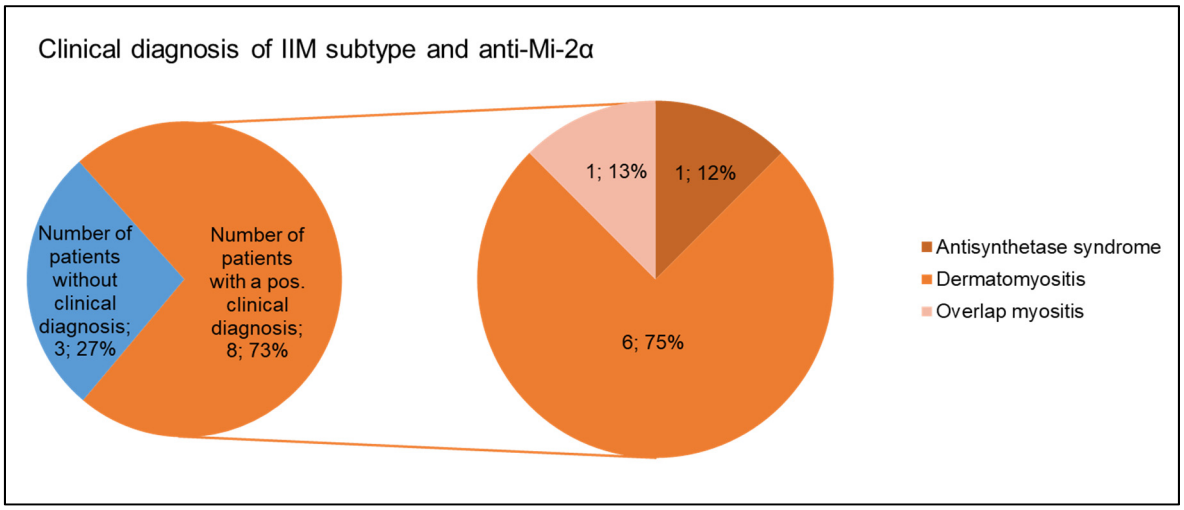

Figure 8: Illustrating the number of patients positive for anti-Mi-2α without and with a documented clinical diagnosis. Furthermore, the IIM diagnoses present in this patient group are listed.

Approximately one quarter of patients with a positive antibody test result for anti-Mi-2β have an IIM diagnosis documented in their medical records. However, all of those patients have DM diagnosed as it can be observed in Figure 9.

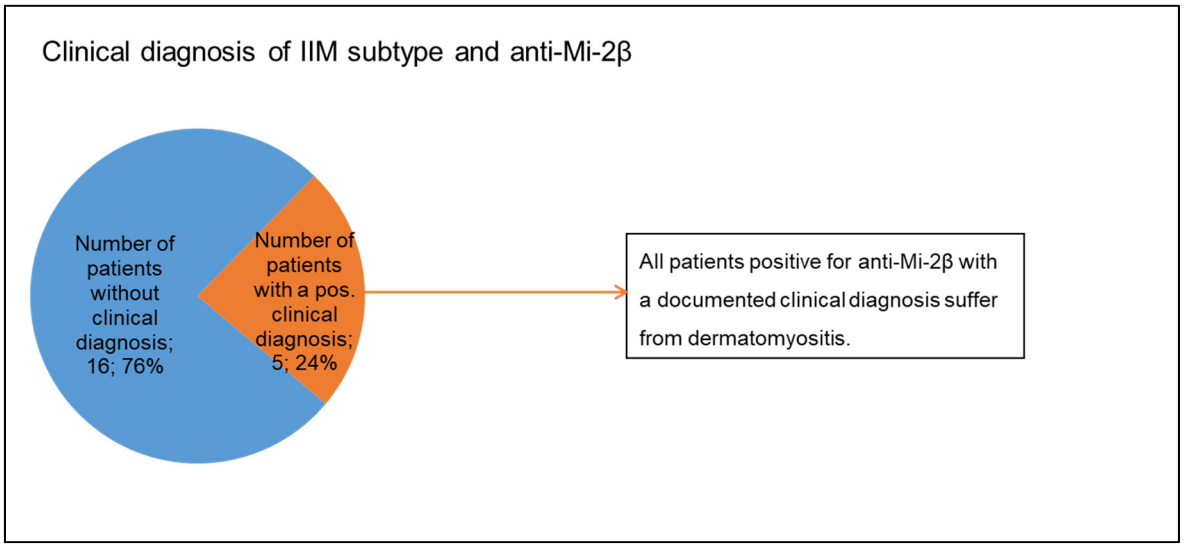

Figure 9: Illustrating the number of patients positive for anti-Mi-2β without and with a documented clinical diagnosis.

Remarkably, 75% of patients with a positive anti-PL-7 titer do not have an IIM diagnosed whereas 25% show some subtype of IIM. Interestingly, five out of six patients (83%) with a positive PL-7 antibody test result have the antiphospholipid-syndrome listed in their medical records.

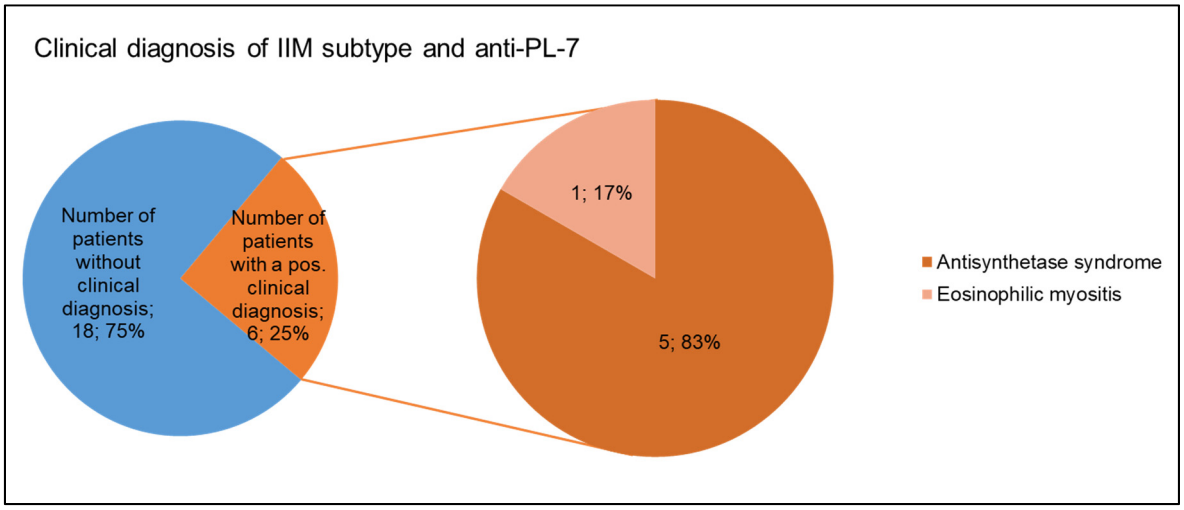

Figure 10: Illustrating the number of patients positive for anti-PL-7 without and with a documented clinical diagnosis. Furthermore, the IIM diagnoses present in this patient group are listed.

Similar to anti-Mi2β and anti-PL-7, approximately one quarter of patients tested positive for anti-SRP have an IIM diagnosis documented in the past. Notably, only in one patient out of 6 patients with a positive medical history concerning IIM, necrotizing autoimmune myopathy has been identified as it can be observed in Figure 11.

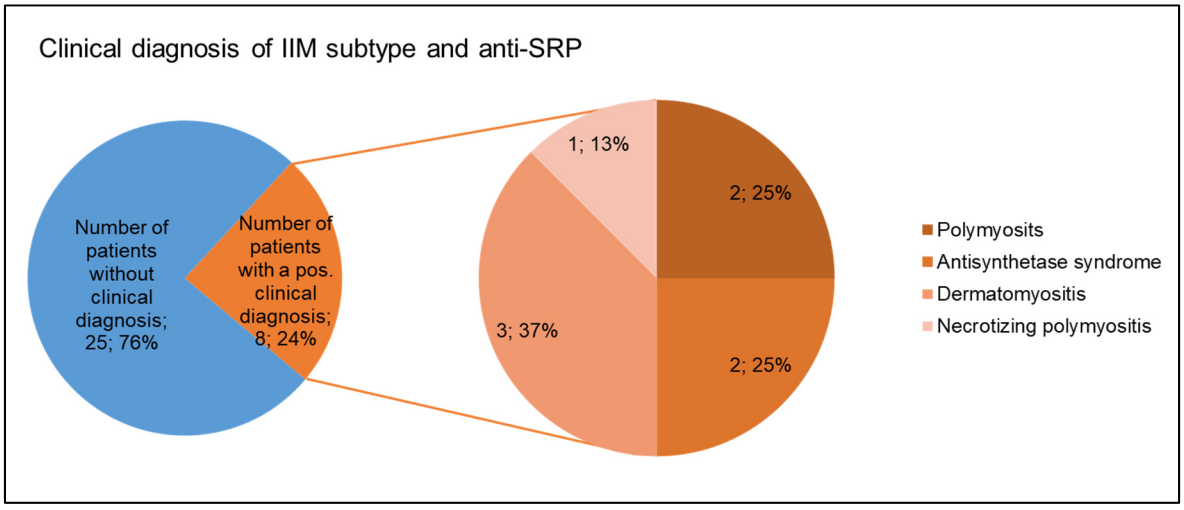

Figure 11: Illustrating the number of patients positive for anti-SRP without and with a documented clinical diagnosis. Furthermore, the IIM diagnoses present in this patient group are listed.

4.3 Myositis-Specific/Associated-Antibodies and the Occurrence of Malignancies

Since connections between positive antibody test results and the occurrence of malignant diseases have been reported(11,22), both the absolute number and the relative frequencies of negative and positive history of malignancy were calculated and the results of those calculations are depicted in Table 11. Furthermore, the positive predictive values for malignancy are shown in Table 11.

Table 11: Overview of the frequencies of malignant diseases in positive tested patients for a specific antibody.

Antibody	Total number of analyzed positive patients	Patients with negative history of malignancy (relative frequency [%])	Patients with positive history of malignancy (relative frequency [%])	PPV for malignancy [%]
Jo-1	19	15 (79)	4 (21)	21
TIF1γ	19	18 (95)	1 (5)	5
MDA-5	13	11 (85)	2 (15)	15
NXP-2	11	10 (91)	1 (9)	9
SAE	3	2 (67)	1 (33)	33
PM-Scl100	25	20 (80)	5 (20)	20
PM-Scl75	65	61 (94)	4 (6)	6
PL-7	24	21 (88)	3 (13)	13
EJ	1	1 (100)	0 (0)	0
OJ	3	3 (100)	0 (0)	0
PL-12	27	25 (93)	2 (7)	7
SRP	33	29 (88)	4 (12)	12
Mi-2α	11	10 (91)	1 (9)	9
Mi-2β	21	17 (81)	4 (19)	19
Ku	24	21 (88)	3 (13)	13

The highest positive predictive values for having a malignant disease are obtained for anti-Jo1 (21%), anti-PM-Scl-100 (20%) and anti-Mi2β (19%). In the following figures (Figure 12, Figure 13 and Figure 14), the different malignancies occurring in those patient groups with a positive antibody titer for anti-Jo 1, anti-PM-Scl-100 and anti-Mi2β are listed. Since a few patients with positive antibody titers have more than one malignant diseases, the number of patients with a positive history of malignancy is not equal to the number of malignant tumors found within the patient groups (see Figure 12 to Figure 14).

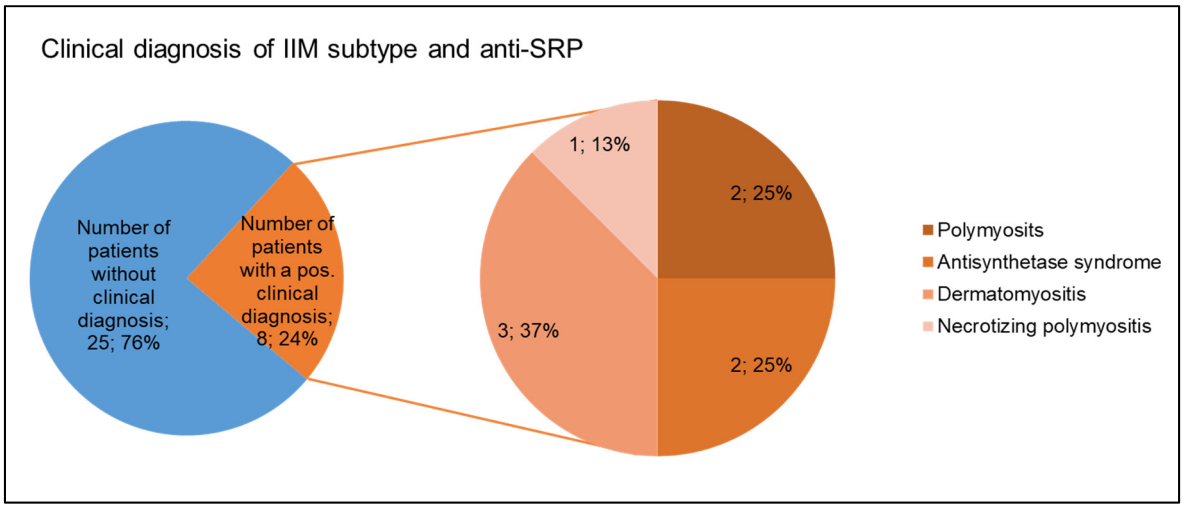

Figure 12: Illustrating the number of patients positive for anti-Jo1 without and with a malignant disease. Furthermore, the malignancies present in this patient group are listed.

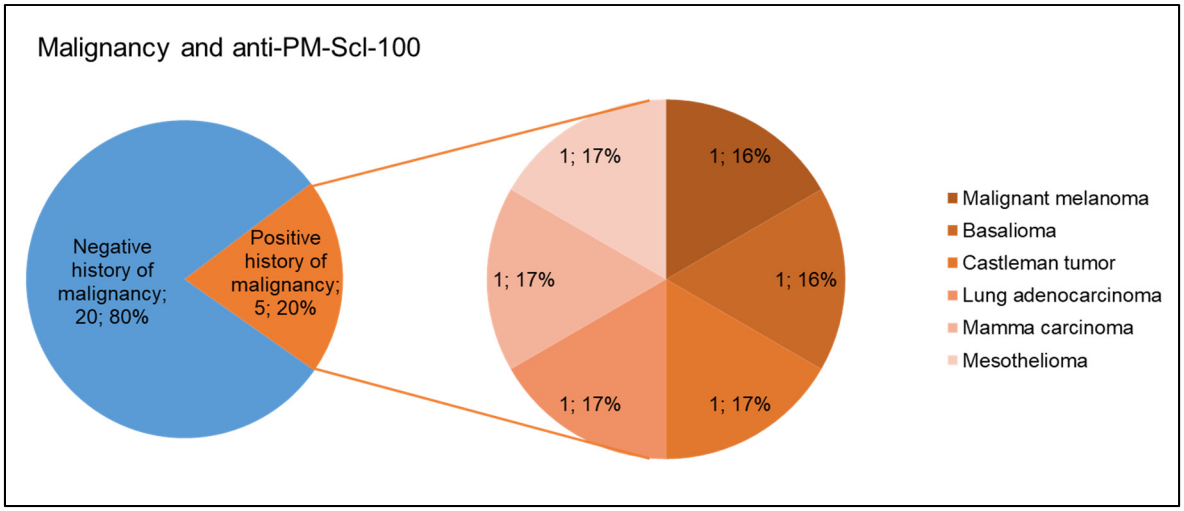

Figure 13: Illustrating the number of patients positive for anti-PM-Scl-100 without and with a malignant disease. Furthermore, the malignancies present in this patient group are listed.

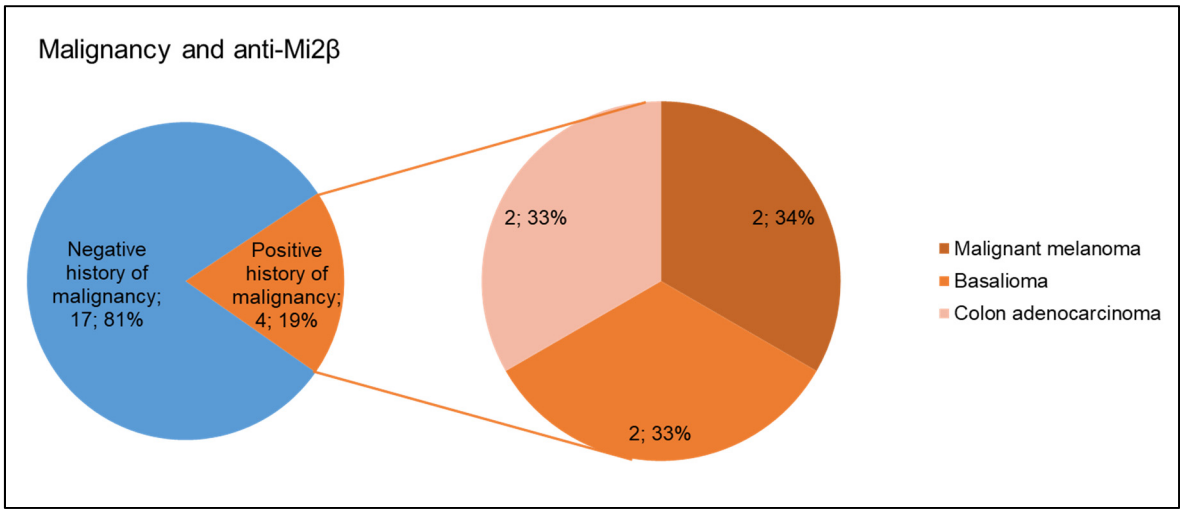

Figure 14: Illustrating the number of patients positive for anti-Mi2β without and with a malignant disease. Furthermore, the malignancies present in this patient group are listed.

4.4 Myositis-Specific/Associated-Antibodies and the Occurrence of Autoimmune Diseases

In Table 12, the absolute numbers as well as the relative frequencies of patients with a positive and negative medical history concerning autoimmune diseases are shown.

Table 12: Overview of the presence of autoimmune diseases other than myositis in positive tested patients.

Antibody	Total number of analyzed positive patients	Number of patients with no history of AI (relative frequency [%])	Number of patients with history of AI (relative frequency [%])	PPV for AI [%]
Jo-1	19	15 (79)	4 (21)	21
TIF1γ	19	10 (53)	9 (47)	47
MDA-5	13	12 (92)	1 (8)	8
NXP-2	11	10 (91)	1 (9)	9
SAE	3	3 (100)	0 (0)	0
PM-Scl100	25	12 48)	13 (52)	52
PM-Scl75	65	39 (60)	26 (40)	40
PL-7	24	13 (54)	11 (46)	46
EJ	1	1 (100)	0 (0)	0
OJ	3	2 (67)	1 (33)	33
PL-12	27	20 (74)	7 (26)	26
SRP	33	27 (82)	6 (18)	18
Mi-2α	11	8 (73)	3 (27)	27
Mi-2β	21	12 (57)	9 (43)	43
Ku	24	16 (67)	8 (33)	33

In column five of Table 12, the PPVs of each antibody for having another autoimmune disease are depicted. The PPVs are rather low except the positive predictive values of anti-TIF1γ, anti-PM-Scl100, anti-PM-Scl75, anti-PL-7 and anti-Mi-2β. For those antibodies with a PPV greater than or equal to 40, the relative frequencies and the absolute number of patients with a positive and negative history of autoimmunity are illustrated in the following figures.

Furthermore, the different autoimmune diseases and also the absolute numbers as well as the relative frequencies of the specific autoimmune diseases that are identified within the patients tested positive for the specific antibodies are shown in

the following illustrations (Figure 15 to Figure 20). As already explained above in sections 4.2 and 4.3, the reason for the difference in the number of patients with a positive history of autoimmunity and the number of diagnosed autoimmune diseases is, that in some cases one patient was diagnosed with two subtypes (see Figure 15 to Figure 20).

In Figure 15 all autoimmune diseases within the patient group positive for anti-TIF1γ antibody are shown. Hashimoto thyroiditis and subacute cutaneous lupus erythematosus are the two most common autoimmune disorders.

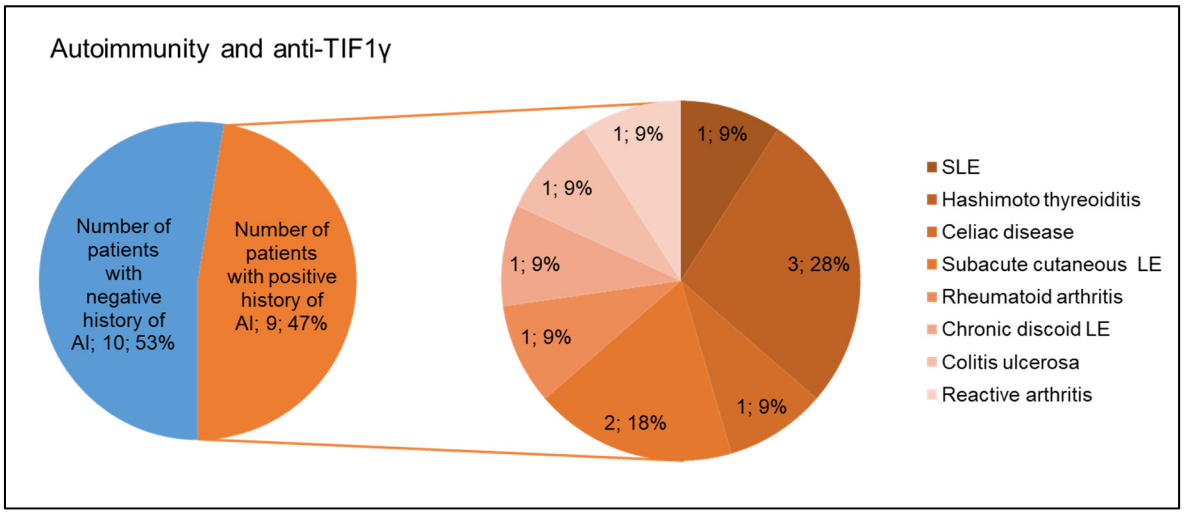

Figure 15: Illustration of autoimmune diseases present in patients positive for anti-TIF1γ.

Figure 16 illustrates how many patients positive for anti-PM-Scl100 actually have an autoimmune disease diagnosed and furthermore, the various types of autoimmune disorders are listed. SLE, polymyalgia rheumatica and systemic sclerosis are the three most common diagnoses within the patients positive for anti-PM-Scl100.

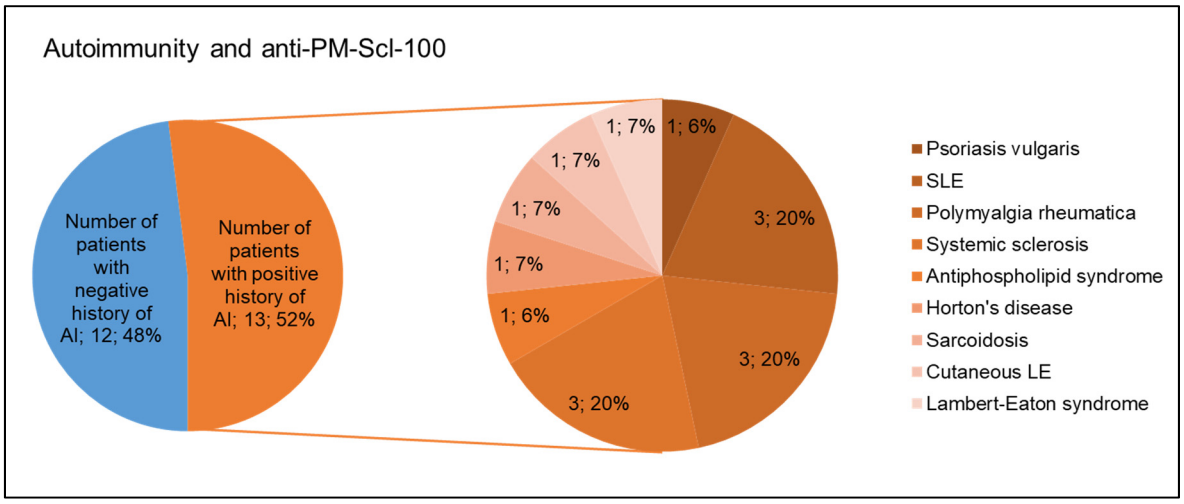

Figure 16: Illustration of autoimmune diseases present in patients positive for anti-PM-Scl-100.

The following Figure 17 depicts the percentage of patients positive for anti-PM-Scl-75 with a positive and negative history of autoimmunity. Moreover, all autoimmune diagnoses found within this patient group are listed and it can be observed that 20% (a total number of 6 patients out of 26) suffer from SLE.

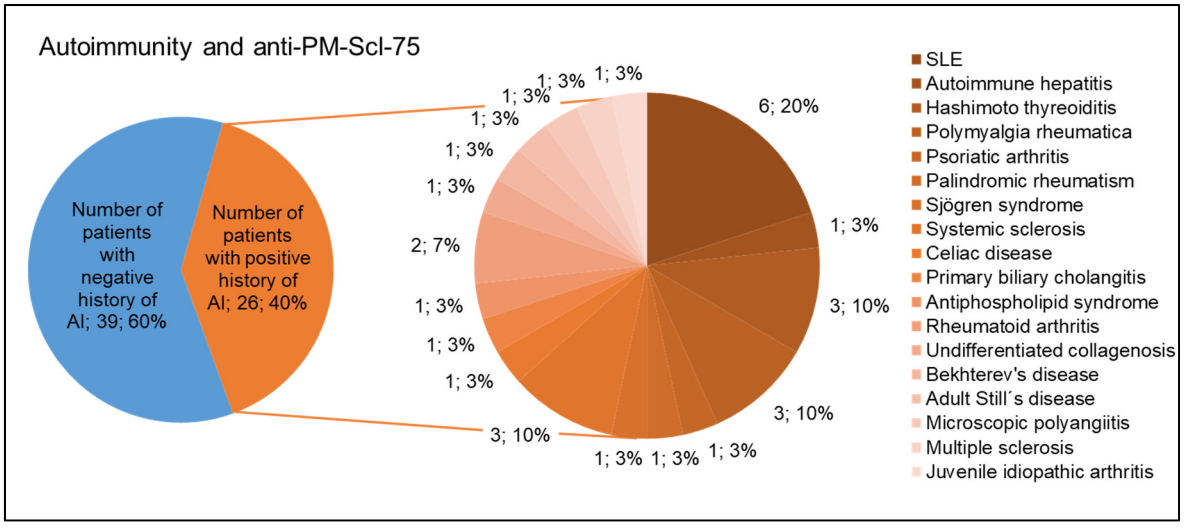

Figure 17: Illustration of autoimmune diseases present in patients positive for anti-PM-Scl75.

In Figure 18, it can be seen that 46% of the patients positive tested for anti-PL-7 suffer from an autoimmune disorder. Obviously, the relative frequency of autoimmunity is high in this patient group, but no specific autoimmune disease was recorded more than once. There are just isolated cases of autoimmune diseases within these patients.

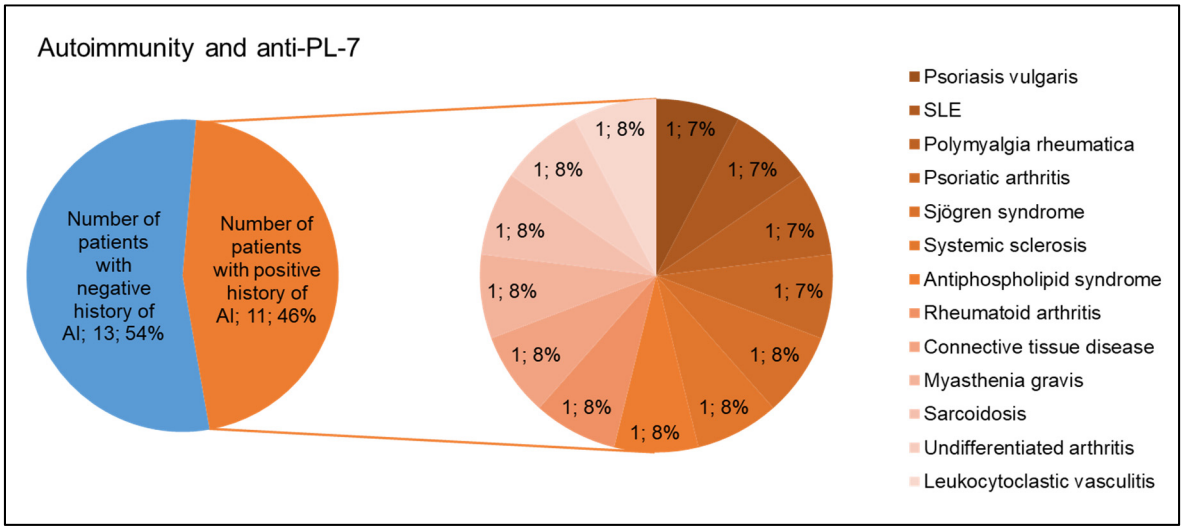

Figure 18: Illustration of autoimmune diseases present in patients positive for anti-PL-7.

Figure 19 illustrates the number of patients positive for anti-Mi-2β with another autoimmune disease than myositis. Rheumatoid arthritis, psoriasis vulgaris and Hashimoto thyroiditis are most commonly detected in this patient group.

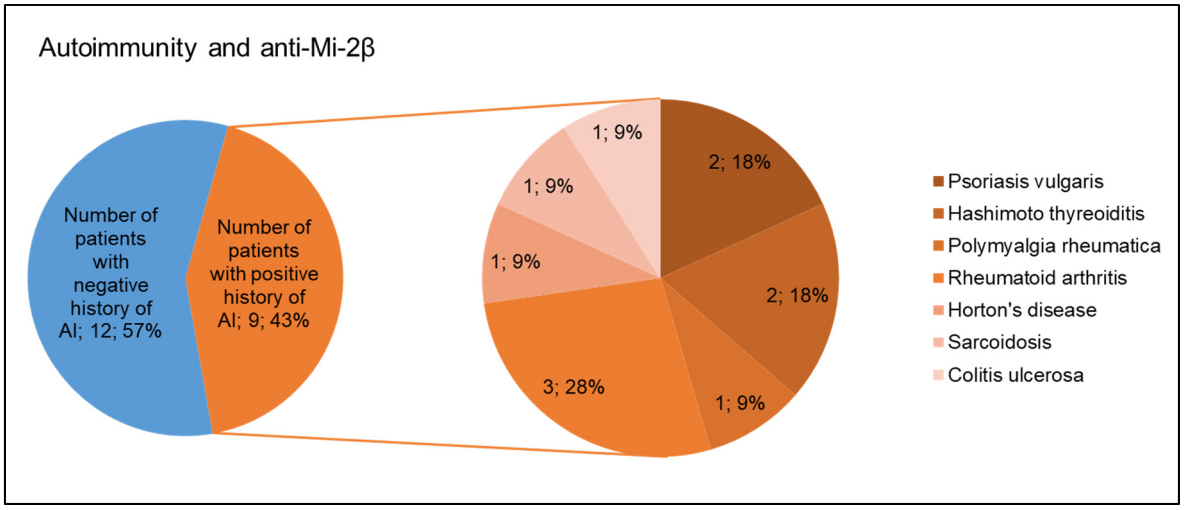

Figure 19: Illustration of autoimmune diseases present in patients positive for anti-Mi-2β.

The calculated PPV of anti-Ku is 33% and obviously lower than 40. Nevertheless, also for the autoantibody anti-Ku the different autoimmune diseases present in patients tested positive for anti-Ku are depicted in Figure 20 since the majority of those patients suffer from SLE. Within the patient group positive for anti-Ku antibody, six out of eight patients with an autoimmune disease suffer from SLE

(46%), followed by antiphospholipid-syndrome (23%) and scleroderma (15%) as shown in Figure 20.

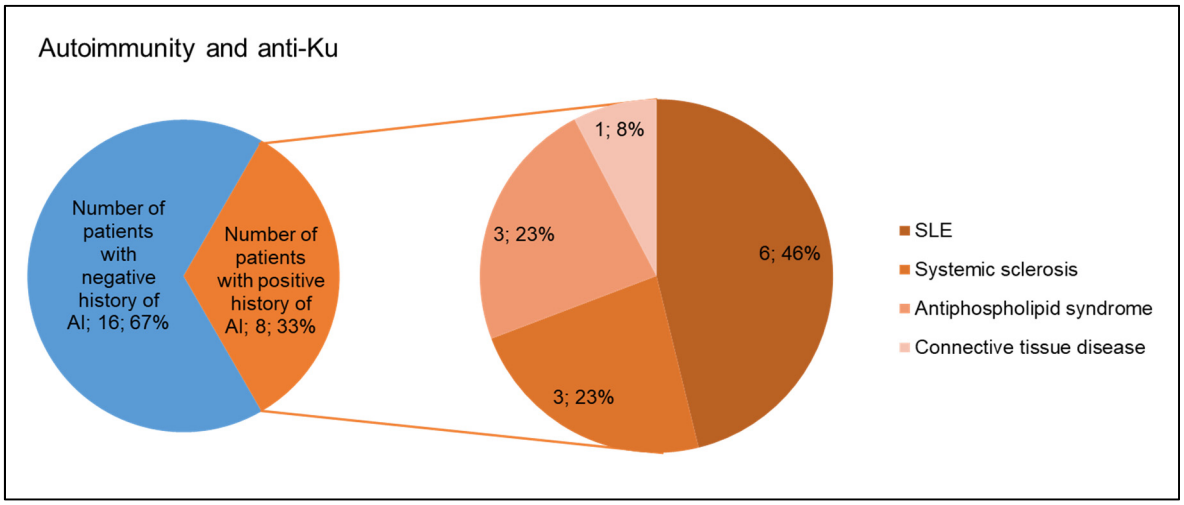

Figure 20: Illustration of autoimmune diseases present in patients positive for anti-Ku.

4.5 Myositis-Specific/Associated-Antibodies and the Occurrence of Interstitial Lung Disease

Finally, the collected data was also analyzed concerning whether the tested antibodies are indicators for the development of interstitial lung disease. Therefore, the absolute numbers of patients suffering from ILD and patients without lung involvement as well as the corresponding relative frequencies were calculated as shown in Table 13. Moreover, the positive predictive value for ILD for each antibody has been determined.

Table 13: Overview of the frequencies of ILD within the patient group tested positive for a specific antibody.

Antibody	Total number of analyzed positive patients	Absolute number of patients without ILD (relative frequency [%])	Absolute number of patients with ILD (relative frequency [%])	PPV for ILD [%]
Jo-1	19	15 (79)	4 (21)	21
TIF1γ	19	19 (100)	0 (0)	0
MDA-5	13	13 (100)	0 (0)	0
NXP-2	11	10 (91)	1 (9)	9
SAE	3	3 (100)	0 (0)	0
PM-Scl100	25	25 (100)	0 (0)	0
PM-Scl75	65	61 (94)	4 (6)	6
PL-7	24	18 (75)	6 (25)	25
EJ	1	1 (100)	0 (0)	0
OJ	3	3 (100)	0 (0)	0
PL-12	27	23 (85)	4 (15)	15
SRP	33	29 (88)	4 (12)	12
Mi-2α	11	10 (91)	1 (9)	9
Mi-2β	21	19 (90)	2 (10)	10
Ku	24	23 (96)	1 (4)	4

ILD was present in few patients. The highest relative frequency of ILD was found within the patients with a positive anti-PL-7 (25%) and anti-Jo1 (21%) antibody detection test. ILD is described as a relevant complication of idiopathic inflammatory myositis and the prevalence within IIM patients is reported to be 40%(2). 39 patients out of 242 have a documented diagnosis of IIM out of which eight patients suffer from ILD. Therefore, the prevalence of ILD in IIM patients in this study is 21%.

4.6 Correlations

Only one significant correlation between the anti-SAE antibody and the anti-MDA5 antibody within the normal range could be detected as shown in Figure 21.

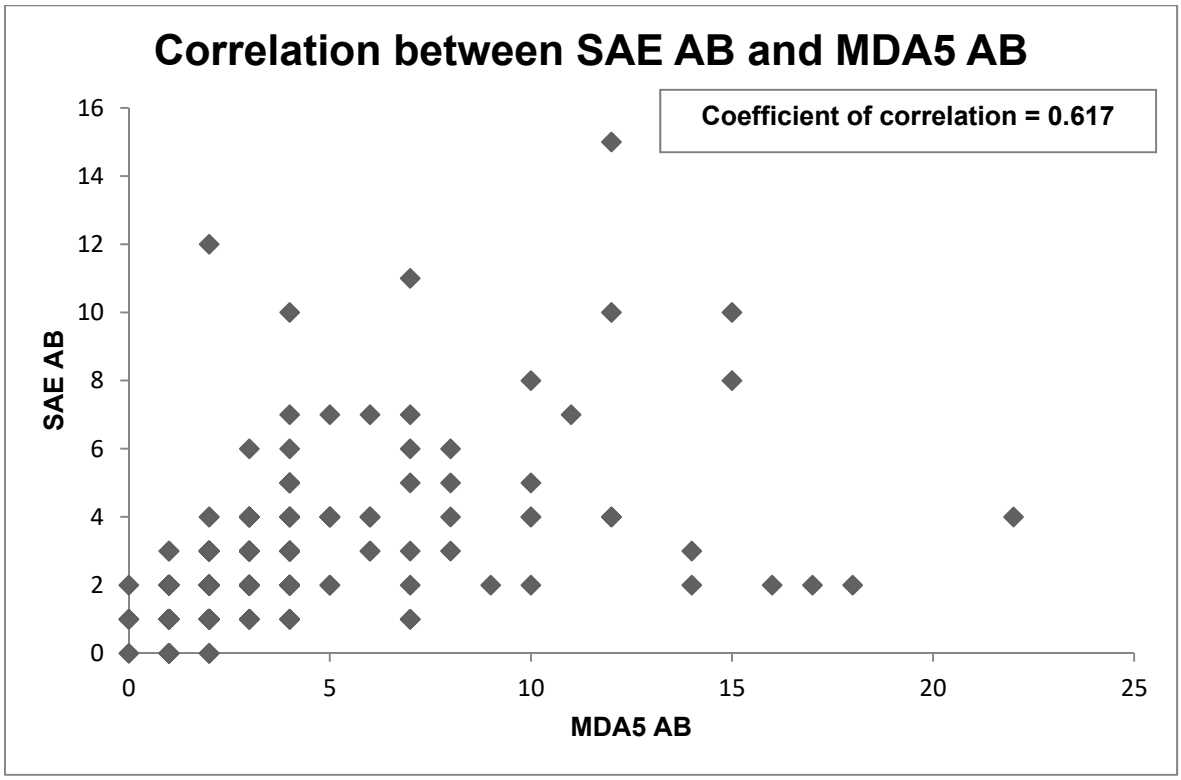

Figure 21: Illustrating the correlation between MDA-5 and SAE antibody.

4.7 Application of the EULAR/ACR Classification Criteria for Adult and Juvenile Idiopathic Inflammatory Myopathies

By applying the EULAR/ACR classification criteria as described in section 1.3.6, seven out of 242 autoantibody positive patients were described as “Definite IIM” and a total number of 15 patients ended up with “Probable IIM” as it can be seen in Figure 22. Moreover, it can be observed that in six out of the seven patients classified as “Definite IIM” a clinical diagnosis is documented in the patient’s medical records. To put it into different words, there is only one patient classified as “Definite IIM” without a clinical diagnosis. By taking the patients classified as “Probable IIM” into consideration, ten out of 15 patients have a clinical diagnosis recorded in the medical record while five patients do not show a myositis subtype diagnosed. Finally, 220 patients are classified as “No IIM”. 197 patients out of

those 220 in fact do not have a clinical diagnosis documented in the medical record, but the remaining 23 patients in fact have IIM subtypes as diagnosis.

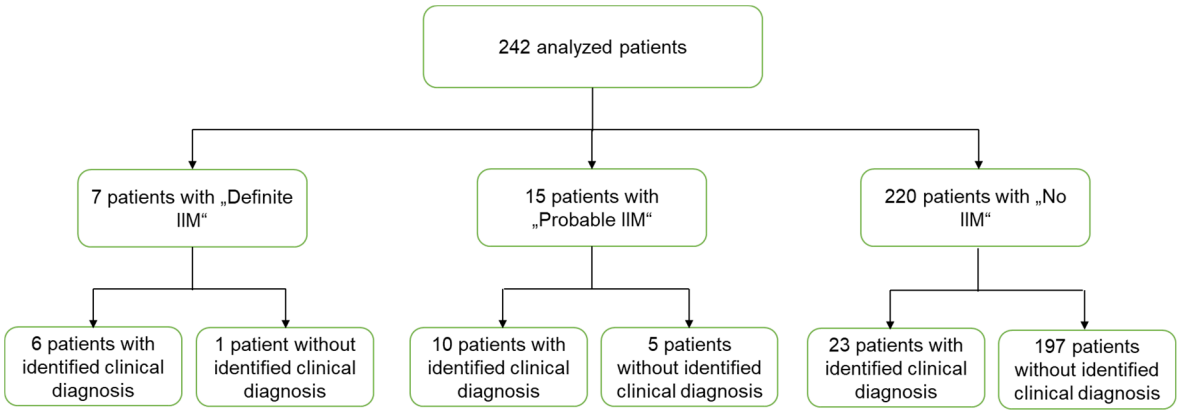

Figure 22: Illustration of how many patients are classified as "Definite IIM", "Probable IIM" and "No IIM" by using the EULAR/ACR classification criteria.

Moreover, by analyzing Figure 22, it can be said that in 213 patients (88%) out of 242, the output of the EULAR/ACR criteria web calculator represents the actual situation concerning existing or missing IIM diagnosis. In 12% of the patients (29 out of 242), the EULAR/ACR criteria do not correlate with the actual existing or missing IIM diagnosis.

Furthermore, by taking a closer look at the seven patients classified as “Definite IIM”, it can be seen that one obvious difference between the six patients with documented IIM diagnosis in the patients’ medical records and the one patient without a determined IIM diagnosis exists. That is whether a muscle biopsy was performed or not. Those six patients classified as “Definite IIM” and having a clinical diagnosis documented in the medical record have all had a muscle biopsy performed and in all patients the muscle biopsy was positive for myositis associated muscular changes. While in the case of the one patient classified as “Definite IIM” without an IIM diagnosis, no muscle biopsy was performed. Another interesting observation is that four out of these six patients with an IIM diagnosis and classified as “Definite IIM” have DM clinically diagnosed. However, two of those four DM patients are sub-classified as PM by the EULAR/ACR classification criteria. Interestingly, in those two cases no skin changes are described in the medical records.

In 39 patients a clinical diagnosis of an IIM subtype was recorded in the patients' medical records. In Figure 23 the different clinical diagnosis identified are shown and in brackets the total number of patients for each diagnosis is given. Furthermore, for each clinical diagnosis the number of patients with "Definite IIM", "Probable IIM" and "No IIM" is specified.

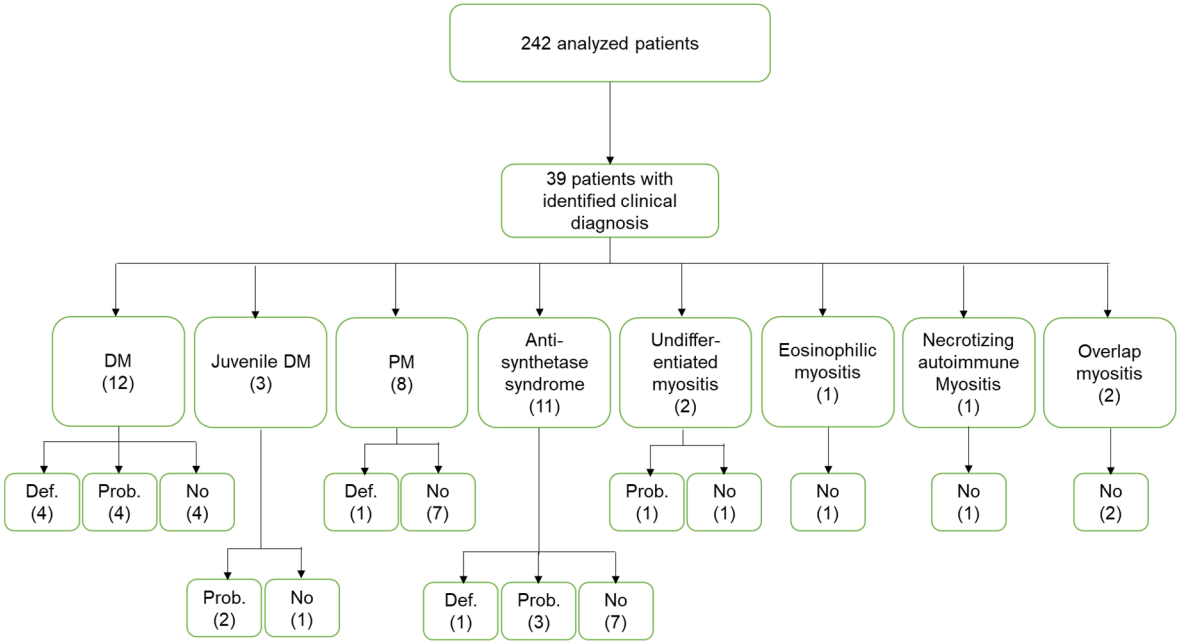

Figure 23: Identified clinical diagnosis.

5 Discussion

5.1 PPV for having IIM

The positive predictive values for having an idiopathic inflammatory myopathy within the 242 analyzed patients tested positive for myositis-specific and/or myositis-associated antibodies are rather low except the PPVs for the antibodies anti-Jo1 with a PPV of 42% and anti-Mi2α with a PPV calculated of 73%. According to these results, the probability that patients with a positive anti-Jo1 titer suffer from idiopathic inflammatory myopathy is 42%. The probability for patients tested positive for anti-Mi2α for actually having IIM is 73%. Hence, if a patient has positive anti-Jo1 or anti-Mi2α titers, the likelyhood of having an IIM subtype is higher than for all other antibodies as it can be seen in Table 10. The higher the PPV is, the more reasonable and important it is to perform further exploration of the patient. Since positive predictive values get influenced by the actual prevalence of the disease within the tested population(32), it makes sense that we obtained rather low PPVs as idiopathic inflammatory myopathies represent a group of rare diseases(4).

The PPV of anti-Jo1 for having the clinical diagnosis of myositis is 42%, but when applying the EULAR/ACR classification criteria, the PPV of anti-Jo1 for probable and/or definite myositis increases from 42% to 58%. An explanation for the increase in the positive predictive value for anti-Jo1 (42% → 58%) when using the EULAR/ACR classification criteria could be that the anti-Jo1 antibody is the only myositis-specific autoantibody that is part of these new classification criteria. Hence, patients that have a positive anti-Jo1 titer achieve a higher score by applying the EULAR/ACR criteria than patients that are positive for other autoantibodies. Therefore, people with a positive anti-Jo1 titer are more likely to be classified as IIM than other patients.

The PPV of anti-Mi2α for having the clinical diagnosis of myositis is 73%, but when applying the EULAR/ACR classification criteria to the patient group tested positive for anti-Mi2α, the PPV gets reduced from 73 to 36%, which means that the probability for really suffering from idiopathic inflammatory myopathy is nearly halved. The decrease in positive predictive values of anti-Mi2α when applying the

EULAR/ACR classification criteria can be again explained by the fact that only anti-Jo1 is part of the criteria while anti-Mi2α and all the other MSAs and MAAs discussed in the thesis are not part of the criteria. Therefore, patients with a positive autoantibody titer different to anti-Jo1 get a lower score by applying the EULAR/ACR classification criteria and apparently, the probability for having myositis, although a positive antibody titer is present, is reduced.

5.2 Positive Antibody Titers and Malignancy

Anti-TIF1γ and anti-NXP2 are typically associated with malignant diseases(11,22). By analyzing Table 11, it can be seen that the relative frequencies of patients with a positive history of malignancy are rather low. The highest value is obtained for anti-SAE (relative frequency of 33%), but it is important to note that in total only three patients positive for anti-SAE underwent analysis. Therefore, the sample size is too small in order to be able to make a valid statement whether this antibody is associated with malignancy or not. Furthermore, the relative frequencies of a positive history of malignancy within the patients tested positive for anti-TIF1γ and anti-NXP2 are even lower. Hence, in this study there is no obvious accumulation of malignant diseases within anti-TIF1γ and anti-NXP2 positive patients which is contradictory to the literature findings. An explanation for this discrepancy could be that the sample sizes for each antibody are too small.

Moreover, the PPVs for having a malignant disease of these antibodies seem to be rather low which means that according to the calculated PPVs the probability for having a malignant tumor when having a positive antibody titer is small. The highest PPVs were obtained for anti-Jo1 (21%), anti-PMScl-100 (20%) and anti-Mi2β (19%). Since the number of patients tested positive for those antibodies vary from 19 to 25 patients, the comparison of the calculated PPVs is reasonable.

Since the prostate specific antigen (PSA) is known as a tumor marker for prostate carcinoma, I checked the literature for the positive predictive value of PSA for having a prostate carcinoma. The PPV of PSA depends on the PSA serum level. Within a range of 4.0 to 10ng/ml PSA, the PPV reported in literature vary from 25 to 30% and above 10ng/ml the PPV of PSA for having a prostate carcinoma varies from 42 to 65%.(33) By comparing the positive predictive values of anti-Jo1, anti-

PMScl-100 and anti-Mi2β to the PPV of PSA within the range of 4.0 to 10ng/ml, it can be seen that the PPVs show similar values. Hence, I conclude that it would make sense to investigate those antibodies in more detail in the context of malignancy. Maybe it will be possible in future to use those autoantibodies as predictors for the occurrence of malignant diseases.

5.3 Positive Antibody Titers and Autoimmunity

The positive predictive values for having another autoimmune disease diagnosed while showing a positive antibody titer were calculated as it can be observed in Table 12. For anti-TIF1γ, anti-PMScl-100, anti-PMScl-75, anti-PL-7 and anti-Mi2β PPVs equal to or greater than 40% were obtained. The highest PPV of 52% was obtained for anti-PMScl-100, which means that the probability for another autoimmune disease in patients with a positive anti-PMScl-100 titer is 52%, a fifty-fifty chance.

Anti-PMScl-100 and anti-PMScl-75 are so-called myositis-associated antibodies and can be detected also in other diseases based on autoimmune processes(11,19). According to Tieu et al.(11), anti-PMScl autoantibodies are described to be mainly found in patients with polymyositis, systemic sclerosis and PM/SSc overlap syndromes. By taking Figure 16 and Figure 17 into consideration, it can be seen that the patients positive for one of the above mentioned antibodies show a big diversity in diagnosed autoimmune diseases. Although 20% of patients with a positive anti-PMScl-100 titer and a positive history of autoimmunity show the clinical diagnosis of systemic sclerosis, autoimmune diseases like SLE and polymyalgia rheumatica both show as well a relative frequency of 20% within that patient group. In total nine different autoimmune diseases are diagnosed within the patient group with a positive anti-PMScl-100 titer (Figure 16). The patients tested positive for anti-PMScl-75 and having an autoimmune disease diagnosed, show even a higher diversity of autoimmune diseases. In total 18 different autoimmune diseases are present within the described patient group (Figure 17). Again systemic sclerosis can be found within the patients that are positive for anti-PMScl-75 (relative frequency of 10%, 3 out of 26 patients), but predominantly SLE (relative frequency of 20%, 6 out of 26 patients) is present, followed by Hashimoto thyroiditis and polymyalgia rheumatica both with a relative frequency of 10%. Muro

et al.(34) described one patient with a positive anti-PMScl antibody titer with Sjögren's syndrome. Also in this study, one patient tested positive for anti-PMScl-75 has Sjögren's syndrome diagnosed. The study by Muro et al.(34) on a Japanese patient cohort shows in general that anti-PMScl autoantibodies are not necessarily specific for DM and/or SSc, but these antibodies can be also detected in different types of other autoimmune diseases, most prevalently found in undifferentiated connective tissue diseases (UCTD)[5]. According to Muro et al.(34), further studies on larger patient groups need to be conducted for the evaluation of the clinical relevance of anti-PMScl autoantibodies in the context of autoimmune diseases. Maybe those two antibodies, anti-PMScl-75 and anti-PMScl-100, can be identified as indicators for the presence of specific autoimmune diseases like SLE or polymyalgia rheumatica in future.
Furthermore, the relative frequency of having an IIM diagnosed within the patient group positive for anti-PMScl-100 is 16% and for anti-PMScl-75 the relative frequency is 5% as it can be seen in Table 10. Therefore, their description as myositis-associated antibodies fits to our findings since they are rather low specific for IIM and they are also found in patients with other autoimmune diseases than IIM(11,19).

As anti-PMScl-100 and anti-PMScl-75 autoantibodies, also anti-Ku antibodies belong to the group of myositis-associated antibodies and are reported to be found also in other connective tissue diseases like systemic sclerosis or systemic lupus erythematosus(19). In Figure 20, it is shown that 33% of patients with a positive anti-Ku titer (8 out of 24 patients) have a positive history of autoimmunity. In all patients tested positive for anti-Ku antibody and having a positive history of autoimmunity, subtypes of connective tissue diseases(35) were diagnosed. The most frequent CTDs found in these patients are SLE, followed by the antiphospholipid syndrome, systemic sclerosis and undifferentiated connective tissue disease. These findings confirm the definition of MAAs(11,19). The accumulation of SLE diagnosis that can be observed within the patients tested

[5] Undifferentiated connective tissue diseases are defined as conditions with signs and symptoms of systemic autoimmune diseases, but UCTDs do not fulfill the classification criteria for specific CTDs like for example SLE, SS, RA etc.(40).

positive for anti-Ku antibody, similar to the findings for anti-PMScl-75/100 antibodies, is remarkable and confirm the findings described by Cavazzana et al.(19).

Anti-TIF1γ, anti-PL-7 and anti-Mi2β are per definition MSAs and should be nearly solely found in IIM patients(19). However, the patients with positive antibody titers for these three antibodies show high relative frequencies of autoimmune diseases other than myositis as it is depicted in Figure 15, Figure 18 and Figure 19 (those relative frequencies can be compared since the sample size for each antibody is similar ranging from 19 to 25 patients). Furthermore, by taking a closer look at the relative frequencies of IIM diagnosis within those patient groups positive for the above mentioned antibodies (Table 10), it can be observed that the relative frequencies of IIM diagnosis are for each of these three antibodies lower than the relative frequencies of other autoimmune diseases than myositis. These findings are contradictory to the definition of MSAs(19).

5.4 Positive Antibody Titers and ILD

ILD is described as a relevant complication of IIMs and the reported prevalence within IIM patients is reported to be as high as 40%(2). However, the calculated prevalence within IIM patients in this study is almost half compared to the prevalence reported in literature. In this study a prevalence of 21% was observed. One possible explanation for this discrepancy could be that sometimes the diagnosis *Interstitial Lung Disease* is not documented and reported properly as “ILD” or “Interstitial Lung Disease” in the patients’ medical records. In some cases, although lung affection is described, it is not further specified which type of lung affection is present. I only included patients with documented diagnosis of interstitial lung disease in the patients’ medical records.
Interstitial lung disease was found in relatively few patients. The highest relative frequency of ILD (25%) was found in patients positive for anti-PL-7. Furthermore, also the PPVs for having ILD are low which means that the likelyhood of having ILD when a positive antibody test result is available is low as it can be seen in Table 13. The highest PPV was obtained for anti-PL-7 (25%), followed by the PPV for anti-Jo1 (21%), anti-PL-12 (15%) and the PPV for anti-SRP (12%). The prevalence of ILD within IIM patients obtained in this study is rather low. Hence it

also makes sense that the PPVs are low as the positive predictive value gets influenced by the prevalence. If the prevalence of a disease in a population is high, also the PPV is high and vice versa((32).

5.5 Correlation of Anti-MDA5 and Anti-SAE

One significant correlation with a Spearman correlation coefficient of 0.617 (Figure 21) between anti-MDA5 and anti-SAE antibodies was detected during this study. The closer this coefficient is to one, the higher is the correlation.
By taking a closer look at Figure 21, it can be seen that the correlation between the two antibodies anti-MDA5 and anti-SAE exists only in the normal range below the cutoff value of ten. Above the cutoff value of ten, the correlation does not anymore exist according to our data and therefore, this correlation is not of clinical relevance.

5.6 Application of the EULAR/ACR Classification Criteria for Adult and Juvenile Idiopathic Inflammatory Myopathies

By applying the new EULAR/ACR classification criteria (3) to a total number of 242 patients, in 213 patients (88%) the output of the EULAR/ACR criteria web calculator (3, www.imm.ki.se/biostatistics/calculators/iim) confirms the actual existing or missing IIM diagnosis. Hence, in only 12% of the patients (29 out of 242) the web calculator result does not correspond to the diagnosis documented in the patients' medical records.
A reason for the mismatch could be that classification criteria are not equivalent to diagnostic criteria. Classification criteria get designed for clinical trials in order to include comparable patients in studies. Hence, the major aim of classification criteria is to create homogenous patient groups, which requires strict criteria.(3) Diagnostic criteria on the other hand get established for medical doctors in order to support them in diagnosing diseases. In this case, the major aim is making the right diagnose.(36) Obviously, classification and diagnostic criteria are designed for different purposes and therefore, the discrepancy between the output of the EULAR/ACR classification criteria and the actual documented diagnosis in the patients' medical records is explainable. Finally, one must be careful in the application and interpretation of classification criteria in the diagnostic pathway of diseases like IIMs.

Since the EULAR/ACR classification criteria confirm in 88% the actual existing or missing IIM diagnosis in our patients, I conclude that these new EULAR/ACR classification criteria represent a good support in diagnosing idiopathic inflammatory myopathies in adults and juveniles.

5.7 Limitations of the Study

In the course of my diploma thesis, I had to retrospectively study all the electronic as well as the paper medical records of the patients included in the study. In general, retrospective studies have advantages and disadvantages. On the one hand by conducting retrospective studies, results can be obtained rather quickly. But on the other hand, this study design has important drawbacks as well. For example, it is possible that the information provided in the patients' medical records is incomplete or maybe even incorrect. Hence, the quality of the outcome of a retrospective study strongly depends on the accuracy and correctness of documentation. Another drawback of retrospective studies is that one cannot determine the documentation guidelines beforehand, since at the time point at which the study is conducted all the documentation is already finished.(37)

Since only patients with positive antibody titers were included in the study, the specificities of the different antibodies were not calculated. For this purpose, also patients with negative antibody test results have to be analyzed.

By applying the new EULAR/ACR classification criteria a problem concerning the evaluation of the muscle strength reduction came up. In literature, there is no value defined, when a significant muscle strength reduction is present in a patient. Therefore, a cutoff value of equal to or less than seven for the manual muscle strength test was agreed on by the specialists of the Rheumatology Department Graz. Another point that has to be taken into consideration concerning the muscle strength assessment is that the evaluation of the muscle strength is dependent to a certain degree on the investigator's opinion. Furthermore, in order to be able to define whether a muscle strength test result indicates a significant reduction in muscle strength one must know the patient's muscle strength in the past. Sportsmen will show maybe muscle weakness more delayed than elderly patients,

losing muscle mass quite fast. Hence, the assessment of the muscle strength reduction should be clearly defined in order to obtain reproducible scores.

5.8 Conclusion

Overall, it can be summarized that the thesis provides essential information for the interpretation of positive MSA/MAA antibody titers by taking their positive predictive values into consideration. The study should support medical doctors in dealing with positive autoantibody titers. Therefore, the positive predictive values of each tested autoantibody for suffering from an IIM subtype, for having a positive history of autoimmunity or malignancy and finally, the PPVs for having ILD should be integrated in the decision making process whether further diagnostic tests have to be performed.

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