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STUDIES ON AUTOANTIBODIES AND INFLAMMATORY MARKERS IN PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES

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Studies on Autoantibodies and Inflammatory Markers in patients with Idiopathic Inflammatory Myopathies

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To my Jessica.

To Bruno, my mother and my father.

"*Aut viam inveniam aut faciam*"

Hannibal

"Then a muleteer told me that you don't have to get there first,
but you have to know how to get there"

(*Después me dijo un arriero que no hay que llegar primero,*
pero hay que saber llegar)

"El Rey", Mexican popular song

ABSTRACT

Idiopathic Inflammatory myopathies (IIM), commonly known as myositis, are chronic autoimmune diseases characterized by low muscle strength and low muscle endurance as main features. However, a high number of patients may develop extra muscular manifestations such as interstitial lung disease, skin rash or arthritis. There are known genetic and environmental risk factors for developing these conditions, but the cause is not fully understood.

To date, it is considered that myositis is driven by an autoimmune component. In this sense, the production of autoantibodies is characteristic in most patients and each of these autoantibodies is strongly linked to specific clinical manifestations. However, the implications of positive autoantibodies as prognostics tools beyond the association with clinical features has not been fully studied. Moreover, different methods have been used to detect these autoantibodies and new methods, usually employed in the daily clinical setting, require validation. In addition to the autoantibodies, the role of inflammatory markers as predictors of subjective health perception has been overlooked in patients with myositis.

The aim of this thesis was to validate an autoantibody assay commonly used in the clinic, a line blot assay, as well as to explore the usefulness of autoantibodies as predictors of response to treatment and organ damage, and to investigate the association of inflammatory markers with patient reported outcomes.

A validation of a line bot test was conducted using an immunoprecipitation-based algorithm as comparator in a cohort of well-characterized patients with myositis. The prevalence of relevant clinical associations with both assays was compared between patients. A moderate agreement between the assays was found, and the clinical features of patients with detected autoantibodies by the line blot assay were consistent with known clinical phenotypes (**Paper I**). By using a longitudinal Swedish electronic database (SweMyoNet), dermatomyositis specific autoantibodies (DMSA) were found as predictors for moderate level of response to treatment; initial doses of glucocorticoids and shorter time lag from first symptoms to diagnosis were also predictors of response to treatment (**Paper II**). In a longitudinal study, based on a large international electronic database (MyoNet), patients with anti-PM/Scl autoantibodies accumulated damage more pronounced than seronegative patients, and patients with DMSA accumulated less pronounced damage than seronegative patients. Furthermore, a strong correlation between severity of muscle damage and functional disability was found, especially in patients with immune-mediated necrotizing myopathies (**Paper III**). By analyzing data from MyoNet, we found a longitudinal correlation between Patient Global Assessment (PatGA) and inflammatory markers, namely C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) (**Paper IV**).

Altogether, the findings of this thesis indicate that the line blot assay is useful to detect the most frequent autoantibodies in patients with a known myositis diagnosis. The results of this thesis also suggest that autoantibodies are useful as predictors of response to treatment and of organ damage, and that inflammatory markers are associated with subjective health perception status measured by the Patient Global Assessment scale in patients with myositis.

Keywords: myositis, autoantibodies, treatment, damage, patient-reported outcomes measures (PROM).

RESUMEN

Las miopatías idiopáticas inflamatorias, comúnmente conocidas como miositis, son enfermedades caracterizadas principalmente por debilidad muscular y resistencia muscular disminuída. Sin embargo, un número importante de patientes pueden llegar a desarrollar manifestaciones clínicas extra musculares tales como inflamación pulmonar o artritis. Se conocen diversos factores de riesgo para desarrollar miositis pero la causa sigue siendo desafortunadamente desconocida.

A la fecha, sin embargo, se considera que un componente autoinmune dirige la patogénesis en miositis. En este sentido, la producción de autoanticuerpos es característica en la mayoría de los pacientes. Más aún, cada uno de estos autoanticuerpos está fuertemente relacionado con manifestaciones clínicas específicas. Hasta ahora, diferentes métodos para detectar estos autoanticuerpos han sido utilizados y nuevos métodos, usualmente empleados en la rutina clínica diaria, requieren ser validados. Así, las implicaciones de pruebas positivas para autoanticuerpos como herramientas pronósticas más allá de la asociación con manifestaciones clínicas no han sido estudiadas a fondo. Además de los autoanticuerpos, el papel de los marcadores de inflamación como predictores del estado autopercibido de salud ha sido pasado por alto.

El objetivo de esta tesis fue validar una prueba de detección de autoanticuerpos (*line blot*) así como explorar el uso de autoantcuerpos como predictores de respuesta al tratamiento, daño orgánico, e investigar la asociación de los marcadores de inflamación con desenlaces reportados por pacientes.

La validación de la prueba de detección de autoanticuerpos fue llevada a cabo usando un algoritmo basado en inmunoprecipitación como comparador usando una cohorte de pacientes con miositis bien caracterizada. La frecuencia de asociaciones clínicas relevantes con ambas pruebas fue comparada entre pacientes. Se encontró una concordancia moderada entre las pruebas, y las manifestaciones clínicas de los pacientes con autoanticuerpos detectectados por *line blot* fueron consistentes con fenotipos clínicos conocidos (**Estudio I**). Usando una base de datos electrónica sueca (SweMyoNet), se encontró que los autoanticuerpos específicos de dermatomiositis (DMSA) fueron predictores de respuesta moderada a tratamiento; también las dosis iniciales de glucocorticoides y menor tiempo desde los primeros síntomas al diagnóstico fueron encontrados como predictores de respuesta a tratamiento (**Estudio II**). En un estudio longitudinal basado en una extensa base de datos internacional (MyoNet), se encontró que los pacientes con anticuerpos anti-PM/Scl acumularon más daño y los pacientes con DMSA acumularon menos daño que los pacientes seronegativos. Asimismo, se encontró una alta correlación entre la severidad de daño muscular y la discapacidad funcional especialmente en pacientes con miopatías necrotizantes (**Estudio III**). Usando la base de datos electrónica MyoNet, se encontró una correlación entre la escala Evaluación Global del Paciente y los marcadores inflamatorios, es decir, proteína C reactiva y velocidad de sedimentación globular (**Estudio IV**).

En conjunto, los hallazgos de esta tesis indican que el método *line blot* es útil para detectar los autoanticuerpos más frecuentes en pacientes con un diagnóstico de miositis. Los resultados de esta tesis sugieren que los autoanticuerpos son útiles como predictores de respuesta al tratamiento y a daño orgnánico, y que los marcadores inflamatorios se asocian con el estado autopercibido de salud medido por la escala Evaluación Global del Paciente en pacientes con miositis.

Plabras clave: miositis, autoanticuerpos, tratamiento, daño, desenlances reportados por el paciente (PROM)

LIST OF SCIENTIFIC PAPERS

This thesis is based on four original papers. These papers are listed below and will be referred to in Roman numerals:

I. **Comparison of autoantibody specificities tested by a line blot assay and immunoprecipitation-based algorithm in patients with idiopathic inflammatory myopathies.**
***Espinosa-Ortega F*,** Holmqvist M, Alexandeson H, Storfors H, Mimori T, Lundberg IE, Rönnelid J. Ann Rheum Dis 2019 Jun;78(6):858-860

II. **Factors associated with treatment response in patients with idiopathic inflammatory myopathies: A registry-based study.**
Espinosa-Ortega F, Holmqvist M, Dastmalchi M, Lundberg IE, Alexanderson H. Arthritis Care Res (Hoboken) 2022 Mar;74(3):468-477

III. **Autoantibodies and damage in patients with idiopathic inflammatory myopathies: A longitudinal multicenter study from the MYONET international network**
Espinosa-Ortega F*, Lodin K*, Dastmalchi M, Vencovsky J, Diederichsen LP, Shinjo SK, Erler A, Danieli MD, Selva-O'Callhgan A, de Visser M, Griger Z, Ceribelli A, Gómez-Martín D, Andersson H, Vázquez Del-Mercado M, Knitza J, Wedderburn L, Chinoy H, Lillerker J, New P, Krogh N, Lundberg IE, Alexanderson H, on behalf of the MyoNet Registry Study Group.
Manuscript

IV. **Patient global assessment and inflammatory markers in patients with idiopathic inflammatory myopathies – a longitudinal study.**
Lodin K*, ***Espinosa-Ortega F****, Dastmalchi M, Vencovsky J, Andersson H, Chinoy H, Lilleker JB, Krogh NS, New P, Shinjo SK, Maurer B, Griger Z, Tavor Y, Ceribelli A, Torres-Ruiz J, Vázquez Del-Mercado M, Leonard D, Erler A, Alexanderson H, Lundberg IE, on behalf of the MyoNet Registry Study Group.
Manuscript

*Equal contribution

SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

I. **Novel insights of disability assessment in adult myositis.**
Espinosa-Ortega HF, Moreno-Ramírez M, Alexanderson H. Curr Op Rheumatol 2017 Nov;29(6):591-97.

II. **Response to: 'Semi-quantitative analysis of line blot assay for myositis-specific and myositis-associated antibodies: a better performance?' by Cavazzana *et al*.**

Rönnelid J, ***Espinosa-Ortega F***, Lundberg IE. Ann Rheum Dis 2020 Nov;79(11):e153.

III. **Response to: 'Comment on: standardisation of myositis-specific antivodies : where are we today?' by Infantino *et al*.**
Rönnelid J, ***Espinosa-Ortega F***, Lundberg IE. Ann Rheum Dis 2021 Jul;80(7):e116.

IV. **Evaluation of a New Skeletal Troponin I assay in patients with idiopathic inflammatory myopathies.**
Bamberg K, Mehtälä L, Arola O, Laitinen S, Nordling P, Strandberg M, Strandberg N, Paltta J, Mali M, ***Espinosa-Ortega F***, Pirilä L, Lundberg IE, Savukoski T, Pettersson K. Appl Lab Med 2020 Mar 1;5(2):320-331

V. **Low health-related quality of life in adult individuals with multiple limb deficiencies compared with population-based reference values.**
Alexanderson H, Frimore L, ***Espinosa F***, Wikström M, Stockselius A. Prosthet Ort Int 2022 Jun 1;46(3):232-238.

TABLE OF CONTENTS

1 Introduction 3
2 Literature review 3
2.1 The concept of idiopathic inflammatory myopathies 3
2.2 Epidemiology 4
2.2.1 Incidence and prevalence 4
2.2.2 Mortality 4
2.3 Risk factors 4
2.4 Diagnosis and classification 5
2.5 Clinical findings 6
2.5.1 Muscular symptoms 6
2.5.2 Extra muscular features 7
2.6 Inflammatory markers in myositis 10
2.7 Autoantibodies 10
2.7.1 Methods of autoantibody detection 11
2.8 Clinical and autoantibody-defined subtypes 12
2.8.1 Dermatomyositis 12
2.8.2 The anti-synthetase syndrome 15
2.8.3 Immune-mediated necrotizing myopathy 17
2.8.4 CTD-myositis 18
2.8.5 IBM phenotype 18
2.8.6 Polymyositis 19
2.9 Medical treatment 20
2.9.1 Glucocorticoids 20
2.9.2 Immunosuppressive agents 20
2.9.3 Biologic therapy 21
2.9.4 Exercise therapy 23
2.10 Prognosis 23
2.11 Outcome measurement 24
2.11.1 Disease activity 24
2.11.2 Definition of Improvement 26
2.11.3 The ACR/EULAR response criteria 27
2.11.4 Organ damage 27
3 Research aims 30
4 Materials and methods 31
4.1 Data sources 31
4.2 Study population 32
4.3 Classification of patients 32
4.4 Methods for detecting autoantibodies 33
4.4.1 Line blot Protocol. 33
4.4.2 Immunoprecipitation Protocol. 33
4.4.3 ELISA Protocol. 33

4.5 Autoantibody-defined subgroups 34
4.6 Clinical data 34
4.7 Participants 36
4.8 Outcomes 36
4.9 Statistical considerations 37
4.10 Ethical considerations 40
5 Main results 42
5.1 Paper I 42
5.1.1 Agreement between IP and LB assays 42
5.1.2 Association between autoantibodies and clinical manifestations 43
5.2 Paper II 43
5.2.1 Autoantibodies and level of response to treatment 43
5.2.2 Predictive factors for treatment response 44
5.3 Paper III. 45
5.3.1 Extent of organ damage 45
5.3.2 Autoantibodies and organ damage 45
5.3.3 Correlation between severity of muscle damage and physical disability 46
5.3.4 Change of damage over time and the effect of autoantibodies 47
5.4 Paper IV 48
5.4.1 Relationship between PatGA and inflammatory markers over time 48
5.4.2 Association between PatGA and inflammatory markers and mediation by functional measures 49
6 Discussion 51
7 Conclusions 60
8 Clinical implications 60
9 Future perspectives 61
10 Populärvetenskaplig sammanfattning 63
11 Acknowledgements 65
12 References 69

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ADM	Amyopathic dermatomyositis
ALAT	Alanine aminotransferase
ASA	Antisynthetase autoantibodies
ASAT	Aspartate aminotransferase
AZA	Azathioprine
BAFF	B-cell activating factor
BILAG	British Isles Assessment Group
CAM	Cancer-associated myositis
CFM	Cyclophosphamide
CI	Confidence interval
CK	Creatin kinase
CRP	C-reactive protein
CSM	Core set measures
CTD	Connective tissue disease
CTLA	Cytotoxic T lymphocyte protein 4
DM	Dermatomyositis
DMSA	Dermatomyositis-specific autoantibodies
ELISA	Enzyme-linked immunosorbent assay
EM	Extramuscular activity
ENMC	European Neuromuscular Centre working group
ESR	Erythrocyte sedimentation rate
EULAR	European Alliance of Associations for Rheumatology
FHL-1	Four-and-a-half LIM domain 1
GC	Glucocorticoids
HAQ	Health Assessment Questionnaire
HLA	Human leukocyte antigen
HMGCR	Hydroxy-3-methylglutaryl-coenzyme A reductase
IBM	Inclusion body myositis
IFN	Interferon
IIM	Idiopathic inflammatory myopathies
ILD	Interstitial lung disease
IMACS	International Myositis Assessment and Clinical Studies Group
IMNM	Immune-mediated necrotizing myopathy
IP	Immunoprecipitation
IQR	Interquartile range
JAK	Janus kinase complex
JDM	Juvenile dermatomyositis
LB	Line blot
LDH	Lactate dehydrogenase
MAA	Myositis-associated autoantibodies
MDA5	Melanoma-differentiation-associated gene 5
MDAAT	Myositis Disease Activity Assessment Tool
MDI	Myositis damage index
MITAX	Myositis Intention to Treatment Index
MMT8	Manual Muscle Test of 8 muscle groups
MRC	Medical Research Council

MRI	Magnetic resonance imaging
MSA	Myositis-specific autoantibodies
MTX	Methotrexate
MYODAM	MDI score of damage severity
NA	Not assessed
NSIP	Non-specific interstitial pneumonia
NXP2	Nuclear matrix protein 2
OMERACT	Outcome Measures in Rheumatology Clinical Trials
OP	Organizing pneumonia
OR	Odds ratio
PIN	Personal Identification Number
PM	Polymyositis
PRO	Patient-reported outcomes
PROM	Patient-reported outcome measures
RA	Rheumatoid arthritis
RIG	Retinoic acid-inducible
RIM	Rituximab in myositis trial
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RP-ILD	Rapidly progressive interstitial lung disease
SAE	Small ubiquitin-like modifier-1 activating enzyme
SD	Standard deviation
SDI	Systemic Lupus International Damage Index
SLE	Systemic lupus erythematosus
SRP	Signal recognition particle
SRQ	Swedish Rheumatology Quality register
SSc	Systemic sclerosis
TIF	Transcriptional intermediary factor 1-gamma
TIS	Total improvement score
TNF-α	Tumor necrosis factor alpha
UIP	Usual interstitial pneumonia
USA	United States of America
VAS	Visual analogue scale

1 Introduction

Idiopathic inflammatory myopathies are rare but very disabling conditions. The paucity of these conditions presents difficulties to study them properly. The Myositis Research group at the Department of Medicine, Division of Rheumatology at Karolinska Institutet is a multidisciplinary team devoted to conduct studies to gain knowledge on the disease mechanisms, risk factors, development of diagnostic and therapeutic tools to improve the quality of life of patients with myositis. This thesis contains some of the studies carried out within this research environment.

Over the last decades, physicians and health professionals have witnessed the rapid progress in the field of therapeutics and diagnostics of patients with autoimmune rheumatic diseases. A better understanding of the pathogenesis of myositis has enabled clinical researchers and scientists to apply the learnt concepts from other rheumatic conditions to innovative solutions for patients living with myositis. Also, an increasing awareness of the impact of myositis on the quality of life of patients has prompted researchers to standardize how we measure the accuracy of diagnostic tests, how we evaluate the efficacy of medical interventions and how we can develop accurate prognostic markers of prognosis. Detectable autoantibodies in the serum of patients with myositis are promising tools that might fill this gap. The serologic tests, however, require a careful evaluation process in order to support their use as prognostic markers. Autoantibodies, but also inflammatory markers, together with their association with prognosis and long-term consequences such as organ damage and patients' subjective health perception is the topic of the present thesis.

Below I have organized a literature review to guide the reader to the knowledge gap I wanted to address with my thesis.

2 Literature review

2.1 The concept of idiopathic inflammatory myopathies

The idiopathic inflammatory myopathies (IIM), which in this thesis is referred to as myositis, is an umbrella concept including a group of acquired chronic muscle diseases that share an inflammatory and autoimmune background. Traditionally, the main clinical subtypes have been polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM). However, as discussed further in this thesis, updated classification criteria have included new subsets of patients according to their serological status as new autoantibodies have been described.

Myositis is considered a systemic inflammatory disease. Clinically, myositis is characterized by proximal muscle weakness, low muscle endurance, severe muscle fatigue, and pain. However, very frequently patients may suffer from manifestations due to extramuscular inflammation, for example, interstitial lung disease, cutaneous lesions, arthritis, dysphagia, and cardiac disturbances. Due to the complexity of the disease the diagnostic approach of patients with suspected IIM is usually quite challenging. 'Idiopathic' refers to the fact that the underlying pathogenic mechanism in these diseases is unknown.

Nevertheless, as discussed in this thesis, a vast amount of literature indicates that many different autoimmune mechanisms, including the production of autoantibodies, may have a role in the pathophysiology of myositis and therefore may have prognostic implications. As a result, some authors have insisted that a proper name for these entities would be 'autoimmune myositis' **(1)**.

2.2 Epidemiology

2.2.1 Incidence and prevalence

A systematic review reported an overall estimated incidence of 7.98 cases/million/year (95% CI 7.38 – 8.66) and an estimated prevalence of 14 cases/100,000 (95% 12.84 – 15.46) between 1966 and 2013 **(2)**. Another contemporary systematic review from Africa showed an estimated incidence 1.2 – 7.5 cases/million/year and prevalence of 8.8 cases/100,000 **(3)**. In Sweden, a nationwide-population study estimated an incidence of 11 cases/million/year (95% CI 10 -12) and a prevalence of 14 cases/100,000 (95% CI 13 – 14) for the whole myositis group including both DM and PM. The age- and sex-adjusted incidence for IBM has been estimated to 0.79 cases per 100,000 (**4**) and the prevalence to 2.01 per 100,000 **(2)**. Epidemiological studies indicate that myositis, except for IBM, is more frequent in women than in men **(5, 6, 7)**.

2.2.2 Mortality

Patients with myositis have a notable high risk for early death. A Norwegian study detected a higher standardized mortality rate (SMR) in DM (2.6), PM (2.4) and in IBM (1.7) over a median follow up of 85 months **(8)**. In that study, disease-related causes of death were frequent (e.g., cancer, pulmonary complications, and infections). A Swedish nationwide register based study found an increased mortality rate in patients with myositis compared with the general population, hazard ratio 3.7 (CI 3.2 – 4.4), especially within the first year after diagnosis (cumulative mortality rate 9% in myositis and 1% in the general population) **(9)**. In this nationwide study, the main causes of death in patients with myositis were diseases related to the respiratory system, circulatory system, and malignant neoplasms especially within the first year after diagnosis. A similar risk has been demonstrated in several other studies **(10, 11, 12**).

Together, these studies show that being diagnosed with myositis is a determinant factor for survival; therefore, it is of utmost importance that clinicians and researchers have accurate tools to early detect patients with myositis, and once the diagnosis is made, to stratify patients according to their risk for complications.

2.3 Risk factors

The human leukocyte antigen (HLA) region has a strong association with autoimmune diseases. Patients with myositis have a particular interconnection with some alleles of the HLA 8.1 ancestral haplotype. Interestingly, some alleles of this ancestral haplotype have specific associations with clinical subtypes of myositis. For example, HLA-DRB1*03:01 with PM, HLA-B*08:01 with DM, and HLA-DRB1*01:01 and HLA-DRB1*13:01 with IBM **(13, 14)**. More recently, however, the association between specific alleles with phenotypes had a better

explanation because of the relationship between those alleles and autoantibodies rather than with clinical phenotypes **(15)**. Also, previous studies have shown that most of myositis patients harbor only one myositis-specific autoantibody and the coexistence of two specific autoantibodies is highly unusual **(16)**. Nonetheless, recent studies using cluster analysis have demonstrated that patients may harbor both specific and associated autoantibodies **(17)** (more on specific and associated autoantibodies in the *Autoantibodies* section). Interestingly, patients within these autoantibody-groups showed a very strong association with specific HLA alleles. An example for this is HLA-DRB1*03 which is more frequent in anti-Jo1+, -Jo1/Ro52+, and -PM/Scl+ patients, but less frequent in anti-Mi-2 and -TIF1γ+ patients **(17)**.

Several non-genetic risk factors for developing myositis have been explored. Smoking, a well-known environmental risk factor for rheumatoid arthritis, has been linked to a high risk for myositis, especially anti-Jo-1+ myositis. Furthermore, an exponential interaction between smoking and the HLA-DRB1*03 allele has been observed **(18)**. Other well-documented environmental risk factors include gastrointestinal and pulmonary infections **(19)**, UV-light exposure **(20)**, and gut dysbiosis **(21)**. The latter is of particular interest due to the association between specific intestinal bacteria and the myositis-specific autoantibodies. Together, this piece of evidence suggests a dynamic interaction between genetic, immune, and environmental factors contributing to the development of myositis, especially through the presence of autoantibodies.

2.4 Diagnosis and classification

The diagnosis of myositis is a construct based on clinical symptoms and signs of skeletal muscle inflammation among other symptoms. Because there are no diagnostic criteria, the treating clinician's diagnosis remains as the correct diagnosis **(22)**. Since Bohan and Peter published their seminal work on the diagnostic and classification criteria in 1975, these criteria have been extensively used for the past four decades **(23, 24)**. These criteria proposed definitions for *definite*, *probable*, and *possible* categories for the main phenotypes for the first time, i.e., PM and DM. Also, non-myositis entities were provided as potential mimickers. An important category for overlap myositis, or connective tissue disease (CTD)-associated myositis was also added. In 1991, Dalakas improved Bohan and Peter's criteria by adding detailed information on expected histological findings on muscle biopsies and by including a new described form of corticosteroid-resistant, progressive myositis with characteristic vacuoles in muscle biopsies, the IBM **(25)**.

At the beginning of my doctoral studies during the Spring of 2017, a decisive shift in the paradigm of classification criteria came when the European Alliance of Associations for Rheumatology /American College of Rheumatology (EULAR/ACR) criteria were published **(26)**. These criteria were developed based on pre-selected variables from previous criteria and as suggested by experts applied to myositis cases and controls. Two sets of models were developed, with and without muscle biopsy, allowing clinicians to accurately classify cases even when a biopsy would not be feasible (e.g., in children with JDM).

The performance of these criteria depends on the use of biopsy information: with biopsy, sensitivity 93% / specificity 88% and without biopsy, sensitivity 87% and specificity 82%. These criteria allow classification of each case into one of the main clinical subtypes: PM, DM (juvenile and adult), amyopathic DM (ADM), and IBM. An available on-line calculator provides the estimated probability of a given case to be classified as myositis. The recommended cut-offs are ≥55% and <90% for *probable* and ≥90% for *definite*; a probability ≥50% and <55% is considered as *possible* myositis. An important contribution of the EULAR/ACR criteria is the inclusion of one myositis-specific autoantibody: the anti-histidyl t-RNA synthetase (anti-Jo-1). This inclusion adds not only semantic and diagnostic value, but it is also a step forward to improve disease stratification and individualized pharmacological therapy.

Regarding IBM, Griggs criteria were the most accepted criteria **(27)**, but this set of criteria relies mainly on the presence of typical histopathological findings, for example, rimmed vacuoles or deposition of amyloid inclusions. In 2013, the European Neuromuscular Centre (ENMC) working group published a set of diagnostic criteria allowing the clinicians to diagnose patients either only by clinical findings or by clinico-pathologically findings **(28)**.

For this reason, it is now a well-recognized phenomenon that IBM patients may develop a typical and complete clinical phenotype without characteristic findings in muscle biopsy, and evidence indicates that both groups have the same disease course **(29)**.

2.5 Clinical findings

In the following section I will outline the clinical signs and symptoms of patients with myositis, both muscular and extra muscular symptoms.

2.5.1 Muscular symptoms

Proximal and symmetrical muscle weakness is the hallmark of patients with myositis. Patients with amyopathic or hypomyopathic dermatomyositis may show no or low muscle weakness whereas patients with immune-mediated necrotizing myopathies (IMNM) may suffer from marked disabling muscle weakness. Patients with IBM, however, may suffer from a special pattern of muscle weakness, mainly involving the quadriceps and finger flexor muscles. Distal muscle weakness is not specific for IBM but may be also present in patients with DM or PM **(30)**. Low muscle endurance, even in absence of muscular weakness assessed by the manual muscle test (MMT), is common in all forms of myositis. Evidence for inflammatory changes in muscle tissue may be detected by electromyography or magnetic resonance imaging (MRI), together with elevated muscle enzymes. Muscle pain, once considered uncommon, is now recognized as a main complaint with an important disease burden **(31)**.

Laboratory findings of muscle injury due to inflammation include abnormal levels of circulating muscle enzymes and muscle proteins. Creatine kinase (CK) in serum is the most widely used marker of muscle injury due to its good diagnostic accuracy, high sensitivity and satisfactory correlation with disease activity **(32)**. CK levels are frequently higher in PM

patients compared to DM patients **(33)**. However, several factors may preclude the elevation of CK levels even after confirmed muscle inflammation, for example, by MRI or biopsy. These factors include 1) the use of glucocorticoids (GC), 2) presence of CK inhibitors in serum, and 3) extensive muscle atrophy **(34)**. CK levels tend to decrease between 3 to 8 weeks after improvement, and they usually tend to increase between 5 to 6 weeks before a flare. The main CK isoenzyme, MM, is mainly responsible for the increase levels of CK, however, also the CK-MB fraction may be released even in the absence of myocardial injury **(35)**. Other muscle enzymes which serum levels correlates well with increased levels of CK are alanine aminotransferase (ALAT), aspartate aminotransferase (AST), aldolase, and lactate dehydrogenase (LD). Myoglobin, a muscle protein, is usually elevated very early during muscle injury and seems to correlate with muscle strength better than muscle enzymes, but due to its diurnal variation, it requires to be obtained at the same time of the day **(34)**. Elevations of serum troponin T, but not Troponin I, are now recognized as good markers of skeletal muscle injury and not necessarily reflect cardiac muscle injury **(36, 37)**.

2.5.2 Extra muscular features

2.5.2.1 Skin features

Skin involvement is the second most frequent manifestation of patients with myositis after muscle involvement (approximately 30% of patients) **(7)**. Indeed, this clinical finding defines the subset DM. Classical skin manifestations include *Gottron's sign* i.e., erythema over areas of stretching, *Gottron papules*, i.e., red papules over the extensor surfaces, and *heliotrope rash* i.e., a pink, red or purplish coloring around the eyes and eyelids. Ulcerative lesions, which may be present mainly in patients with anti-MDA5 antibodies, are considered as DM classical cutaneous features when they appear in typical localizations **(38)**. Less common cutaneous signs are the *shawl sign*, the *V sign*, and the *Holster sign*. Abnormalities in nailfold capillaroscopy have also been described as a characteristic feature of DM **(39, 40)**.

2.5.2.2 Pulmonary features

The next most common extra muscular organ manifestation in myositis is pulmonary disease. Pulmonary disease ranges from 20 to 78% depending on the population studied, diagnostic modality, and, more important, the patient's autoantibody status **(41)**.

High resolution computed tomography (HRCT) is the preferred modality to assess the extent and nature of inflammatory lesions in the pulmonary parenchyma. Inflammatory infiltrates can invade the parenchymal lung tissue, and therefore, can manifest as interstitial lung disease (ILD). The most common pattern of ILD is cellular non-specific interstitial pneumonia (NSIP), but usual interstitial pneumonia (UIP), fibrotic NSIP and organizing pneumonia (OP) are other patterns that may be observed **(41, 42)**. Progression of the active inflammatory process in the lungs may result in lung fibrosis. The fibrotic changes may appear either in early phases of the disease or because of a resolved inflammatory process **(42)**. Intriguingly, the exact mechanism of lung fibrosis in patients with myositis remains unclear.

Table 1. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups.

Variable	Score	
	Muscle biopsy	
	yes	no
Age of onset of first symptoms assumed to be related to the disease ≥18 and <40 years	1.3	1.5
Age of onset of first symptom assumed to be related to the disease ≥40 years	2.1	2.2
Muscle weakness		
Objective symmetric weakness, usually progressive, of the proximal upper extremities	0.7	0.7
Objective symmetric weakness, usually progressive, of the proximal lower extremities	0.8	0.5
Neck flexors are relatively weaker than neck extensors	1.9	1.6
In the legs, proximal muscles are relatively weaker than distal muscles	0.9	1.2
Skin manifestations		
Heliotrope rash	3.1	3.2
Gottron's papules	2.1	2.7
Gottron's sign	3.3	3.7
Other clinical manifestations		
Dysphagia or esophageal dysmotility	0.7	0.6
Laboratory measurements		
Anti-Jo-1 autoantibody present	3.9	3.8
Elevated serum levels of CK or LD or ASAT or ALAT	1.3	1.4
Muscle biopsy features – presence of:		
Endomysial infiltration of mononuclear cells surrounding, but not invading myofibers		1.7
Perimyisial and/or perivascular infiltration of mononuclear cells		1.2
Perifascicular atrophy		1.9
Rimmed vacuoles		3.1

Anti-Jo-1, anti-histidyl-tRNA synthetase; CK, creatin kinase; LD, lactate dehydrogenase; ASAT, aspartate aminotransferase; ALAT, alanine-aminotransferase

Besides ILD, respiratory muscles may be affected by inflammation resulting in muscle weakness leading to mechanical respiratory assistance. This form of pulmonary disease is uncommon compared to ILD and is usually seen in patients with PM **(43)**.

Assessing the progression of ILD is difficult because of the lack of a standardized definitions of radiology findings. Thus, making comparisons between the main radiological patterns in terms of severity and prognosis is still a challenge. Nonetheless, the clinical course of ILD may fall into one of the following four categories: 1) asymptomatic ILD, 2) Rapidly Progressive-ILD, 3) sub-acute/chronic ILD, and 4) chronic progressive-fibrosing ILD. The latter is considered more consistent with fibrotic histopathologic findings (*pulmonary fibrosis*) and thus, less susceptible to response to immunosuppressive treatment **(41)**.

2.5.2.3 *Cardiovascular features*

Cardiovascular disease in patients with myositis occurs either due to disease-specific active inflammation or due to coronary atherosclerosis. The frequency of heart disease varies according to the type of disease, but also depending on the diagnostic modality **(44)**. In the case of active inflammation, myocarditis is the most important manifestation of cardiac disease, but ventricular dysfunction, both systolic and diastolic, is often recognized **(45)**. Arrhythmia and conduction disturbances are commonly reported. Recently, a study showed that a prolonged QTc interval was associated with the presence of certain autoantibodies (anti-Mi-2 and anti-PL7) in a Danish-Swedish cohort of patients with myositis **(46)**. Concerning atherosclerotic disease, patients with myositis have a high risk for cardiovascular events, both arterial and venous, particularly within the first year after the diagnosis and with a malignancy background **(47)**.

2.5.2.4 *Gastrointestinal features*

The inflammatory process in myositis may also involve the gastrointestinal tract. Involvement of upper tract in form of dysphagia is the most common symptom: a study showed that up to 85% of patients with anti-TIF1γ may develop this clinical manifestation and it is associated with poor prognosis as it may appear in association with malignancy **(48)**. Also, patients with IBM and positive anti-cN1A autoantibodies have a more severe course of dysphagia than anti-cN1A-negative patients **(49)**. Less frequent is the involvement of the lower tract, usually in form of dysmotility **(50)**, gastrointestinal hemorrhage **(51)**, and intestinal perforation **(52)**.

2.5.2.5 *Cancer*

For decades, epidemiological studies have demonstrated a clear association between myositis and malignancy, particularly among patients with DM phenotype **(53)**. Recent studies have confirmed that this epidemiological association persists, particularly at the time a patient is diagnosed with myositis or right after the diagnosis **(54)**. Older age, dysphagia, presence of anti-TIF1γ or anti-NXP2 autoantibodies, elevated C-reactive protein (CRP), elevated erythrocyte sedimentation rate (ESR), and presence of ovoidal palatal patches (i.e., asymptomatic, well-demarcated,

erythematous patch on the posterior hard palate) are other risk factors for myositis-associated cancer **(55, 56, 57)**.

2.6 Inflammatory markers in myositis

Several laboratory abnormalities besides muscle enzymes may be found in patients with myositis as expected for an autoimmune systemic disease. These laboratory markers may reflect the level of severity of the inflammatory response and are easily accessible from blood samples.

C-reactive protein (CRP) is an acute-phase reactant and a well-known marker of inflammation albeit very unspecific. Causes of elevation of CRP levels include infection, cancer, rheumatic conditions and even cardiovascular events. Extreme high levels of CRP may be observed in patients with giant cell arteritis, rheumatoid arthritis, and vasculitis during active disease, and thus it is used as biomarker of inflammatory activity **(58)**. In patients with myositis, however, this association is not as strong as in other inflammatory rheumatic conditions. An exception though is the coexistence of ILD and cancer. Indeed, it has been proposed as an independent predictor of mortality in patients with ILD, particularly in anti-MDA5+ patients **(59)**. Another acute-phase reactant is the erythrocyte sedimentation rate (ESR). This test is elevated in 50% of patients with myositis but does not correlate with overall disease activity **(34)**. However, a meta-analysis showed that ESR is independently associated with ILD in both PM and DM **(60)**. Other inflammatory markers, for example, serum IL-2R and ferritin levels, have been shown to correlate with more systemic inflammation in patients with myositis **(61, 62)**.

Acute-phase reactants are included in the work-up of patients with RA or giant cell arteritis and used as surrogate markers of disease activity. Because these markers are more frequently found elevated in the context of lung inflammation in patients with myositis, they have been neglected as a biomarker of the inflammatory burden, and therefore are not part of the recommended core set measures to follow up patients. Several different cytokines together with acute-phase reactants contribute with the inflammatory response, both acute and chronic. Systemic inflammation, occurring in the context of an infectious or non-infectious disease, takes part of a highly coordinated body response known as sickness behavior. Sickness behavior is a coordinated pattern of behavioral changes aiming primarily to combat the “aggressor”. The effect of over-exaggerated sickness behavior is a well-known phenomenon in other chronic inflammatory diseases, for instance, asthma **(63)**. Even in healthy individuals, circulating cytokines has an impact on subjective health perception **(64)**. In myositis, however, the impact of inflammatory markers, specifically CRP, on self-reported outcomes has remained unexplored.

2.7 Autoantibodies

Autoantibodies are considered as the mainstay in autoimmune diseases, but their role in the pathogenesis of myositis has been a subject of debate for decades. Although they are not the sole mechanism involved in the rupture of the immune tolerance, they may play a role in the pathogenesis and perpetuation of sustained autoimmunity, but they can be just an

epiphenomenon. Yet, autoantibodies are often used as biomarkers in autoimmune rheumatic diseases, for example, rheumatoid factor or anti-neutrophil cytoplasmic antibodies. Over the past two decades, the discovery of new autoantibodies has revolutionized the diagnostic approach in the field of rheumatology, particularly in myositis.

By the time I began my first doctoral project in 2017, at least 15 different autoantibodies, specific to myositis, had been described **(65)**. Additionally, other *co-existent* autoantibodies are frequently observed. Myositis autoantibodies are now grouped into two main categories: myositis-specific autoantibodies (MSA) and myositis-associated autoantibodies (MAA). The group MSA is considered as highly disease-specific, that is, these autoantibodies are found almost exclusively in patients with myositis. One group of MSA is the so-called anti-synthetase autoantibodies (including anti-Jo-1, anti-PL12, anti-PL7, anti-EJ, anti-OJ, and anti-Ks). Additional MSA are anti-Mi-2, and anti-TIF1γ. Together, these autoantibodies may be found in approximately 50 percent of patients with myositis.

On the other hand, MAA is a group of autoantibodies that is also frequently found in serum of patients with other rheumatic conditions, for example systemic sclerosis or Sjögren's syndrome. There is no unique phenotype associated with these autoantibodies, but rather patients may present clinically as overlap syndromes (*See CTD-myositis*). Some of the MAA are anti-PM/Scl autoantibody, anti-Ro (both Ro52 and Ro60), and anti-KU. Conversely to MSA, MAA may coexist with MSA, for instance, anti-Jo-1 and antiRo52 which is the most common combination observed.

Detecting autoantibodies brings different advantages in the clinical setting. First, the presence of autoantibodies may help clinicians to differentiate autoimmune myositis from other forms of neuromuscular myopathies, for example, muscular dystrophies. Second, once the diagnosis has been established, the implications of the presence of autoantibodies go beyond the power of classification. The strong association between MSA, and to a less degree MAA, with well-defined phenotypes may predict the clinical course of the disease (prognosis) (**Table 2 phenotypes of Ab**). Third, the myositis autoantibodies may be useful to predict response to treatment (precision medicine). Still, despite of the evidence showing clear relationship between positivity for autoantibodies and clinical features, the diagnostic accuracy of MSA/MAA is not completely well-defined **(66)**. Therefore, there are currently no evidence-based guidelines recommending using the detection of autoantibodies as a diagnostic tool, and their use as prognostic factors is not fully explored.

2.7.1 Methods of autoantibody detection

Detection of autoantibodies in patients with rheumatic diseases has been performed by means of immunoprecipitation technique, historically considered as the gold standard. The principle of this assay is to isolate a target from a solution, for example, a protein or a cell lysate, by using an autoantibody that identifies this target. This antibody-complex is precipitated by centrifugation,

and then coupled to a solid substrate where the complex can be detected **(67)**. This assay may be protein- or RNA-based, and thus, it can detect native antigens. Unfortunately, only few research centers have the technology to carry out these tests due to their high cost and complex techniques. Also, there is a lack of standardization which makes it difficult to employ this technique on a routine basis.

New assays have emerged as a possibility to test for autoantibody positivity in the daily clinical setting. One of these assays is the line blot (LB) test. LB is a multispecific test, i.e., employs several antigens at the same time spotted over nitrocellulose membranes. In this way, the procedure saves time and costs. Another commercial test, though monospecific – capable of detecting only one antigen at a time – is the enzyme-linked immunosorbent assay (ELISA). The basic principle of ELISA is to detect antigens attached to a surface by means of an antibody that can bind the antigen. The antibody used is linked to an enzyme; in a final step, the enzyme's substrate is added. If there is a reaction, a signal is detected. One of the main pitfalls of these tests is that the antigen used may not be native, and thus, conformational changes in the structure may potentially have a diagnostic impact **(68)**.

The increasing availability of these new techniques and assays for detecting autoantibodies routinely has raised issues that include validation of these assays in the clinical context, quality assurance, accuracy of the tests as well as standardization. Standardization is the process of implementing a standard preparation to achieve uniformity of test-results **(69)**. In this context, there is an urgent need of appropriately conducted studies capable of determining the true value of new autoantibody assays.

2.8 Clinical and autoantibody-defined subtypes

2.8.1 Dermatomyositis

Typical skin symptoms define a clinical subset of patients of myositis, that is, DM. However, this clinical framework might be supported by histopathological and serological features specific to DM. The main histological features of DM, regardless of autoantibody status, are perifascicular atrophy and perivascular inflammation **(70)**. Additionally, it has been proposed that DM is a B-cell mediated microangiopathy as deposition of membrane attack complex (c5b-c9) has consequently been observed **(71)**. Also, studies have shown that patients with DM have an increased expression of molecules of the type I interferon pathway, the so-called *interferon signature* **(72)**.

In 2018, the ENMC group reached consensus about DM classification and established the importance of subclassifying DM patients according to their autoantibody status **(38)**. Dermatomyositis-specific autoantibodies (DMSA) include anti-Mi-2, anti-TIF1γ, anti-NXP2, anti-MDA5, and anti-SAE. In the next section, I will describe each DMSA and its clinical implications in detail.

2.8.1.1 Anti-Mi-2 autoantibody

The Mi-2 antigen was characterized from a dermatomyositis patient (Mi patient) for the first time in 1985 by Ira Targoff. This protein is a component of the nucleosome remodeling-deacetylase complex, which has a main role in the regulation of chromosomic transcription **(73, 74)**. This complex consists of 8 protein components, and sera from anti-Mi-2 positive patients react against one of these proteins: a 240 kDa. To date, two different, although highly similar proteins have been cloned resembling the 240 kDa: the Mi-2α and Mi-2β. The current line blot assays can detect antibodies against both proteins. The prevalence of this antibody ranges from 2 to 38% in adult DM patients **(75)**. A previous study had suggested that anti-Mi-2 positive patients had mild muscle weakness, but recent studies have shown that these patients present with more profound weakness than anti-Mi-2 negative patients **(76)**. In the same study, anti-Mi-2+ patients were usually not affected by fever or interstitial lung disease, typical manifestations of antisynthetase syndrome and, therefore, had better prognosis.

2.8.1.2 Anti-TIF1γ autoantibody

The target of anti- TIF1γ autoantibodies is a 155 kDa nuclear protein, the tripartite motif 33 protein, also known as transcriptional intermediary factor 1-gamma **(77)**. This protein has been found in varying levels in skin and muscle tissue and has been implicated in several physiological processes. Some of these processes include to act as a promoter of the transcription of DNA, regulator of posttranslational proteins, and regulator of the transforming growth factor beta (TGF-β) pathway. Anti-TIF1γ autoantibodies were described in 2006 for the first time. The overall estimated prevalence of these autoantibodies is 7-32% in all forms of myositis, especially in DM **(55, 78)**.

Only a small number of anti-TIF1γ positive patients develop pulmonary disease (16%) as well as arthritis and Raynaud's phenomenon compared with anti-TIF1γ negative patients **(79)**. However, the most striking feature of anti-TIF1γ patients is their association with malignancy, especially breast, ovary and lung cancer. Several epidemiological studies have shown that patients with myositis have a strong association with cancer-associated myositis (CAM), but this phenomenon is by far more frequent in patients with dermatomyositis **(55)**, particularly among adult patients positive for anti-TIF1γ (Odds ratio 23, 95% CI 5.2 – 101.2).

2.8.1.3 Anti-NXP2 autoantibody

Anti-NPX2 autoantibodies target a 140 kDa protein, the nuclear matrix protein, which is involved in p53 regulation. This autoantibody was first described in children with juvenile dermatomyositis (JDM), as anti-MJ autoantibody **(80)**; later also detected in adult patients **(81)**. The frequency of these autoantibodies ranges from 2 to 25% of patients with myositis, depending on the population studied.

Table 2. Autoantibody-defined phenotypes

Autoantibody	Frequency	Muscle involvement	Skin involvement	Lung involvement	Cancer	Other
Myositis specific autoantibodies						
Anti-Jo-1	15 – 30%	++/+++	+++	+++	-	Raynaud's phenomenon, arthritis, mechanic's hands, fever
Other anti-synthetase	<1 – 15%	+	++	+++	-	Pulmonary > muscular symptoms
Anti-Mi-2	2 – 38%	+++	+++	+/-	-	Skin disease
Anti-TIF1γ	7 – 32%	++	+++	-	+++	Cancer
Anti-NXP2	2 – 25%	+	+++	-	+++	Calcinosis
Anti-MDA5	5 – 20%	+/-	+++	+++	-	Skin vasculitis, RP-ILD
Anti-SAE	1 – 8%	++	+++	-	+	Dysphagia
Anti-SRP	5 – 15%	+++	+	+/-	-	Severe muscle weakness
Anti-HMGCR	5 – 10%	+++	-	-	-	Statin-related myositis
Myositis associated autoantibodies						
Anti-PM/Scl	8 – 10%	++	+++	+++	+/-	Sclerodactyly, dysphagia, Raynaud's
Anti-Ro52	10 – 40%	++	++	++	-	Raynaud's phenomenon
Anti-U1RNP	10%	+++	++	++	-	Puffy hands, lung fibrosis, myositis
Anti-KU	<2%	+/++	++	++/+++	-	Overlap syndrome
Anti-FHL1	14 – 27%	+++	+/-	-	-	Severe muscle weakness, muscle atrophy, vasculitis, dysphagia
Anti-cN1A	4-21%	+++	+/-	-	-	Association with IBM, SLE and Sjögren's

Other anti-synthetase: anti-PL7, anti-PL12, anti-OJ, anti-Ej, anti-Ko, anti-Ha, anti-Ks. RP-ILD: rapidly progressive interstitial lung disease

+/- unclear data, + uncommon, ++ common, +++ very common, - not known.

A distinctive clinical feature of anti-NXP2 positive patients is the elevated risk for suffering from calcinosis, in both JDM and DM patients compared to anti-NXP2 negative patients **(82, 83).** Anti-NXP2+ patients have been reported to have muscle atrophy, large-joint arthritis, and dysphagia **(80)**. Lung disease, on the other hand, is rather infrequent among anti-NXP2 patients **(57)**. Like anti-TIF1γ positive patients, it has been shown that adult anti-NXP2 positive patients, particularly at older age, may have a higher risk for cancer **(84)**.

2.8.1.4 *Anti-MDA5 autoantibody*

Anti-melanoma-differentiation-associated gene 5 (MDA5) antibodies target a retinoic acid-inducible (RIG)-like receptor: IFN induced with helicase C domain protein 1 (IFIH1) **(85)**. The frequency of these autoantibodies varies from 10-48% of Asian DM patients, but in approximately 2% of patients from a European cohort **(17)**.

Besides the classical DM skin rashes, anti-MDA5+ patients may have cutaneous ulcers, digital necrosis and palmar papules. A distinctive characteristic of these patients is alopecia, which tends to wean off once disease activity is controlled **(38)**. Another prominent clinical feature of anti-MDA5+ patients is the strong association with interstitial lung disease **(86)**, especially a life-threatening subtype known as RP-ILD. This form of ILD is defined as worsening of radiographic interstitial changes and progressive dyspnea occurring within 3 months of the onset of respiratory symptoms **(87)**. In contrast with the common extramuscular features, anti-MDA5+ patients present with minimal or no muscular symptoms, and the clinical picture is dominated by the cutaneous disease. This is why this form of DM subset is known as clinically amyopathic DM (ADM), which is now included as a subset in the EULAR/ACR criteria.

2.8.1.5 *Anti-SAE autoantibody*

Anti-small ubiquitin-like modifier-1 activating enzyme (SAE) antibodies were first described in 2007. The targeted antigen is a heterodimer composed of two subunits of 37kDa and 71kDa molecular weights (SAE1/SAE2, respectively). The SAE enzyme has an active function involving the regulation of chromatin and its interactions with other proteins, and chromatin accessibility and gene activation **(88)**. Between 1 to 8% of patients with DM are positive for this autoantibody.

Besides severe skin rashes with no special distribution, usual clinical symptoms include dysphagia (40-80%) and ILD with variable degree of radiographic patterns and severity. Patients with ILD usually have a course with normal or mild restrictive pulmonary functional tests **(89)**. A very interesting phenomenon in anti-SAE+ patients is the high risk for suffering from a hydroxychloroquine-triggered skin rash compared with anti-SAE- patients (OR 8.43; 95% CI, 1.98-49.19) **(90)**.

2.8.2 The anti-synthetase syndrome

The anti-synthetase syndrome (ASyS) is a subtype of the myositis essentially defined by the presence of autoantibodies targeting different aminoacyl-tRNA synthetase enzymes. The main function of these enzymes is to attach a sequence of amino acids to their corresponding cognate transfer-RNA (tRNA). At the beginning of my doctoral studies, eight different autoantibodies against the diverse aminoacyl- tRNA-synthetases had been described (**Table 3**). The prevalence of these autoantibodies among patients with myositis varies depending on the population studied and the assay used for their detection. Overall, the frequency of these autoantibodies represents approximately 20% of all patients with myositis. These anti-synthetase autoantibodies (ASA)

may coexist with the myositis-associated antibodies (MAA), but ASA are usually mutually exclusive.

Table 3. The different anti-tRNA synthetase antibodies and their frequency in patients with myositis.

Antibody subtype	Target antigen	Prevalence in myositis
Jo-1	Histidyl	20-25%
PL12	Alanyl	5%
PL7	Thronyl	5%
OJ	Isoleucyl	<5%
EJ	Glycyl	>5%
KS	Asparaginyl	<1%
Zo	Phenylalanyl	<<1%
YRS/Tyr	Tyrosyl	<<1%

The exact definition of ASyS is still under revision **(91)**, but the most widely used is the Connors's definition **(92)**. This definition classifies a patient as having ASyS if an anti-synthetase autoantibody is present plus one among the following: myositis, ILD, arthritis, unexplained fever, Raynaud's phenomenon and/or mechanic's hands. The most common clinical triad (myositis, ILD and arthritis) is present in about 20% of ASyS patients, and about 40% of patients may have concomitant occurrence of both ILD and myositis. However, more usual it is the prevalence of either myositis or ILD alone.

Muscle weakness or evidence for muscle inflammation ranges from 57% to 75% of anti-synthetase positive patients **(93, 94)**. This prevalence, however, depends on the method used to assess muscle involvement (e.g., MRI, electrophysiology, muscle biopsy, etc.). Anti-PL7+ and anti-PL12+ patients often show better muscle strength and lower levels of CK than anti-Jo1+ patients.

Pulmonary disease is the most common manifestation of the anti-synthetase syndrome, affecting approximately 75% of patients **(93)**. The radiographic patterns of ASyS-ILD are not specific for ASyS, as they may develop in other forms of myositis, but three main types of distribution are recognized: cellular/fibrosing Non-Specific Interstitial Pneumonia (NSIP), Usual Interstitial Pneumonia (UIP), and organizing pneumonia (OP). However, NSIP is the leading pattern in ASyS patients, accounting for 60-77% of patients with ILD **(95)**. Only few studies have analyzed the long-term prognosis of patients with ASyS-ILD, and prognosis of such patients is still controversial. Consequently, large longitudinal studies with international collaborations are needed to better understand the prognosis of lung disease in anti-synthetase patients.

Polyarthralgia is more common in anti-Jo-1+ than in other anti-synthetase patients (71% vs. 45%), but polyarthritis seems to have same distribution among other anti-synthetase groups (anti-PL7+ and anti-PL12+ (20%) **(93, 94)**. However, up to 65% of anti-EJ+ patients may have joint involvement. Interestingly, some anti-Jo-1+ patients have shown to harbor anti-citrullinated

protein autoantibodies, but their clinical significance is still inconclusive **(96, 97)**. Other clinical findings, for example pericarditis, are more common in anti-PL7+ patients **(98)**.

2.8.3 Immune-mediated necrotizing myopathy

Immune-mediated necrotizing myopathy (IMNM) is an emerging subtype of the inflammatory myopathies. For decades, this group of IMNM was considered as part of the PM spectrum, because of the clinical presentation and course. Currently, two autoantibodies highly specific for this entity has been identified: anti-anti-3-hydrozy-3methylglutaryl-coenzyme A reductase (HMGCR) and anti-signal recognition particle (SRP) antibodies. Yet a seronegative form of IMNM is also recognized and is associated with malignancy **(99)**.

Anti-SRP autoantibodies, described in 1986, have the signal recognition particles as their target. These proteins are cytoplasmic complexes of small RNA; they are involved in the functions of the endoplasmic reticulum during protein synthesis **(100)**. In 2010, the antigen for the anti-HMGCR antibodies was identified in a group of patients with biopsy-confirmed necrotizing myopathy who had been exposed to statin therapy **(101)**. Indeed, previous exposition to statins has been suggested as the main risk factor for inducing the formation of anti-HMGCR autoantibodies (between 44% to 66% of anti-HMGCR associated IMNM). Together, the anti-HMGCR and the anti-SRP autoantibodies are found in 6% to 15% of patients with myositis.

Anti-SRP+ patients are often younger at the time of disease onset than anti-HMGCR+ (mean age 39.9 vs. 63.5 years, respectively) **(102)**. Both groups of patients tend to have a more severe muscle involvement, both inflammatory and atrophic changes in MRI, compared to DM and PM. However, anti-HMGCR+ patients seem to have less severe muscle involvement than anti-SRP+ patients **(103)**. Extremely high levels of CK in IMNM patients, ranging from 3000 to 25000 UI/L, may be observed, and usually correlate well with muscle weakness. Some extramuscular manifestations in patients with IMNM include ILD, which varies in frequency, from none to less than 20% patients, and usually with a mild course **(99)**. Also, anti-SRP autoantibody was associated with cardiac involvement in an old study **(100)**.

An essential pathological feature of IMNM muscle biopsies is the absence or low number of inflammatory infiltrates. In 2018, the ENMC group published a consensus on the pathological features that must be observed to diagnose IMNM in the appropriate clinical context: 1) presence of necrotic fibers with scattered distribution, 2) necrosis and macrophagocytosis at different stages, but also regeneration, and 3) macrophage predominant infiltrates associated with low frequency of lymphocytes **(99)**. Noteworthily, muscle biopsies from IMNM patients lack the typical findings of PM muscle biopsies (non-necrotic inflammatory anti-CD8+ cells with perimysial distribution) or DM muscle biopsies (perivascular inflammatory infiltrates and perifascicular atrophy) **(102)**.

2.8.4 CTD-myositis

This subgroup of patients is defined as the combination of myositis and the presence of typical features of other connective tissue diseases (CTD), for example, systemic sclerosis (SSc) or systemic lupus erythematosus (SLE) **(104)**. Patients with an overlap phenotype should have a full picture of a CTD that fulfills classification criteria for that specific condition and should also fulfill IIM classification criteria. Additionally, similarly to patients with MSA, there are some associations between MAA and CTD-myositis **(78)**.

One of these overlap syndromes is the scleromyositis phenotype associated with anti-PM/Scl autoantibodies. These patients may present with myositis, Raynaud's phenomenon, dysphagia, arthritis, a variable degree of interstitial lung disease, but not with increased risk for malignancy or scleroderma renal crisis **(105)**. Another autoantibody initially associated with SSc is the anti-Ku autoantibody. Anti-Ku+ patients may present with signs of myositis and symptoms similar to anti-PM/Scl, but with no signs of cutaneous sclerotic changes. Importantly, anti-Ku+ patients without myositis may present with a CTD, for instance, SLE, Sjögren's syndrome or rheumatoid arthritis **(106)**. Among the MAAs, the most frequent in patients with myositis is the anti-Ro/SSA autoantibody, ranging from 20-30% of all patients with myositis **(104)**. This autoantibody consists of two components, the 52kDa and the 60kDa polypeptides. Anti-Ro antibodies are common in patients with Sjögren's syndrome, SLE, SSc, and in rheumatoid arthritis but also in other non-rheumatic autoimmune diseases for example biliary cirrhosis. Antibodies against the Ro52 antigen, also known as TRIM21, are particularly relevant in myositis because of their association with MSA, especially with anti-Jo-1 autoantibodies, and in lesser degree with other non-Jo-1 autoantibodies. The presence of anti-R052 autoantibodies in presence in serum of anti-Jo-1+ or anti-MDA5+ autoantibodies seems to confer a worse prognosis **(107)**. Lastly, the fourth MAA clinically relevant in myositis is the U1-RNP autoantibody. Positivity for anti-U1-RNP autoantibodies is part of the classification criteria for mixed connective tissue disease (MCTD). Puffy hands, myositis, pulmonary fibrosis and sclerodactyly occur as clinical manifestations of anti-U1RNP+ patients **(108)**, but isolated myositis as the first manifestation is unusual.

2.8.5 IBM phenotype

Inclusion body myositis (IBM) is now considered under the umbrella of IIM. Patients with IBM tend to be older than 50 years at onset of symptoms. It is more common in men than women. Characteristically IBM develops slowly with progressive, severe, often asymmetrical muscular weakness and atrophy, especially in the finger flexors **(109)** and the quadriceps. Other muscle groups frequently involved include foot plantar- and dorsiflexors and paraspinal muscles, and the latter can result in a head drop syndrome **(110)**. Up to 84% of women and 74% of men with IBM **(111)** may develop dysphagia at some point over the course of the disease often as a result of dysfunction of the cricopharyngeal sphincter **(110)**.

Most of patients with IBM do not harbor MSA, except for autoantibodies against the cytosolic 5'-nucleotidase 1A (NT5C1A) antigen. The prevalence of this autoantibody, although not specific

for IBM, ranges from 33-70% of these patients, and they are particularly useful in identifying patients with this form of myositis from PM. In addition, these autoantibodies may be useful as predictors of severe disease is still matter of discussion **(112)**.

2.8.6 Polymyositis

Finally, despite of being one of the major categories of myositis in old criteria such as the Peter and Bohan's, PM is nowadays more often regarded as an exclusion diagnosis. Indeed, several studies have evidenced that patients previously categorized as PM were re-classified as either DM, ASyS, IMNM, or IBM based on serologic, histopathological and clinical data **(113, 114)**. A group of special consideration is PM patients negative for autoantibodies, both MSA and MAA (i.e., seronegative). The initial clinical debut of these patients may share similarities with several different mimickers (e.g., endocrinological myopathies, channelopathies, muscle dystrophies, among others), and thus require a careful diagnostic approach. For this reason, some experts believe that PM does not exist anymore as a category of IIM **(115)**. Still, patients clinically classified as PM may have some pathological differences compared with other forms non-immune myopathies such as inflammatory infiltrates with perivascular distribution, as well as CD8+ cytotoxic cells surrounding healthy, non-necrotic fibers that have high expression of major histocompatibility class (MHC)-1 molecules cytotoxic T cell response **(116)**.

To sum up, testing patients for autoantibodies might help to better define different phenotypes of the myositis spectrum. This in turn might be useful to recognize patients with myositis earlier in the disease course, that potentially could impact the level of response to treatment. However, it is not completely explored if the presence of autoantibodies is useful as predictors of the level of response to pharmacological treatment or further irreversible complications, for example, chronic or persistent damage.

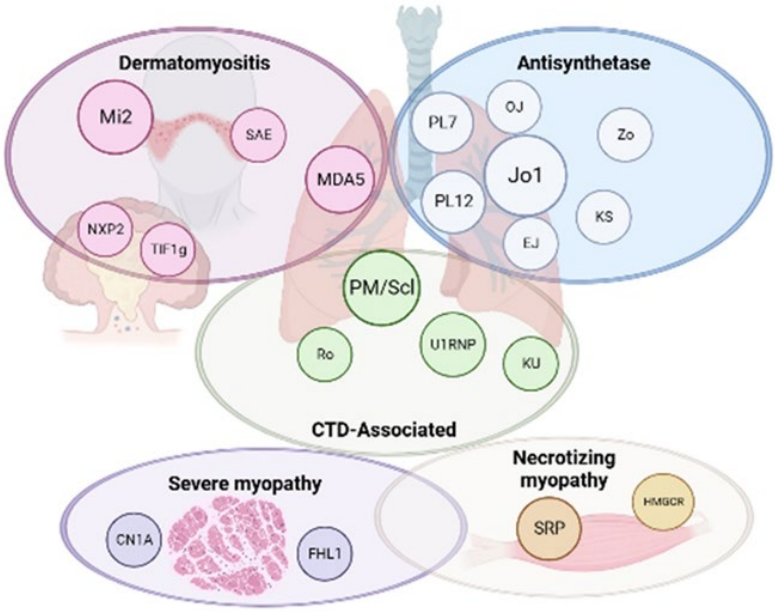

Figure 1. Clinical and serological subgroups of idiopathic inflammatory myopathies. Myositis-specific and myositis-associated autoantibodies have strong associations with clinical manifestations. CTD-Associated: Connective tissue disease associated myopathy *Created with Biorender.com*

2.9 Medical treatment

The evidence suggesting that the adaptative immune system is implicated in the pathophysiology of myositis is the rationale for using immunosuppressive drugs, as well as glucocorticoids (GC), as the cornerstone of medical therapy.

2.9.1 Glucocorticoids

Since the description of the first case of corticotropin as medical therapy in a 5-year-old boy with DM **(117)**, clinicians and researchers have continuously focused on the use of GC as treatment for rheumatic diseases. Although there are no available controlled trials testing the use of GC in patients with myositis, this therapy is considered as the mainstay of treatment for patients with myositis. Conventionally, patients are treated with the common 1 to 0.75 mg/kg, followed by a "taper" approach. Nonetheless, some experts recommend that for very severe disease, such as ILD, patients should be given intravenous pulse therapy (between 500 to 1000 mg per day for three days). The ideal length of a glucocorticoid course is not well defined but the general consensus is to strive to reach a stable dose of 5 to 10 mg after 6 months of the initial doses **(118)**. Yet, a formal comparison between different GC doses has not been done.

2.9.2 Immunosuppressive agents

Although GC therapy currently works as an anchor medical strategy for patients with myositis, several adverse side effects preclude their chronic use. Therefore, the addition of GC-sparing drugs is required to maintain the immunosuppressive effect achieved by intensive GC treatment.

To date, there are only few studies, most of them case series, that have tested the efficacy of conventional immunosuppressive drugs on muscular and extramuscular disease. A study demonstrated a better effect on muscle endurance after adding Methotrexate (MTX) or cyclosporine A (CyA) over prednisone alone **(119)**. Another study found that patients who were given MTX had a higher probability of complete response than patients who were given Azathioprine (AZA) **(120)**. Azathioprine showed no further benefit on muscle strength over treatment with prednisolone alone after three months of therapy, but a long follow-up (three years) of the same study showed that the combined therapy (AZA + GC) had a better impact on functional status than prednisolone alone **(121, 122)**. In patients with myositis-ILD, the use of AZA was associated with a better efficacy profile and a better GC-sparing effect than mycophenolate mofetil (MMF) **(123)**. However, another study showed that either AZA or MTX have similar rates of improvement in pulmonary function tests in patients with ASyS **(124)**. Mycophenolate mofetil has shown to be a safe alternative for juvenile and adult patients, however, in most of the studies patients had a clinical dermatomyositis phenotype, thus these reports should be taken with caution when patients with other clinical subtypes are encountered **(125, 126, 127)**.

For patients with refractory disease, calcineurin inhibitors have been proved to be an effective option. Most of the evidence, however, comes from retrospective studies from Japan, where the use of tacrolimus has been particularly assessed in patients with MDA5-associated ILD **(128, 129)**. Nonetheless, a small report from the United States of America (USA) described that tacrolimus might be a first-line drug especially for patients with anti-SRP or anti-Jo-1 autoantibodies and with ILD **(130)**. Another study from the USA, though retrospective, showed that tacrolimus was effective in ILD, in special in DM, independent of the autoantibody status **(131)**. Finally, another option for severe cases is cyclophosphamide, which is nowadays reserved for severe ILD, usually with doses between 0.3 to 1.5 g/m2 with monthly intervals for 6 to 12 months **(132)**.

2.9.3 Biologic therapy

The incoming of biologic therapy opened the door for new possibilities for patients living with myositis. Since the advent of these therapies, several mechanisms of action have been tested in different subtypes of myositis.

Tumor necrosis factor alpha (TNF-α) inhibitors have shown to be effective in treating patients with rheumatoid arthritis and spondylarthritis. Only infliximab, adalimumab and etanercept have been tested in myositis but with conflicting results **(133, 134, 135)**. On the one hand, a patient with DM and lung involvement was treated with adalimumab, and after three-month treatment, skin rashes, muscle weakness and ILD improved **(135)**. On the other hand, an open pilot study showed that patients treated with infliximab had no improvement; moreover, an increased production of blood type I interferon together with a clinical flare in some patients were observed **(133)**. Anakinra, a recombinant blocker of the interleukin-1 (IL-1) receptor, was studied in a group of 15 patients, and showed that seven of patients improved clinically and some improved

their muscle endurance **(136)**. The effect of abatacept, a fusion protein of the cytotoxic T lymphocyte protein 4 (CTLA4) and the Fc portion of human IgG1 that blocks T cell co-stimulation, was investigated in an open label randomized controlled trial **(137)**. This study showed that 40% of patients on abatacept improved in muscular and extramuscular measures at both 3 and 6 months after the randomization. Unfortunately, the authors could not identify any clinical phenotype as predictor for response. Tocilizumab, an IL-6-receptor inhibitor, useful in treating patients with RA, was tested in patients with refractory PM/DM in a multicenter randomized placebo-controlled trial **(138)**. In this trial, tocilizumab failed to show an improvement in different clinical measures compared to the placebo group after 6 months of treatment. Inhibition of the Janus kinase complex (JAK) is a relatively novel therapeutic option for patients with RA, spondylarthritis, and other non-rheumatic conditions. The first JAK-inhibitor, tofacitinib, was studied in a small open-label study including patients with DM and demonstrated good clinical efficacy in skin disease **(139)**. A long-term follow-up study (96 months) showed the treatment is safe and well tolerated **(140)**. Tofacitinib has been also investigated in patients with MDA5-related ILD with promising results **(141)**. One of the main concerns of the JAK inhibition especially tofacitinib is however, the high frequency of thromboembolic and cardiovascular complications **(142)**.

Rituximab is the best studied biologic therapy for patients with myositis and therefore deserves especial mention. This drug downregulates the frequency of B-cells by targeting the anti-CD20 receptor. The effect of this drug is temporary, and the restitution of the B-cell populations occurs typically between six to twelve months after the infusion. The efficacy of this drug was assessed in the RIM (Rituximab in Myositis) study, a randomized, placebo-controlled trial by showing clinical benefit in more than 80% of patients **(143)**. Besides the RIM trial, several small studies have assessed the effect of rituximab and suggest that this drug may be useful in some specific subgroups of patients such as the IMNM and in other forms of disease beyond muscle symptoms (ILD) **(144)**. There is indeed an especial interest on exploring the potential predictive value of autoantibodies and other biomarkers as prognostic tools to help clinicians to guide medical therapy.

A randomized clinical trial in only patients with DM was conducted using intravenous immune globulin (IVIg) as active treatment arm. This study reached its primary endpoint, that is, minimal improvement (Total Improvement Score $\geq$ 20 out of 100) in the active treatment arm **(145)**. This drug is now approved by the Food and Drug Administration agency for treatment of DM, especially with severe cutaneous disease. International experts also recommend its use in patients with IMNM **(146)**. One of the caveats of IVIg, nonetheless, is that it has not been tested for severe muscular disease or other extra muscular manifestations, which makes difficult to extrapolate the findings to patients with other phenotypes such as ASyS.

Most of the aforementioned studies on pharmacological treatment have focused on patients with long-standing diagnosis, that is, patients with well-established disease and sometimes refractory to first-line medication. Unfortunately, these studies cannot be extrapolated to patients with a

recent diagnose or an early disease. Thus, there is a lack of knowledge on biomarkers and/or predictors for response to treatment in patients during the early course of their disease.

2.9.4 Exercise therapy

Exercise as a therapeutic intervention for patients with myositis has been a striking progress, and now is part of the rehabilitation of these patients. Since the first trials assessing the safety of some training programs during the 1990's, it is now recognized that exercise improves muscle endurance, muscle function, and quality of life **(147)**.

At the molecular level, exercise can downregulate genes implicated in inflammation and fibrosis **(148)**, and improve muscle aerobic capacity by decreasing the lactate levels and increase mitochondrial enzyme activity **(149)**. It is therefore advisable that patients with patients with myositis, especially early after they receive the diagnosis, should start up a training program always supervised by an experienced physiotherapist.

2.10 Prognosis

The meaning of this term refers to the possibility of predicting or foreseeing the risk of a certain outcome or a future situation. In medicine, it is usually considered that researchers strive to predict the course of an illness, but in fact, what is relevant is the ability to predict the course of an illness in a particular patient. In the case of research in myositis there is still a broad meaning of this term. However, we could consider that by using this concept the intentional meaning is in general the long-term course or long-term state of a given patient.

As commented in the Epidemiology section, myositis disorders are uncommon conditions with significant impact on daily activities, participation and quality of life. Although it could be considered that death is the ultimate organ damage, patients that may survive the critical first year after diagnosis may experience a tremendous burden of disability due to the disease itself, complications of fibrosis/scarring processes, and/or adverse effects of the intense immunosuppressive therapy. For example, a prospective study from the late nineties showed that the disease course of 257 patients with PM and DM differed depending on the age at diagnosis: patients older than 60 years at diagnosis had a more rapidly increase of physical disability than patients younger than 60 years, and this increase was more accentuated if they had experienced an avascular necrosis or vertebral compression fracture **(150)**. Another study demonstrated that myositis diagnosis is strongly associated with limited work ability, and physical disability correlates with less chance of doing paid work **(151)**. Unfortunately, most of long-term studies have focused on patients with the juvenile variant of myositis and there is lack of studies of natural history in patients with adult myositis that have investigated the course of patients depending on the different autoantibody groups. Also, it is unknown how potential associated factors can help to improve the prediction of response to treatment especially in patients with recent diagnosis.

2.11 Outcome measurement

Taking care of patients with myositis requires that the medical professionals can apply adequate tools to evaluate the effect of medical therapies, follow up long-term outcomes and self-reported health assessment. But before describing the different standardized measures, I will provide a brief notion of the constructs that have given rise to these outcomes.

The total set of pathological features due to the inflammatory process, as well as its extent and severity, which are susceptible to reversibility and potentially able to return to normal is known as *disease activity* **(152)**. On the other hand, the persistent and/or permanent change in anatomy, physiology, pathology or function that may appear after the diagnosis of the disease is known as *damage* **(152)**.

2.11.1 Disease activity

To achieve the goal of evaluating these constructs, an international network including physicians, physiotherapists, statisticians, and patients as research-partners have standardized the way of measuring and reporting different dimensions of health care of patients with myositis. This group, the International Myositis Assessment and Clinical Studies Group (IMACS), defined first a set of a minimum measures to be included in the assessment of disease activity **(153)**. These core set measures (CSM) include the physician and patient global assessment of disease activity, muscle strength, physical function, laboratory assessment, and global disease activity including extramuscular assessment. Also, although not included in the CSM, the IMACS recommends the use of the Short Form-36 for the evaluation of health-related quality of life (HRQoL) in adult patients.

Physician global assessment (PhyGA) is measured either on a 0 to 10 cm visual analogue scale (VAS, where 0 means no disease activity and 10 cm is very high disease activity) or on a 5-point Likert scale (0 means no disease activity and 4 means extremely high disease activity). This measure is based on the physician's judgement supported by medical history, physical examination and laboratory assessment. PhyGA has a good correlation with muscle strength and levels of muscle enzymes, and has good discriminant validity (i.e., the change in the scale is higher in patients who are responders to treatment than in non-responders) **(153)**.

Patient-reported outcome measures (PROM) are measurements directly taken from the patients' own perspective of their disease, the status of their well-being without the modification of the medical staff. The *patient global assessment* (PatGA) is measured in the same way as the PhyGA, and these two measures, PhyGA and PatGA, correlate moderately **(154)**. In order to capture this dimension, adult patients (or parents to children) are asked to consider the total effect that the rheumatic disease has on their body including muscles, skin, lungs, etc. The usual recall period for assessing this domain is between the previous 2 to 4 weeks.

Several other dimensions might possibly be captured within the domain of PatGA, for example, fatigue and pain. The Outcome Measures in Rheumatology Clinical Trials (OMERACT)

Myositis Special Interest Group (SIG) conducted a set of interviews in three different countries aimed to explore the life impact of myositis. This study showed that muscle symptoms, fatigue, interaction with health-care providers, side effects of medications and pain were the top five domains **(155)**. These results have encouraged clinicians and researchers alike to explore deeper into importance of these PROs.

Muscle weakness is the hallmark of myositis and thus it is of outmost importance to quantify the level of muscle strength during the diagnostic approach. Moreover, it is crucial to assess the muscle strength, defined as the voluntary isometric strength in a particular muscle group, in order to evaluate the effect of therapeutic interventions during the follow-up. The IMACS recommends the use of the *Manual Muscle Test* of 8 muscle groups (MMT8) **(156)**. The muscle groups included are the neck flexors (axial muscles), deltoids middle, biceps brachii, gluteus maximus, gluteus medius, quadriceps (proximal muscles), wrist extensors, and ankle dorsiflexors (distal muscles). By using this test, the examinator scores the muscle strength by using either the Medical Research Council (MRC) or the Kendall grading scale. The Kendall scale uses a 10 cm scale for each muscle group where 0 corresponds to no contraction whereas 10 reflects a sustained position against strong pressure. Then, each of the eight scales are summed up into a maximal total score of 150 if both sides are included or 80 if only the dominant side is evaluated. MMT8 has good discriminant validity **(154)**, however, there is no consensus for an absolute value reflecting a meaningful and relevant difference. The IMACS Rehabilitation and Exercise Scientific Interest group recommends the use of a muscle endurance measure to complement the MMT8 **(157)**. The Functional Index 2 or 3 is now used in Sweden and other countries in the follow-up and/or clinical work-up, which has less ceiling effects than the MMT **(158)**.

Physical impairment in patients with myositis may be assessed by using several different questionnaires **(159)**. These tools tried to capture the impact of the disease on different aspects of the daily life of patients. The most common used questionnaire in rheumatology is the *Health Assessment Questionnaire (HAQ)*. Initially, this tool was designed to be used in patients with rheumatoid arthritis, but its use has now spread to be applied in other conditions, for example, myositis. In fact, the HAQ is included in the CSM, although not thoroughly validated for myositis. This questionnaire correlates moderately with muscle strength measured using the MRC scale **(154)** and mildly but significantly with disease activity **(154)**. The HAQ score ranges from 0 to 3, where 0 reflects no or mild physical dysfunction, <0.125 to 0.25 mild dysfunction, and >1.0 moderate to severe disability **(154)**.

Muscle enzymes additional to CK may be elevated when inflammation is present in muscle tissue as described in Muscular symptoms Section. These enzymes correlate moderately with each other, and they are not redundant measures **(153)**. Because CK levels and other enzymes may show variations that do not mirror disease activity, these measures have the lowest weight in the 2016 ACR/EULAR Response criteria **(160)**.

Because of the very frequent extramuscular involvement beyond the skeletal muscle in patients with myositis, the IMACS has created two tools to assess this domain: the Myositis Intention to Treatment Index (MITAX) and the Myositis Disease Activity Assessment Tool (MDAAT). Firstly, the MITAX index is basically a modification of a similar tool derived from patients with SLE, the BILAG. It is based on the principle of the physicians' intention to treat **(152)**. The evaluator assigns different values/scores depending on the presence of active disease in each organ or system. Lastly the MDAAT score is a set of 10 cm visual analogue scales (like PhyGA higher score reflects higher disease activity) and is completed by the evaluator. The organs/systems that are assessed are constitutional, pulmonary, gastrointestinal, cardiovascular, cutaneous and articular **(152)**. Both assessments express the total disease activity mainly in the extramuscular organs affected and may be used alone or combined.

2.11.2 Definition of Improvement

The median minimum of improvement for global assessments (physician's, patients', and extramuscular) is at least 20% to be considered as clinically meaningful. For physical function and muscle strength, a median minimum of 15% has been agreed in order to be considered as improved. Serum levels of muscles enzymes, however, need to achieve at least a median change of 30% to score as improved **(156)**. Additionally, the IMACS group published several definitions of improvement in the CSM, and the definition with highest rate is the one requiring at least 20% of improvement in at least 3 out of the 6 CSM, with no more than 2 CSM worsening greater than 25% (not including muscle strength) (**Table 4**).

Table 4. Core set measures and the minimum percentage of change in each measure to classify as clinically improved

Core set domain	% Change for improvement	Measurement
Physician global assessment	20	VAS or Likert scale (0 – 10 cm)
Patient global assessment	20	VAS or Likert scale (0 – 10 cm)
Muscle strength	20	Manual muscle test (8 muscle groups)
Physical function	20	Health Assessment Questionnaire
Muscle enzymes	30	Elevated serum activity of muscle enzymes
Extramuscular activity	20	MDAAT

VAS: visual analogue scale; MDAAT: Myositis Disease Activity Assessment Tool

2.11.3 The ACR/EULAR response criteria

In 2017, a new set of criteria for assessing the response to treatment in patients with myositis, both adult and children, was published **(160)**. These standardized criteria were obtained from data-driven cases and resulted in a hybrid set of criteria, that is, as categorical or continuous scales, for both adult and juvenile patients (**Table 5**). These outcomes are generated from the Total Improvement Score (TIS) on a scale from 0 to 100. This score is the sum of the improvement in each of the six CSM. However, the absolute change in each individual CSM is "transformed" by using a "weight" depending on the importance or relevance of each CSM, thus the CSM considered more important result in higher contribution to the TIS.

As mentioned before, these criteria may be used as a continuous outcome due to a greater sensitivity to change or as a categorical outcome. In the latter case, an improvement of equal or greater than 20 in the TIS is considered as minimal, an improvement equal or greater than 40 is considered as moderate, and an improvement equal or greater than 60 as major. At the beginning of my PhD studies, no study had validated the use of these criteria.

2.11.4 Organ damage

To be considered as a permanent or persistent change in anatomy, physiology or function, a change must have occurred after established diagnosis of myositis and persisted for at least 6 months even after initiation of treatment. Therefore, only items present since date of diagnosis should be considered.

The two available measures specifically designed for evaluating damage in patients with myositis are the Physician Global Damage score and the Myositis Damage Index (MDI). The former is applied in a similar way as PhyGA but considering the total organ damage. The MDI tool is a modification of the Systemic Lupus International Damage Index (SDI). The MDI is composed by two complementary parts assessing 11 organ or systems: muscle, skeletal, cutaneous, gastrointestinal, pulmonary, cardiovascular, peripheral vascular, endocrine, ocular, infection, and malignancy **(154)**. The first component, MDI extent of damage score evaluates the presence (or absence) of a given item e.g., muscle atrophy. The total possible score in children is 0 – 35, in teenagers 0 – 37 and in adults 0 – 38. The second component, MDI severity of damage score, is proposed as a sum of the 10 cm VAS scores for each of the eleven individual organ and systems, and therefore the possible score ranges from 0 to 110. In addition, the categories of Other damage and Global Damage may be included but are not part of the severity score. These two components have strong correlation, but they are not redundant measures **(161)**.

Table 5. ACR/EULAR criteria for minimal, moderate and major improvement in adult IIM

Core set measure	Level of improvement	Improvement score
Physician global activity	Worsening to 5%	0.0
	>5 – 15% improvement	7.5
	>15 – 25% improvement	15.0
	>25 – 40% improvement	17.5
	>40% improvement	20.0
Patient global activity	Worsening to 5%	0.0
	>5 – 15% improvement	2.5
	>15 – 25% improvement	5.0
	>25 – 40% improvement	7.5
	>40% improvement	10.0
Manual muscle testing	Worsening to 2%	0.0
	>2 – 10% improvement	10.0
	>10 – 20% improvement	20.0
	>20 – 30% improvement	27.5
	>30% improvement	32.5
Health Assessment Questionnaire	Worsening to 5%	0.0
	>5 – 15% improvement	5.0
	>15 – 25% improvement	7.5
	>25 – 40% improvement	7.5
	>40% improvement	10.0
Enzyme (most abnormal)	Worsening to 5%	0.0
	>5 – 15% improvement	2.5
	>15 – 25% improvement	5.0
	>25 – 40% improvement	7.5
	>40% improvement	7.5
Extramuscular activity	Worsening to 5%	0.0
	>5 – 15% improvement	7.5
	>15 – 25% improvement	12.5
	>25 – 40% improvement	15.0
	>40% improvement	20.0

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The IMACS has endorsed and recommended the use of the MDI score. However, the clinical meaning of the MDI and its individual components has not been established. Because this tool is derived from the SDI, evidence on the variables contributing to organ damage comes from studies assessing the accrued damage in patients with systemic lupus erythematosus. Thus, some of these variables have been evaluated in patients with myositis, for example, age and high disease activity at diagnosis are known determinants of organ damage **(162, 163)**. Nonetheless, many other factors, some of them inherent to patients with myositis like the presence of autoantibodies, have not been explored.

To sum up, the role of autoantibodies as prognostic markers in patients has not been completely studied. The lack of validation and harmonization of new assays for detecting autoantibodies, particularly line blot, is still of concern. The value of myositis autoantibodies, both MSA and MAA, as predictors for response to treatment in patients with recent diagnosis and the role of autoantibodies in predicting organ damage has not been explored. Additionally, the relationship between inflammatory markers and self-reported health has not received enough attention and therefore has not been studied. With this thesis, I have tried to make some steps forward to fill these knowledge gaps at least to some extent.

3 Research aims

The overall aim of this thesis was to explore the prognostic value of autoantibodies and inflammatory markers in patients with idiopathic inflammatory myopathies.

The specific aims of this thesis were:

1. To compare a line blot assay containing 16 specificities with an immuneprecipitation-based algorithm as an attempt to validate the former assay, and to characterize clinical associations with the autoantibody specificities in order to recognize the clinical usefulness of the line blot assay.

2. To explore the prognostic value of autoantibodies and other clinical features for predicting response to treatment in a group of patients with recent diagnosis of myositis by using a standardised set of criteria after one year of treatment.

3. To investigate the patterns of extent of accrued damage in patients with myositis as well as the trajectories of damage over time depending on the autoantibody status.

4. To evaluate the correlation between Patient Global Assessment with systemic inflammatory markers, CRP, ESR and CK levels, in patients with myositis as well as to explore if this correlation could be explained by functional measures such as muscle strength, functional disability and overall disease activity.

4 Materials and methods

4.1 Data sources

Different sources of data were used to obtain patient data to carry out the four different studies.

The Karolinska Myositis Cohort.

Since 2003 patients with diagnosis of inflammatory myopathies have been prospectively followed at the Myositis Clinic Karolinska University Hospital, Stockholm, Sweden. At diagnosis, patients are asked for their consent to blood samples for diagnostics and research purposes (including serum and DNA and these samples are stored in a biobank). At the first meeting with the healthcare team, patients have a full assessment of muscle strength, muscle function and muscle endurance, and are given an individually adapted exercise program followed by a team conference soon after the introduction of the medical treatment. Most patients also meet the occupational therapist for evaluation of hand function, activity limitation and an individual hand exercise program. Then, patients are followed in the clinic according to a local healthcare myositis plan at 3, 6 and 12 months and then yearly. At each time point, patients are clinically examined, and blood samples are drawn. In addition, patients are invited to be followed in the SweMyoNet and the MyoNet registries described below.

Swedish Rheumatology Quality register (SRQ)

The Swedish society has an historic tradition of keeping registers since the 1600's when the Swedish church started to keep notations of parish members **(164)**. Nowadays, the welfare system in Sweden, which is mostly tax-funded, makes use of the Personal Identification Number from the Total Population Register. Following this tradition, the SRQ was created in 1995 by the Swedish Society for Rheumatology **(165)**. The aim of this register is long-term follow up of patients with rheumatic diseases to evaluate the effects of treatment. In this registry, there is a specific module for patients with myositis, the SweMyoNet, where clinicians and health professionals register data on patients' disease activity, symptoms, laboratory tests and treatment as well as standardized scores, for example MDAAT and MDI scores. After giving their consent to be included at diagnosis, patients are followed up prospectively. Patients are included via either the outpatient or inpatient facilities.

The MYONET consortium

In 2008, through a European Union-funded project, investigators created an international web-based registry, EuroMyositis, with the starting centers in Manchester, UK, Prague, Czech Republic and Karolinska Institutet, Stockholm, Sweden. This was later expanded to include centers both within and outside EU: including Belgium, Brazil, China, Germany, Hungary, Italy, Mexico, Norway, Spain, and Switzerland. This consortium, initially named EuroMyositis (http://euromyositis.eu), has recently changed its name to MyoNet to express that also non-European participating centers have contributed with patients in the registry. Physicians and

researchers from participating centers enroll patients who are assigned an individual anonymized code. To date, more than 6000 patients with a diagnosis of myositis from more than 30 centers are registered in MyoNet. The registry has collected information on demographic data, disease activity, symptoms, laboratory tests, treatment, outcome measures and data on autoantibodies. Several centers have also longitudinal (follow-up) data for their patients **(7)**.

4.2 Study population

In **Paper I**, patients were retrospectively retrieved from the Karolinska Myositis Cohort between 2013 and 2017. Demographic and clinical data were obtained from the SweMyoNet registry and when needed, medical records were reviewed to complete missing data. A total of 110 patients and 60 healthy controls were included in this study.

For **Paper II**, patients were retrospectively selected from the SweMyoNet registry (n=411 patients) if they had been registered within 12 months (range 0.2 to 11.3 months) from the diagnosis, between January 2003 and December 2015. For this study, patients with JDM and IBM diagnoses were excluded. The date of inclusion to the SweMyoNet was considered as the *index date*. A total of 156 patients were included in this study.

For **Paper III**, the entire dataset of MyoNet (n=4961 patients) on February 1, 2021, was exported, after having anonymized each patient with individual codes. Fourteen centers from 10 different countries contributed to the final dataset. From the entire dataset patients with an available autoantibody test plus registered data on the Myositis Damage Index (MDI) were included. A total of 769 patients met the inclusion criteria. The *index date* was defined as the time point for the first registered MDI assessment.

In **Paper IV**, the entire MyoNet dataset as for Paper III was used (n=4961 patients), and patients were included if they had registered information on Patient Global Assessment (PatGA) at index date. The total number of patients included in this study was 1333 patients. The *index date* was considered as the time of inclusion in the MyoNet registry.

4.3 Classification of patients

All possible candidates to be included in **Paper I-IV** were retrospectively classified as myositis according to the 2017 EULAR/ACR criteria **(26)**. Patients were included if the probability for myositis was ≥ 50% and thus patients with possible (≥50% and <55%), probable (≥55% and <90%) and definite (≥90%) myositis were included. Next, if available information, patients were subclassified into DM, ADM, JDM, PM, IBM.

In **Paper III**, patients with probability for myositis <50% (2017 EULAR/ACR criteria) but had a positive test for either MSA or MAA were included. For **Paper III-IV**, patients fulfilling the criteria for systemic lupus erythematosus **(166)**, systemic sclerosis **(167)**, rheumatoid arthritis **(168)**, Sjögren's syndrome **(169)** and mixed connective tissue disease **(170)** were classified accordingly and categorized as CTD-myositis. Patients with DM, ADM, or PM who fulfilled the

Connor's criteria for anti-synthetase syndrome **(92)** or ENMC criteria for IMNM **(99)** were reclassified accordingly. Patients who were initially registered by the recruiting clinician as ASyS and patients who were reclassified as ASyS retrospectively if they tested positive for any anti-aminoacyl-tRNA synthetase autoantibody were pooled.

4.4 Methods for detecting autoantibodies

In **Paper I**, available sera from consecutive patients with myositis collected between 2013 and 2017 and followed in the Karolinska Myositis Cohort were analyzed. The same serum samples were analyzed by IP in a collaboration with Prof. Mimori, Kyoto University, Japan and by LB assay in collaboration with the Immunology Department at Uppsala University. In addition, sera from 60 healthy controls were tested for autoantibodies using the LB assay.

4.4.1 Line blot Protocol.

Sera were analyzed by using a commercial line blot assay (EUROLINE Autoimmune Inflammatory Myopathies 16 Ag (IgG) with semiquantitative evaluation of staining intensity for 16 specificities at the Department of Clinical Immunology, at the Uppsala University Hospital. The results were reported according to the manufacturer's instructions as negative or positive (0 to 10 vs. ≥ 11 densitometry units). Every strip included recombinant human proteins for Mi-2 (α and β chains), TIF1γ, MDA5, NXP2, SAE1, Ku, PM/Scl (subunits 100kDa and 75kDa), SRP, Jo-1. PL-7, PL-12, EJ, and OJ. In the LB, the anti-Ro52 autoantibody is also included, however, this was not analyzed in Paper I as this specificity is not detected by IP.

4.4.2 Immunoprecipitation Protocol.

As for IP methods, anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, and anti-SRP autoantibodies were detected by RNA-IP (immunoprecipitated RNAs are detected by silver staining after gel preparation), and the rest of the specificities (PM/Scl, Ku, NXP2, MDA5, TIF1γ, and Mi-2) were detected by protein-IP using ^{35}Metlabelled cell extract (immunoprecipitated proteins are detected by autoradiography after gel preparation) **(171)**.

4.4.3 ELISA Protocol.

An in-house **anti-MDA5 ELISA,** performed in Japan, was used to confirm anti-MDA5 antibody in this study. Briefly, the C-terminal half (520aa) recombinant MDA5 protein containing His-tag (using PCR-cDNA from HeLa mRNA and pET151 Directional TOPO™ as a vector, ThermoFisher Scientific) was expressed in E. coli (BL21. Star-DE3) and purified by a nickel column (Ni-NTA Superflow Cartridge, QIAGEN). 0.45ug/100ul solution of the recombinant MDA5 was coated on the 96-well ELISA plate (NUNC-IMUUNO PLATE 442404, ThermoFisher Scientific) (at 4ºC for overnight). The plate was blocked with 1% bovine serum albumin in PBS. 100ul of patient sera (diluted 1:100 and absorbed by E. coli) were reacted at RT for 1h, washed 5 times with PBS-0.1% Tween20, and incubated with HRP-conjugated anti-human IgG (1:10,000 dilution, Promega) at RT for 1h. TMB (3, 3', 5, 5'-tetramethylbenzidine)

was used for substrate of HRP and absorbancy at 450nm was measured. Sera from five of the anti-Jo1 LB+/IP- patients were also evaluated with a commercial **anti-Jo-1 ELISA** (Profile, Phadia, Freiburg, Germany).

In **Paper II**, the first two aforementioned protocols were used to test for autoantibodies. Seventy patients were tested by line blot and eighty-six patients by immunoprecipitation. Anti-HMGCR autoantibodies were analyzed by Dr. Andrew Mammen of the U.S. National Institute of Health using a combined protocol of immunoprecipitation followed by an ELISA assay **(172)**.

The assay used for testing autoantibodies in **Paper III** was dependent on local practice. Of the 769 cases included, 266 (35%) were determined by immunoprecipitation, 162 (21%) by line blotting, 70 (9%) by ELISA, and 14 (2%) by other methods. In 257 (33%) cases, the method was unknown/not registered.

4.5 Autoantibody-defined subgroups

In **Paper II**, patients were categorized into subgroups depending on the presence of autoantibodies as follows: 1) ASyS (anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ), a) DMSA (anti-MDA5, anti-TIF1γ, anti-Mi-2 and anti-SAE), 3) autoantibodies associated with IMNM (anti-SRP and anti-HMGCR), 4) MAA without any MSA (anti-PM/Scl, anti-U1-RNP, anti-Ro, anti-Ku), and seronegative (negative to any of these autoantibodies).

In **Paper III**, patients were sub grouped depending on their autoantibody status as follows: 1) ASyS (anti- ASyS (anti-Jo-1, -PL-7, -PL-12, -EJ, -OJ), 2) DMSA (anti-MDA5, -TIF1γ, -Mi-2, -SAE, -NXP2), 3) IMNM- autoantibodies (anti-SRP, -HMGCR), 4), MSA- and MAA+ (anti-Ro-52, -U1RNP, -Ku), 5) anti-PM/Scl, and 6) negative to any of these autoantibodies (seronegative).

4.6 Clinical data

Duration of symptoms prior to diagnosis are self-reported and registered in SweMyoNet. These data are confirmed by revision of medical records.

Disease activity was assessed by using the six item core set measures (CSM), namely, Physician and Patient Global assessment (PhyGA and PatGA respectively), manual muscle test of 8 muscle groups (MMT8), Health Assessment Questionnaire Disability Index (HAQ-DI), levels of serum muscle enzyme CK, and global extramuscular assessment (MDAAT score) **(153)**. In **Paper III-IV**, the *levels of CK* were standardized as a ratio of the upper limit of normal; this was performed due to the different scales used at different centers. Inflammatory markers, *C-reactive protein* (CRP, reference value < 5mg/L) and *erythrocyte sedimentation rate* (ESR, reference value <15 mm/h in men and <20 mm/h in women) were retrieved.

Comorbidities. In **Paper I-IV** the definition of MyoNet for the different comorbidities was used **(7)**. Any malignancy within ± three years of myositis diagnosis was defined as *myositis-associated cancer*. *Heart involvement* related to myositis is recorded if there is presence of: a) pericarditis (inflammation of the pericardium defined clinically or by electrocardiogram), b) myocarditis (inflammation of the myocardium defined clinically or with echocardiographic or other objective evidence), or c) arrythmia (clinical or electrocardiographic evidence of irregular heartbeat. *Interstitial lung disease* was defined by means of radiologic examination (chest X-ray or chest computed tomography scan) as documented inflammation or scarring of the parenchyma of the lung and abnormal pulmonary function tests attributable to inflammatory process or pulmonary fibrosis. *Dysphagia* was defined as difficulty with swallowing, chewing or eating documented by clinical symptoms or by barium swallow examination, manometry, or other objective measure. *Smoking* was defined as having ever smoked at least one cigarette per day for more than one year.

Treatment. In **Paper II**, data about treatment were obtained from the SweMyoNet register. At Karolinska University Hospital, Stockholm, Sweden, treatment is individualized by the treating physician and is based on the local guidelines for treatment (*Vårdprogram myosit* [document in Swedish]). Pharmacological therapy is usually started with high-dose glucocorticoids (0.75 – 1 mg/kg/day prednisolone (but not more than 80 mg/day) for approximately 4 to 6 weeks and then being tapered. At the same time, immunosuppressive treatment is given usually with methotrexate (15 – 20 mg/week), azathioprine (1.5 – 2 mg/kg/day), or mycophenolate mofetil (2 – 2.5 g/day). Cyclophosphamide and any biologic therapy are given for severe or refractory disease. For **Paper II**, the use of glucocorticoids, methotrexate, azathioprine, mycophenolate, cyclophosphamide, and any biologic drug (either rituximab or abatacept) during follow-up was recorded as dichotomous variables. The prednisolone dose (or equivalent) at index date was recorded as a continuous variable.

4.7 Participants

A summary of the demographic data and diagnosis in each study is presented in **Table 6**.

Table 6. Summary of the characteristics of patients included in Paper I-IV

	Paper I	Paper II	Paper III	Paper IV	
Number of patients	110	156	769	Women 940	Men 393
Age at diagnosis, mean (SD)	55.7 (18) ***	57 (14)	51 (16)	50.9 (16.4)	52.8 (16.8)
Women	72 (65)	104 (67)	523/760 (69)	50.9 (16.4)	52.8 (16.8)
Diagnosis					
ADM	4 (4)	8 (5)	11 (1.4)	11 (1.2)	5 (1.3)
Dermatomyositis	36 (32)	62 (40)	248 (32.3)	277 (29.5)	128 (32.6)
Polymyositis	52 (47)	86 (55)	114 (14.9)	243 (25.9)	98 (24.9)
Overlap with CTD	-	-	81 (10.5)	159 (16.9)	40 (10.2)
IBM	16 (14)	NA	37 (4.9)	21 (2.2)	30 (7.6)
ASyS	-	-	214 (27.4)	150 (16.0)	59 (15.0)
IMNM	-	-	52 (6.9)	31 (3.3)	12 (3.1)
JDM	3 (3)	NA	12 (1.6)	16 (1.7)	5 (1.3)
Comorbidities					
Interstitial lung disease	#	52(34)	300/712 (42)	300 (38.9)	110 (33.2)
Dysphagia	#	57 (36)	339/697 (48)	353 (42.1)	152 (43.4)
Cancer	#	26 (17)	122/769 (16)*	-	-
Heart involvement	NA	9 (6)	90/644 (14)	76 (9.7)	43 (13.2)

Note: * Any malignancy. **Presented in the Autoantibody-defined subgroups in the published paper. ***Age at sera collection; #Depending on the assay.

4.8 Outcomes

In **Paper I** the agreement between the LB and IP assays was investigated. In addition, the correlation between the positivity for the different autoantibodies in each assay and clinical manifestations was analyzed.

In **Paper II**, the ACR/EULAR response criteria were used for evaluating improvement to treatment after one year of follow-up. In short, each of the six IMACS CSM of disease activity was evaluated and the absolute per cent change was calculated ([final value – initial value]/range of the measure x 100). Then, an improvement score is assigned for each CSM based on the calculated absolute percent change. Next, these improvement scores are summed up into the total improvement score (TIS). The three categories of improvement were calculated (threshold of ≥20 for minimal, ≥40 for moderate and ≥60 for major improvement were used). A TIS of <20 was considered as *non-responder*.

The potential effect of the initial values for each CSM at index date was analyzed because it is known that initial values in each CSM can potentially affect the follow-up values. The potential effect of each autoantibody subgroup independent of the initial values in each CSM was assessed and an additional interaction effect between each autoantibody subgroup and initial values on the

follow-up values was also investigated. Potential predictors for achieving minimal, moderate and major response were investigated, with special focus on autoantibody-defined groups.

In **Paper III**, the *Myositis Damage Score* was defined as the dependent variable. The *MDI score of Extent* was investigated at Index date (Early) and at 5-year follow-up (Late). The ten most common individual items of the MDI score of extent were determined at both time points (Early and Late). Differences between the autoantibody-defined groups were analyzed.

Also, the correlation between the *MDI score of severity* (overall and muscle domain) and the level of physical disability measured by the HAQ-DI score was analyzed as an attempt to explore the clinical relevance of the level of accumulated damage over time. The trajectories of the change in the MDI score of Extent over time depending on autoantibody status were investigated.

In **Paper IV**, the measure Patient Global Assessment Association (PatGA) was used as the dependent variable. This measure is used in daily clinical practice to capture the patient-subjective experience of the disease activity. The versions of PGA used in this study varied slightly in concept, wording and reference period ("today" or "last week") between the countries. The wordings most commonly used were: "Considering all the ways your rheumatic disease/myositis has affected you, how do you feel today?" or "past week?" or "How have you been feeling in general this past week, in relation to your rheumatic disease/myositis?". **Paper IV** aimed to explore the association of inflammatory markers (CRP, ESR and CK, the latter as a marker of muscle injury) on the PatGA, and to determine if this association could be mediated by functional measures such as muscle strength, functional disability or extra-muscular activity.

4.9 Statistical considerations

For all the statistical analyses in **Paper I-III**, the statistical software R versions 3.5.0 and 4.1.2 were used **(173)**. For **Paper IV**, STATA1 16.0 (StataCorp, LP, Texas, USA) was used for all analyses.

Descriptive statistics

For all papers included in the thesis, descriptive statistics are presented as median values and interquartile ranges (IQR) or means and standard deviations, depending on the nature of distribution of the variable (parametric or non-parametric). In **Paper II**, a Kruskal-Walli's test was used to analyze differences between the autoantibody-defined groups. Also, a Wilcoxon Rank test was used to analyze comparisons for dependent samples. In **Paper III-IV**, statistical differences in continuous variables between groups were tested using Student *t*-test or Mann-Whitney U-test for continuous data Categorical variables are presented as percent values. To analyze differences between categorical variables, a X^2 or Fisher's exact test were used (depending on the number of expected values).

Kappa statistics

When scientists want to know the level of agreement between two observers (or the agreement between two tests), they need to answer two questions: 1) what is the expected level of agreement between the two observers that is expected by chance alone? and 2) what is the level of improvement that could be expected over the agreement only by chance alone? In 1960, Cohen approached these two questions by calculating the kappa coefficient (κ) **(174)**. This calculation of this coefficient is better expressed in the following equation:

$$\kappa = \frac{\text{(percent agreement observed)} - \text{(percent agreement expected by chance alone)}}{100\% - \text{(percent agreement expected by chance alone)}}$$

A κ greater than 0.75 is considered as an excellent agreement and below 0.4 represents a poor agreement. However, more detailed levels are proposed: values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement **(174)**.

Logistic regression

This type of regression analysis compares a binary outcome (e.g., level of response) and certain exposure (e.g., autoantibody-defined group). In order to calculate the odds of achieving the outcome variable, this variable is transformed into a *logit* variable i.e., log of the odds of outcome (therefore the name logistic). Thus, the general equation for comparing two exposure groups is:

$$\log \text{odds of outcome} = \log (\text{Baseline}) + \log (\text{Exposure odds ratio}) \textit{ or}$$

$$\log \text{odds of outcome} = \beta_0 + \beta_1 x_1$$

x_1 (exposure variable) equals 1 for the group that is exposed and 0 for the unexposed group. Besides categorical exposures, continuous variables can be included in the equation and therefore the predicted odds for each increase of 1 unit of the continuous independent variable results in an increase of the odds for the outcome variable.

Mixed effects models for repeated measurements

Sometimes scientists want to analyze a variable observed over a period (longitudinal data). There are two different sorts of approach: scientists consider that the observations in a sample are either *independent* from each other, or observations are clustered somehow, i.e., are *dependent* from each other.

For the first assumption, a student's *t*-test may be used. But for clustered observations, mixed model regression is an option. The advantage of using this sort of analysis is that the approach assumes that an observed variable would tend to be more similar within the clusters compared to other clusters. In longitudinal analysis, the cluster may be at individual level: each individual is taken as a cluster, i.e., the observed variable would be similar over time within the same individual

compared to other individuals. By doing so, a certain amount of uncertainty is decreased. Another advantage of this method is that the available information of the variable at all time points may be included, allowing the possibility of having missing data (compared to other statistical methods that require existing data at all time points).

In general, the most common approach when using mixed models is to use random effects models, meaning that the scientist explicitly fits a model by estimating the differences between the individuals. In other words, the model allows for variation in the average response to vary at the individual level (between clusters).

Mediation analysis

Mediation analysis is used when the investigator wants to answer the question of whether one variable transmits the effects of a predictor variable on a certain outcome variable. To visually express the relationship between potential explanatory variables and the outcome variable, a *path diagram* may be used:

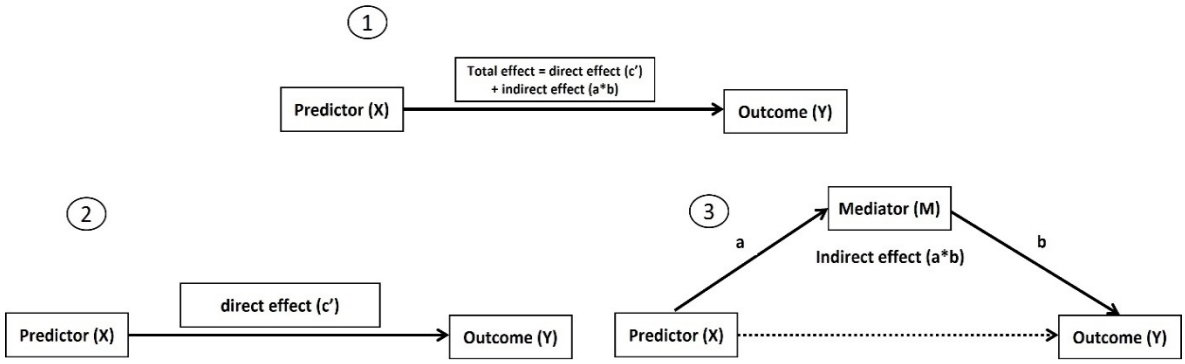


Figure 2. Visual representation of a path diagram used in mediation analysis. ***Panel 1*** describes the total effect of a given predictor (X) over the outcome (Y), including both direct (c') and indirect (a*b) effects. ***Panel 2*** describes the direct effect of a given predictor over the outcome, considering that it might be other potential intervening factors (mediators). ***Panel 3*** describes the indirect effect of the predictor (x) via the mediator variable (M) over the outcome variable (Y); in this pathway, the effect occurs by including the a and b effects, i.e., indirect effect (a*b).

In mediation analysis, the total effect of a given potential predictor (X) over an outcome (Y) is analyzed assuming that it might be complete or partial mediation via another third variable (the mediator variable). A complete mediation assumes that the total effect occurs completely via the mediator; on the contrary, a partial mediation assumes that the total effect occurs via direct and indirect effects (i.e., through the mediator). In this context, direct effect is the directional relation between two variables and indirect effect is the relation between two variables through a mediator (or intervening variable). To test for the presence of mediation, we estimate the amount of indirect effect and whether it is significant. Different methods may be used, but one of the most used is the Sobel test **(175)**. (**Figure 2**)

Table 7. Summary of the statistical methods used in this thesis.

	Studies			
	I	**II**	**III**	**IV**
Kappa statistics	X			
X^2 or Fisher's exact test	X	X	X	X
Kruskal-Wallis		X		
Wilcoxon-Rank test		X		
Spearman Correlation			X	X
Logistic Regression		X		
Mixed effects for repeated measures			X	X
Mediation analysis				X

4.10 Ethical considerations

All the studies that conform this thesis were approved by the Karolinska University Hospital Ethics Committee in Stockholm, Sweden (**Paper I-II**: Dnr 2008/1457-31, Dnr. 2012/736-32; **Paper III-IV**: Dnr. 2008/1919-31/3; Dnr. 2009/1934-32; Dnr. 2013/1390-32; Dnr. 2017/922-32; Dnr. 2019-01593; Dnr. 2023/00244-02). Ethical approvals were also collected at each of the participating international centers included in the MyoNet.

As commented previously, data on demographic, clinical, laboratory and physical activity has been collected in the SRQ register as part of the standard medical care offered to patients. However, this valuable information collected as part of the regular attention is also valuable for answering research questions. At the first visit at the myositis clinic in the Karolinska University Hospital, every patient is asked to contribute to research by signing an informed consent that allows the clinician to upload the patient's data to the electronic registry that can later be used for research purposes. As an important amount of these data is sensitive, every piece of information is stored and maintained properly protected by password and stored on secure servers, and only by an approved application a researcher can have access to such information.

It is mandatory for every researcher that four fundamental statements must be observed: a) benefit patients, b) not damage them, c) respect their autonomy and d) justice **(176)**. In this context, obtaining an informed consent was conceived to ensure that the principle of autonomy was respected for those individuals who were involved in clinical and/or manifest interventions (standard case). There are, however, studies that might have been designed after certain clinical data (or biological samples) have already been obtained from study participants for a previous trial. Such information might also be important to address a specific new research question and it has been debated on the best approach to 1) obtain the informed consent and 2) how to proceed in such situations.

According to Ludvigsson *et al* **(164)**, the current Swedish legislation does not require a specific informed consent for the use of national register data for research purposes. Researchers, however, are expected to protect the integrity and dignity of such information obtained. Nonetheless, the raw data are obtained by using the Personal Identification Number (PIN) and thus requires an approval by an Ethics review board. This amount of data is protected by replacing the original PIN by a unique serial number before delivering the data to the researchers. In my doctoral research, patients with myositis consented to participate in several projects entitling researchers to use information derived from blood samples and correlate them with clinical data derived from the SweMyoNet. Such projects were clearly stated when submitting the study protocol some years ago. In this way, patients were informed about the aims of future studies that would be developed after the time of blood sampling and data collection. So, patients signed a "broad informed consent" but only after all their questions were answered and pertinent explanation was offered.

After the cruelty and horror inflicted to millions of European citizens through medical experiments during World War II, the Declaration of Helsinki was issued after the Nuremberg trials. This attempted to ensure that the four main principles previously mentioned were observed when conducting research in human beings. The overall aim of my doctoral projects stands specifically for two statements of the Declaration: the benefit of human society by understanding the causes of disease, improving diagnostic interventions and protecting the privacy and confidentiality for each of the participants (6^{th} and 24^{th} principles, respectively).

5 Main results

5.1 Paper I

5.1.1 Agreement between IP and LB assays

Both assays were able to detect the presence of any autoantibody with similar frequency: the IP detected 45/110 (41%) and LB detected 48/110 (43%) (P=0.79). Among the LB positive patients (LB+), 85% were positive for MSA. The most frequent detected MSA were anti-Jo-1 (LB+ n=18, 16%; IP+ n=12, 10%), anti-TIF1γ (n=8, 7%; IP+ n=12, 11%) and anti-Mi-2 α or β (LB+ n=8, 7%; IP+ n=2, 2%), followed by anti-SAE (LB+ n=4, 4%; IP+ n=3, 3%) and anti-SRP (LB+ n=3, 3%; IP+ n=4, 4%). Patients with inclusion body myositis as well as controls were negative for anti-Jo-1, -PL7, -SRP, -MDA5 and -TIF1γ specificities by both assays. Among the controls, only three of the sixty were positive for either anti-Ku, anti-NXP2 or anti-Mi-2α (mean 20 units).

The overall concordance between the LB and IP assays was 78% for any autoantibody with a moderate agreement (κ = 0.54). The concordance and agreement (kappa coefficient) for each specificity is summarized in **Table 8**.

Table 8. Comparison between immunoprecipitation and line blot assays in 110 patients with inflammatory myopathies according to EULAR/ACR classification criteria.

Antibody	IP N (%)	LB N (%)	Cohen's kappa	Concordance on positive sera (%) *	Concordance on negative sera (%)
Anti-Jo-1	18 (16)	12 (11)	0.7	11 (10)	92 (83)
Anti-PL-12	1 (1)	0	0.0	NA	NA
Anti-PL-7	0	0	NA	NA	NA
Anti-Ej	0	0	NA	NA	NA
Anti-OJ	0	1 (1)	0.0	NA	NA
Anti-SRP	3 (3)	4 (4)	0.85	3 (3)	107 (96)
Anti-PM/Scl	3 (3)	5 (5)	0.48	3 (2)	105 (95)
Anti-Ku	4 (4)	3 (3)	0.85	3 (3)	107 (96)
Anti-SAE	4 (4)	3 (3) #	0.85	3 (3)	107 (96)
Anti-NXP2	2 (2)	0	0.0	NA	NA
Anti-MDA5	1 (1)	3 (3)	0.49	1 (1)	108 (97)
Anti-TIF1γ	8 (7)	12 (11)	0.56	6 (5)	97 (87)
Anti-Mi-2(α/β)	8 (7)	2 (2)	0.38	2 (2)	103 (93)

*The percentage represent the proportion among the 110 patients. #The IP assay showed 40/90kDa bands in ¾ of patients, suggesting a positivity for SAE1. Note: EULAR: European Alliance of Associations for Rheumatology; ACR American College of Rheumatology; LB: line blot; IP: immunoprecipitation,

Anti-Jo-1 autoantibodies were detected in a total of 18 patients, where 11 were LB+ and IP+ (double positive), 7 LB+ but IP- and only one was LB- but IP+. None of IBM patients was anti-Jo-1+. To further characterize the "LB false positive" anti-Jo-1, sera from five of these patients were evaluated by an ELISA assay. Three of these were positive, one was borderline, and one was negative. Anti-TIF1γ autoantibodies were detected in 14 patients; 6 patients were double positive (43%), 2 were LB+ but IP- (14%), and 6 were LB- but IP+ (43%). Anti-Mi-2 autoantibodies were more frequently detected by LB (n=8) than by IP (n=2). Two patients were double positive (25%) and 6 were LB+ but IP- (75%). Anti-SAE autoantibodies were detected by LB in four patients and among these, IP detected two 40kDa and 90kDa proteins that were considered consistent with both subunits of the SAE antigen, indicating that the LB assay was able to detect autoantibodies in 75% of the sera reactive to SAE.

5.1.2 Association between autoantibodies and clinical manifestations

Several different relevant clinical manifestations were explored. A significant higher number of anti-Jo-1+ patients had ILD (LB: 83% vs. 23%; IP: 92% vs. 26%) and arthritis (LB 67% vs. 20%, P<0.001; IP: 83% vs. 21%, P<0.001) compared to anti-Jo-1 negative patients. Skin rash was more frequent in anti-TIF1γ+ than patients negative for this autoantibody (LB: 75% vs. 19%, P<0.001; IP: 75% vs. 17%, P<0.001). This association was not found in anti-Mi-2+ patients, neither by LB nor by IP. Dysphagia was less frequent in anti-Jo-1+ than in anti-Jo- patients (LB: 17% vs. 57%, P=0.004; IP: 17% vs. 54%, P=0.04). Malignancy was more common in anti-TIF1γ+ patients than in patients negative for this autoantibody (LB: 63% vs. 17%; IP: 58% vs. 15%).

5.2 Paper II

5.2.1 Autoantibodies and level of response to treatment

All patients included in this study (n=156) had active disease at index date. A total of 39 (25%) patients had anti-synthetase autoantibodies, 28 had DMSA (18%), 9 had IMNM autoantibodies (5%), 35 (22%) had MAA and 45 (30%) were seronegative. No differences in duration of symptom prior to diagnosis were found between the autoantibody-defined subgroups. Patients with anti-synthetase autoantibodies had higher frequency of ILD (67%, P<0.001) and patients with DMSA had higher ESR at index date (P=0.008).

After one year from index date, the median (IQR) total improvement score (TIS) was 27.5 (10 – 51) of maximal 100. No differences in the TIS between the autoantibody-defined subgroups were observed. The absolute change (percentage points) in each CSM among autoantibody-defined subgroups was analyzed at index date and one year after index date. At index date, patients with ASyS had higher MMT8 score than the other autoantibody-defined groups (P=0.01) and patients with DMSA had higher extramuscular score than the other groups (P=0.002). After one year of follow-up, patients with ASyS still had higher MMT8 score than the other groups (P=0.038), and patients with IMNM autoantibodies had higher CK levels than the other groups (P=0.001).

A further analysis was conducted to investigate whether the autoantibody subgroups had an effect independent from the initial values of each CSM and whether there was an effect of the autoantibody subgroups on the TIS depending on the initial values (*interaction variable*). The ASyS subgroup had a higher TIS after adjusting for the initial MMT8 score, but lower TIS after adjusting for initial PhyGA (P=0.006 and P=0.003, respectively). Also, the DMSA subgroup had a lower TIS after adjusting for the initial value of PhyGA (P=0.01).

Among the 156 patients, 62% (n=96) met the criteria for minimal response, 38% (n=60) met the criteria for moderate response, and 19% (n=30) met the criteria for major response. Only patients with DMSA were associated with a moderate response compared with the seronegative (P=0.034). No other significant associations between the autoantibody defined subgroups and levels of response to treatment were observed.

5.2.2 Predictive factors for treatment response

A multivariate logistic regression analysis showed that the DMSA subgroup was associated with moderate response to treatment (OR 4.2, CI 95% 1.2 – 16.5). Time from first symptoms to diagnosis (OR 0.86, CI 95% 0.7–0.96 for major response) and dysphagia (OR 3.02, CI 95% 1.3–7.7 for minimal response and OR 3.2, CI 95% 1.2–9.5 for major response) were independently associated with response to treatment. Also, an independent association between the initial dose of glucocorticoids at index date and all levels of response to treatment was observed (**Table 9**). Finally, a fitted model as a sensitivity analysis (i.e., patients who died during the following up) showed similar results.

Table 9. Univariate and multivariate regression models testing predicting factors for achieving minimal, moderate and major

	Univariate model			Multivariate model		
	Minimal	**Moderate**	**Major**	**Minimal**	**Moderate**	**Major**
Seronegative	1.0	1.0	1.0	1.0	1.0	1.0
ASyS	2.05 (0.84 – 5.16)	1.33 (0.5 – 3.4)	1.38 (0.4 – 4.7)	2.3 (0.6 – 8.5)	0.95 (0.26 – 3.3)	1.6 (0.4 – 7.3)
DMSA	2.28 (0.85 – 6.5)	**4.12 (1.5 – 11.6)**	**3.5 (1.13 – 11.8)**	3.9 (0.99 – 18.3)	**4.2 (1.2 – 16.5)**	3.01 (0.7 – 13)
IMNM	0.45 (0.1 – 1.96)	0.76 (0.1 – 3.7)	0.014 (0.01 – 2.2)	0.6 (0.1 – 3.6)	1.19 (0.1 – 7.4)	2.8 (0.8 – 5.3)
MAA	1.75 (0.7 – 4.45)	2.24 (0.9 – 5.9)	1.6 (0.4 – 5.4)	1.3 (0.4 – 3.9)	2.13 (0.7 – 6.6)	1.25 (0.3 – 5.2)
Initial GC dose	**1.05 (1.03 – 1.07)**	**1.04 (1.02 – 1.06)**	**1.05 (1.03 – 1.08)**	**1.04 (1.02 – 1.07)**	**1.04 (1.02 – 1.07)**	**1.04 (1.01 – 1.07)**
Symptoms to dx*	0.98 (0.95 – 1.00)	0.98 (0.94 – 1.00)	0.86 (0.75 – 0.95)	0.97 (0.95 – 1.0)	0.99 (0.96 – 1.01)	**0.86 (0.7 – 0.96)**
Dysphagia	2.22 (1.1 – 4.64)	2.1 (1.05 – 4.0)	2.4 (1.1 – 5.4)	**3.02 (1.3 – 7.7)**	2.1 (0.9 – 5.1)	**3.2 (1.2 – 9.5)**
Initial ESR	1.02 (1.0 – 1.04)	1.04 (1.01 – 1.06)	1.03 (1.01 – 1.05)	1.01 (0.98 – 1.03)	1.03 (1.0 – 1.05)	1.01 (0.99 – 1.04)
Use of CFM	3.3 (1.3 – 9.2)	2.05 (0.9 – 4.6)	1.9 (0.7 – 4.5)	1.1 (0.28 – 4.6)	1.23 (0.38 – 4.15)	6.0 (1.6 – 2.1)

Note: Highlighted values stand for statistically significant. OR: odds ratio. 95% CI: 95% confidence interval. ASyS: Antisynthetase antibody group, DMSA: dermatomyositis specific antibodies group, IMNM: Immune-mediated necrotizing antibodies group. MAA: Myositis associated autoantibodies group. GC: glucocorticoid. ESR: erythrocyte sedimentation rate. CFM: cyclophosphamide. *Duration of symptoms prior to diagnosis (months).

5.3 Paper III.

A total number of 769 patients were included in the study. The most common autoantibodies were anti-Jo-1 (22%, n=174), -PM/Scl (7.5%, n=58), -Mi-2 (6.4%, n=49), -TIF1γ (5.5%, n=42), and -SRP (4.3%, n=33).

5.3.1 Extent of organ damage

At index date, the mean (SD) MDI score of extent was 3.2 (2.2) out of maximal 38. The median disease duration at the index date was 1.67 years (IQR 0.5 – 5.0). The ten most common items of damage at index date and at five-year follow-up are shown in **Figure 3**.

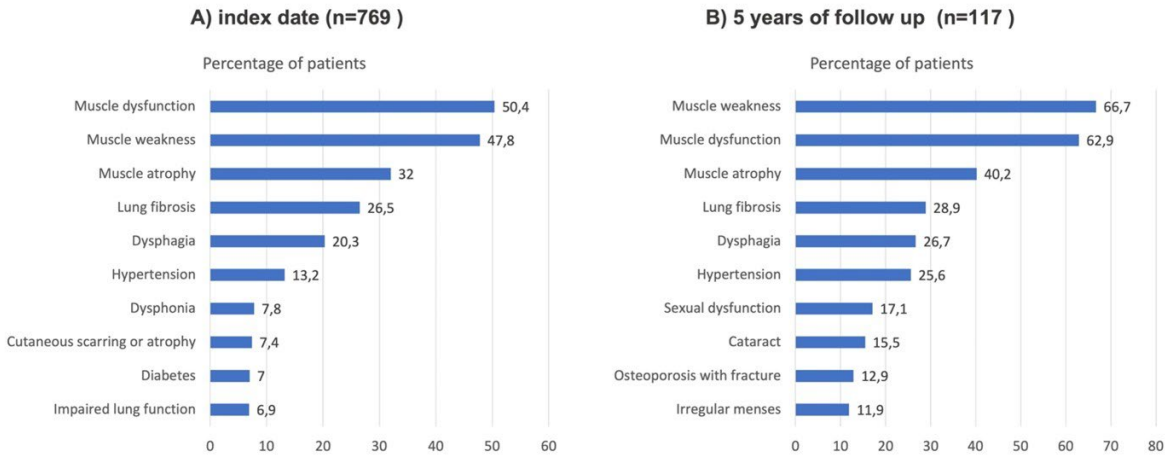


Figure 3. Ten most common items of organ damage in at index date (A) and at five-year follow-up (B).

5.3.2 Autoantibodies and organ damage

At index date, patients with DMSA had lower MDI score of extent than the other autoantibody-defined groups altogether 2.72 (2.6) vs. 3.26 (2.8) (P=0.03). There were no differences in the disease duration from diagnosis to index date between the autoantibody-defined groups. Compared with all the other groups, patients with ASyS were less likely to have muscle dysfunction (40.3% vs. 54.3%, P<0.001), muscle atrophy (22.2% vs. 35.8%, P<0.001), muscle weakness not attributable to active disease (40.6% vs. 50.5%, P<0.05), and dysphagia (10.4% vs. 24.1%, P<0.001) but more likely to have lung fibrosis (55% vs. 15.5%, P<0.001) and impaired lung function (10.6% vs. 5.5%, P=0.02).

Patients with DMSA were less likely to have muscle atrophy (23.3% vs. 34.8%, P<0.001) and lung fibrosis (10.9% vs. 29.6%, P<0.001) than all the other groups. Patients with IMNM autoantibodies were more likely to have muscle weakness not attributable to active disease (65.2% vs. 46.6%, P<0.05) and less likely to have lung fibrosis (9.1% vs. 27.5%, P<0.05) than all the other groups.

Seronegative patients were more likely to have muscle dysfunction (57.4% vs. 47.1%, P<0.01), muscle atrophy (46% vs. 25.8%, P<0.001), dysphonia (11 vs. 6.4%, P<0.05), and dysphagia (28.3% vs. 16.7%, P<0.001) but less likely to have lung fibrosis (14.7% vs. 31.7%, P<0.001) and impaired lung function (3.4% vs. 8.5%, P<0.05) than all the other groups. No other differences between the autoantibody-defined groups and the items of organ damage were found.

At five-year follow-up, patients with DMSA were less likely to have muscle weakness not attributable to active disease than all the other groups (35.3% vs. 70.9%, P<0.01). Patients with ASyS were more likely to have lung fibrosis (61.3% vs. 18%, P<0.001) and less likely to have dysphagia (6.7 vs. 33%, P<0.01) than all the other groups. Patients with MAA were more likely to have muscle dysfunction than all the other groups (100 vs. 58.1%, P<0.01). Seronegative patients were more likely to have muscle atrophy (55% vs. 32.5%, P<0.05) and dysphagia (47.5% vs. 15.2%, P<0.001) than all the other groups. No differences were found in arterial hypertension, sexual dysfunction, cataracts, osteoporosis with fracture, or irregular menses.

5.3.3 Correlation between severity of muscle damage and physical disability

The correlation analysis between the overall MDI score of severity (MYODAM) and physical disability (HAQ-DI score) as well as the analysis for severity in muscle damage and for each autoantibody-defined group are presented in **Table 10**.

Table 10. Spearman correlation analyses between MDI severity score (MYODAM) and severity of muscle damage with HAQ-DI score.

	Index date	5 years
MYODAM overall*	0.37 (P<0.001)	0.61 (P<0.001)
seronegative	0.275	0.46
MAA	0.563	0.76
ASyS	0.370	0.57
DMSA	0.403	0.60
IMNM	0.336	0.86
PM/Scl	0.555	0.31
Severity of Muscle damage	0.42 (P<0.001)	0.72 (P<0.001)
seronegative	0.44	0.85
MAA	0.38	0.43
ASyS	0.53	0.67
DMSA	0.35	0.51
IMNM	0.19	0.72
PM/Scl	0.46	0.17

Note: the number stand for Spearman's rank correlation (rho). ASyS: antisynthetase syndrome, DMSA: dermatomyositis-specific autoantibodies, IMNM: immune-mediated necrotizing myopathy antibodies, MAA: myositis-associated autoantibodies. *MYODAM overall score from 0 to a maximal of 110 cm.

5.3.4 Change of damage over time and the effect of autoantibodies

Available data for MDI items at both index date and at five-year follow-up were available for 117 patients. After five years of follow up, only cataract increased in proportion compared with the number of patients having this item at index date (15.5% vs. 6.2%, P=0.04). No other significant changes in the proportion of other items were found.

The fitted linear mixed-effects regression model for repeated measurements showed that the reference group, i.e., seronegative, had an average increase of 0.12 units in the MDI score of extent per year since the diagnosis was estimated. When analyzing the effect of the autoantibody-defined groups, the model showed that the MDI score of extent increased by an average of 0.21 (0.12 + 0.09) units per year since the diagnosis for the anti-PM/Scl group compared to the reference group and an increase by an average of 0.06 (0.12 – 0.06) per year since the diagnosis for patients with DMSA. An independent effect for each year of age after the diagnosis was also estimated. The fitted model found no effect of the sex in the change of MDI score of extent over time (**Table 11** and **Figure 4**). Two additional fitted models were carried out. The first model restricted the follow-up period to no longer than 10 years and found no differences in the estimations. The second fitted model was conducted after excluding patients with IBM and it showed similar results except for the DM-specific autoantibodies.

Table 11. Results of the general linear mixed model for prediction of MDI extent score over time

variable	β coefficient	95% CI*	P value
Intercept**	**3.66**	3.33, 3.99	**<0.001**
ASyS group	-0.38	-0.94, 0.16	0.17
DM specific	-0.27	-0.91, 0.35	0.39
IMNM	-0.47	-0.98, 0.57	0.33
MAA	0.40	-0.37, 1.18	0.31
PM/Scl	0.31	-0.55, 1.18	0.48
Sex§	-0.12	-0.5, 0.25	0.53
Time since diagnosis (years)	**0.12**	0.08, 0.15	**<0.001**
Age at diagnosis	**0.04**	0.03, 0.05	**<0.001**
ASyS*Time since diagnosis	0.01	-0.04, 0.06	0.73
DMSA*Time since diagnosis	**-0.06**	-0.12, -0.01	**0.04**
IMNM*Time since diagnosis	0.04	-0.04, 0.11	0.38
MAA*Time since diagnosis	-0.06	-0.14, 0.02	0.14
PM/Scl*Time since diagnosis	**0.09**	0.003, 0.18	**0.04**

* 95% of confidence intervals. ** The estimated intercept coefficient is the expected MDI extent of score for the seronegative group, in women, with an average time of follow-up, and with average age at diagnosis.
§Women as reference group.
ASyS: antisynthetase syndrome, DMSA: dermatomyositis specific autoantibodies, IMNM: immune-mediated necrotizing myopathy, MAA: myositis-associated autoantibodies. Highlighted text stands for significant values.

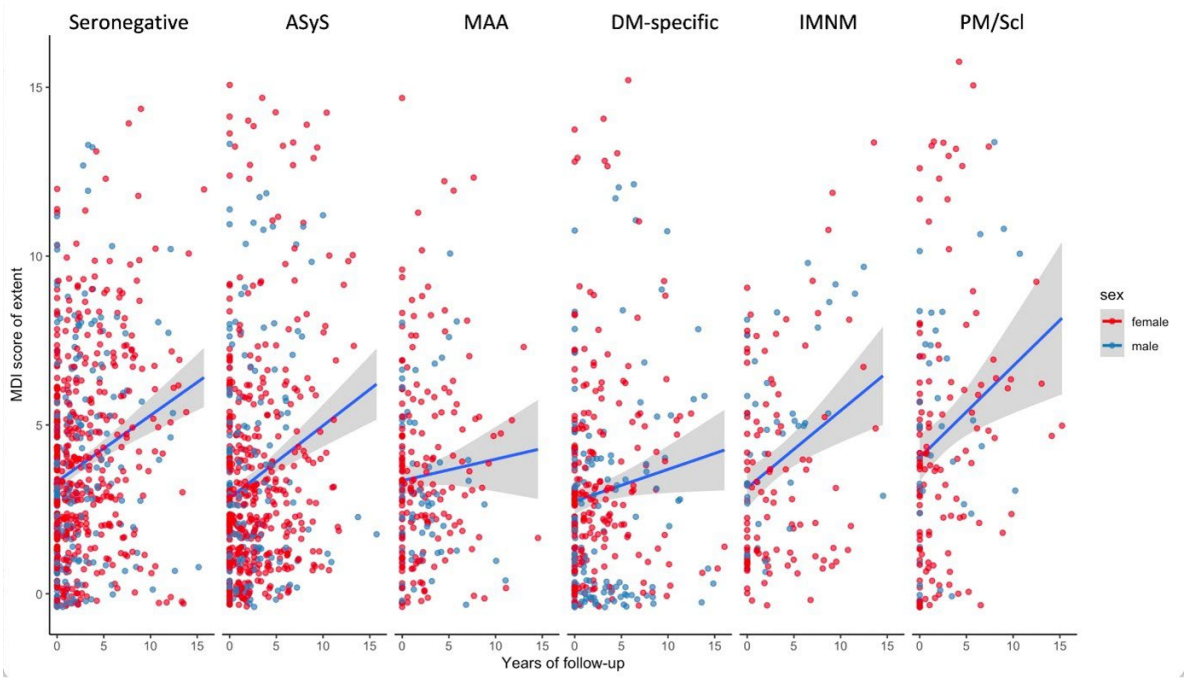


Figure 4. Predicted trajectories of the change over time for the MDI score of extent by autoantibody-defined groups and by sex. NOTE: Each point represents a patient. The blue lines represent the predicted trajectory of the change in the MDI score of the extent using the added β coefficients from the linear mixed effects regression for repeated measures. The grey shadow represents the confidence interval. Years of follow-up: after index date. MAA: myositis-associated autoantibodies, IMNM: immune-mediated necrotizing myopathies autoantibodies, DM-specific: dermatomyositis-specific autoantibodies, ASyS: antisynthetase syndrome.

5.4 Paper IV

At index date, women reported higher PatGA (median 50, IQR 22 – 69) than men (median 41, IQR 14 – 65) (P<0.001). Women had higher ESR (median 16.0, IQR 8.0 – 29.0) than men (median 12.0, IQR 6.0 – 22.5) (P<0.001), but lower levels of CK (median 0.8, IQR 0.4 – 3.8) than men (median 1.2, IQR 0.5 – 4.2) (P=0.037). No differences in the levels of CRP were found between women and men. Compared with men, women had lower MMT8 score (median 69, IQR 58 – 77 vs. median 74, IQR 63 – 80, P<0.001), higher HAQ score (median 0.88, IQR 0.38 – 1.63 vs. median 0.63, IQR 0.0 – 0.13, P<0.001), and higher extramuscular activity VAS (median 15, IQR 5 – 30 vs. median 10, IQR 0.0 – 26, P=0.009).

5.4.1 Relationship between PatGA and inflammatory markers over time

We found a significant decrease in the PatGA and in the levels of ESR, CRP, and CK during the first year of follow-up after the index date. However, PatGA scores increased after the second year of follow-up, and never reached the levels observed at index date during the entire follow-up period.

A significant positive association between PatGA and both ESR and CRP was found for women and men over time of follow-up (**Table 12**). In women, a significant positive association was also found between PatGA and levels of CK, but not in men. After adjustment by different CSM

and overall disease activity, the associations between these measures and inflammatory markers and CK levels were different in women and in men. In women, PatGA remained statistically associated with the levels of inflammatory markers and CK even after adjustment for muscle strength, disability, extra-muscular disease activity, and overall disease activity. These observations suggest that the association could not be fully explained by muscle strength, physical disability and extra-muscular activity.

In men, the association between ESR remained statistically associated with PatGA after adjustment by muscle strength and overall disease activity, but this association was not significant after adjustment for disability and extra-muscular activity. These findings suggest that the overall association between inflammatory markers and PatGA could be explained by disability and extra-muscular disease activity in men. Also, in men the association between CRP and PGA could also be explained by muscle strength and overall disease activity.

When the analyses were adjusted by diagnostic subgroups, the associations were still statistically significant for all the diagnosis subgroups except for PM in women. In men, only patients with PM diagnosis had a significant association between PatGA and levels of CRP.

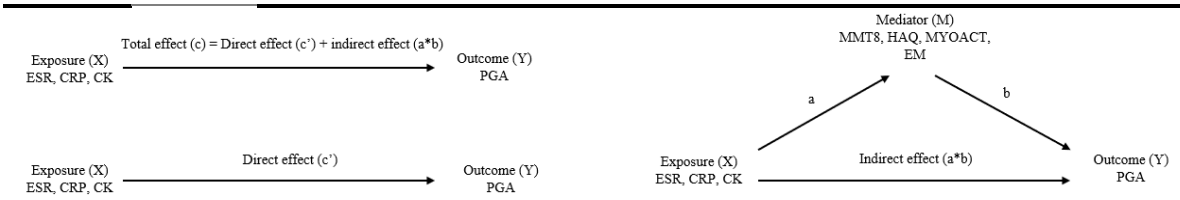
5.4.2 Association between PatGA and inflammatory markers and mediation by functional measures

Next, we conducted a correlation analysis between the change in PatGA and change in ESR, CRP and CK levels during the first year after index date: Significant associations were found in women for all markers (ESR, CRP and CK). For men, however, ESR was not correlated with change in PatGA. Further, an analysis to evaluate mediation by functional measures for significant associations was conducted. Because no association between PatGA and ESR was found in men, mediation analysis was limited to CRP and CK.

The association between change in inflammatory markers and change in PatGA during the first year of observation was partially mediated by improvements in all functional measures (MMT8, HAQ, extra-muscular activity, and MYOACT) in both women and men (**Table 12**). This means that changes in inflammatory markers are associated with functional measures, and these functional measures are in turn associated with PatGA (*indirect effect*). The mediation analyses showed, nonetheless, that a direct effect between inflammatory markers and PatGA was still found (**Table 12**).

Table 12. Mediation analysis for the association between PGA and inflammatory markers between index date and one year of follow-up.

Exposure	Outcome	Mediator	a	b	c Total effect	c' (direct effect)	a*b (indirect effect)	%
women								
ESR	PatGA	MMT8	-0.031	-1.037***	0.246***	0.213***	0.032	13.2%
		HAQ	0.009***	18.942***	0.272***	0.107**	**0.165*****	60.7%
		MYOACT	0.001***	127.835***	0.264***	0.173***	**0.091*****	34.5%
		EM	0.190***	0.749***	0.269***	0.127**	**0.142*****	52.7%
CRP		MMT8	-0.080**	-1.084***	0.319***	0.232***	**0.087****	27.3%
		HAQ	0.010***	19.292***	0.287***	0.101*	**0.185*****	64.7%
		MYOACT	0.001***	124.768***	0.301***	0.167**	**0.134*****	44.5%
		EM	0.209***	0.679***	0.312***	0.170**	**0.142*****	45.5%
CK		MMT8	-0.198***	-0.960***	0.719***	0.529***	**0.190*****	26.4%
		HAQ	0.023***	18.403***	0.719**	0.304***	**0.415*****	57.7%
		MYOACT	0.002***	120.888***	0.671***	0.420***	**0.251*****	37.4%
		EM	0.176***	0.615***	0.669***	0.560***	**0.108*****	16.2%
men								
CRP		MMT8	-0.105**	-1.177***	0.301***	0.177*	**0.124****	41.1%
		HAQ	0.008**	19.488***	0.315***	0.153*	**0.162****	51.4%
		MYOACT	0.001***	127.060***	0.311***	0.169*	**0.142*****	45.7%
		EM	0.213***	0.691***	0.299***	0.152*	**0.147*****	49.1%
CK		MMT8	-0.008	-1.140***	0.049**	0.039**	0.009	18.7%
		HAQ	0.001	19.781***	0.049**	0.038**	0.011	22.7%
		MYOACT	-0.000	134.950***	0.053***	0.053*	-0.000	0.10%
		EM	-0.003	0.671***	0.052**	0.055***	-0.002	4.50%



*P<0.05, **P<0.01, ***P<0.001 %: Proportion of the total effect that is mediated. ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, CK: creatin kinase, PatGA: patient global assessment, MMT8: manual muscle test 8 groups, HAQ: Health Assessment Questionnaire, MYOACT: myositis activity overall, EM: extramuscular activity assessment.

6 Discussion

The overall aim of this thesis was to explore the value of autoantibodies and inflammatory markers as prognostic tools in patients with myositis. We investigated if the LB assay is a reliable test for detecting autoantibodies compared with the IP assay. We addressed the value of autoantibodies as predictors for response to pharmacological treatment. We also explored the usefulness of autoantibodies as predictors of development of organ damage over time. Finally, we looked into the association between inflammatory markers and patient global assessment, and examined if this association might be explained by functional measures.

Validation of the line blot assay

In **Paper I**, we intended to validate the use of a LB assay using an IP-based algorithm in a group of patients with a well-defined diagnosis of myositis who were classified as idiopathic inflammatory myopathies according to the 2017 criteria. Our study found that the LB assay was able to detect autoantibodies in 43% of patients compared with 41% detected by IP. Our results are similar to previous studies. For example, Ghirardello et al. **(177)** reported that the LB assay detected autoantibodies (MSA/MAA) in 47% patients with myositis; the prevalence decreased to 38% after excluding anti-SSA/Ro52 autoantibodies. In another study, Cruellas et al. **(178)** found that the LB assay detected 34% of MSA and 42% of MAA in a cohort of Brazilian patients with PM and DM. Other studies have reported prevalence of autoantibodies detected by LB, between 34 – 72% making our results comparable to those studies **(179, 180, 181, 182, 183)**.

Another aspect of the validation of the LB in **Paper I** required to investigate the agreement between a LB assay and an IP-based algorithm in order to address the question if the LB may be a reliable test for detecting autoantibodies. In our study, the overall agreement between the two assays was moderate (78%), which is comparable with previous reports ranging from 77 to 91% **(177, 180)**. When analyzing individual specificities, however, the agreement between the two assays differed significantly. For the anti-Jo-1 autoantibodies, for example, the agreement was good ($\kappa = 0.69$), although these autoantibodies were detected more frequently by LB than by IP (16% vs. 11%, respectively albeit not significant). If patients with IBM were excluded, the prevalence increased up to 19% and 13%, respectively. Moreover, the frequency of clinical features of anti-Jo-1 patients detected by LB (arthritis, ILD, and Raynaud's phenomenon) is in line with the frequency of these features in anti-Jo-1 positive patients. This suggests that the LB is more sensitive to detect anti-Jo-1 autoantibodies than the IP, at least in European patients **(78)**.

For the anti-Mi-2 autoantibodies, these were detected by the LB assay more frequently than by IP (7% vs. 2% respectively), and therefore the agreement between the assays was fair ($\kappa = 0.38$). This level of agreement is lower than the reported by Mahler et al **(184)** and Richards et al. **(185)**, ($\kappa = 0.62 – 0.77$, respectively). One can speculate that a reason for this discrepancy may be attributed to aspects inherent to the assays, such as the nature of the antigens used in both assays: while the IP may use native proteins (or RNA), the LB kits usually include recombinant proteins.

Another highly technical characteristic inherent to the assay may be the room temperature when incubating the samples: the level of reactivity is sensitive to changes in the room temperature **(186)**. In addition to these considerations, a biological explanation might be that different autoantibodies may target different segments of the Mi-2 antigens (α or β) as suggested by Hengstman et al. **(187)**. Interestingly, our study found that more patients with PM subtype than DM patients were positive for this specificity. Indeed, skin rashes were not associated with this autoantibody in our study by neither LB nor IP. This finding is intriguing because anti-Mi-2 autoantibodies are usually considered within the spectrum of DMSA **(38)**. Our findings showed similar associations as those reported by Vulsteke JB et al. **(188)**. They found that anti-Mi-2 positivity was not associated with DM phenotype in their analysis of 144 patients with myositis, neither by LB test nor by other multispecific assays (Alphadia, Trinity BioTech), indicating that anti-Mi-2 positive patients do not tend to have DM skin manifestations, at least in European populations. Another multicenter European study reported that up to 40% of anti-Mi-2 positive patients had a PM phenotype, and therefore, that this autoantibody could not be taken as marker of any specific subtype of myositis **(187)**. Nonetheless, the conclusions in the latter study were drawn from tests conducted for only the Mi-2β fragment alone detected by ELISA, making it difficult to compare it with the findings in our study. Therefore, our study seems to confirm the notion that the prevalence of anti-Mi-2 autoantibodies, less associated with DM subtype, may follow a gradient depending on the geographical latitude, as previously suggested by Ejaz et al. **(189)**

Our study found that among the fourteen anti-TIF1γ positive patients, only 43% were double positive; fourteen percent were only LB positive and 43% were only positive by IP, and the agreement between LB and IP was moderate (κ = 0.62). Prior studies have found that among the most marked discrepancies between assays are the differences in the reactivities for anti-TIF1γ, but the agreement between both assays were found close to those observed in our study (κ =0.71 and 0.70) **(184, 190)**.

One of the main concerns of our study, however, was the sensitivity of the LB assay for detecting myositis-associated cancer in anti-TIF1γ positive patients. The main clinical relevance of anti-TIF1γ specificity is the association with an underlying malignancy **(54, 55, 191)**. In **Paper I**, malignancy was significantly more common in anti-TIF1γ+ patients than in patients negative for this autoantibody detected by both assays (LB: 63% vs. 17%, P=0.009; IP: 58% vs. 15%, P=0.003). This variation has been subject of debate due to lacking standardization and harmonization on detecting these autoantibodies, and more important, due to the ethical issues regarding the following approach after having detected these autoantibodies in a specific patient. Several reasons may explain this variability. First, anti-TIF1γ autoantibodies usually target conformational epitopes **(192)** making it difficult to compare the accuracy of IP assays with LB assays which uses recombined and/or denatured proteins. Second, the association of anti-TIF1γ autoantibodies with CAM patients has a large variation depending on the population analyzed:

some subtypes of myositis may dilute the association of these autoantibodies with cancer due to the low frequency of malignancy such as antisynthetase syndrome or IBM. In our study, we included patients with different myositis subtypes while other reports have focused exclusively on DM patients and CAM patients **(193)**. Third, the prevalence of malignancy in anti-TIF1γ positive patients is variable depending on the assay used. For example, a recent study from our group showed a higher sensitivity of an ELISA assay (58%) than a LB assay (40%) to detect DM patients with CAM, but a similar sensitivity compared with immunoprecipitation **(193)**. In the latter study, however, the agreement between LB and IP was comparable to our results. In another study, Chinoy et al. **(194)** showed a sensitivity of 50% for immunoprecipitation to detect CAM, and the accuracy improved when this assay was combined with a routine of antibody testing including other more common autoantibodies such as anti-Jo1. Fourth, other factors not inherent to the assay used may affect the accuracy, for example, smoking status, the time when the blood samples are drawn (e.g., at time of diagnosis vs. during the follow-up), immunosuppressive treatment (rituximab has shown to produce and impact on the levels of autoantibodies), or even the stage of the underlying malignancy. All these considerations clearly illustrate the urgent need for a standardized approach to detect autoantibodies in patients with myositis, particularly in patients with high risk for malignancy. Under the light of these considerations, we suggest that patients with high risk for malignancy, but negative for anti-TIF1γ autoantibodies tested by LB, should be re-tested by a more accurate assay such as IP or ELISA.

The next question in **Paper I** was related to the clinical significance of the agreement between assays. There are several concerns about when multispecific tests, like the LB assay, should be conducted in patients with myositis. If this test is intended to be used for diagnostic purposes, a group of positive controls (patients with other forms of myositis and/or other autoimmune diseases) and a group of negative controls (healthy individuals) should be included. In our study, we showed that only 3 individuals out of 60 healthy controls were positive for myositis-autoantibodies and the levels of reactivity were low. This resulted in a specificity of 99,7% (when having the number of MSA measurements as the denominator), but it decreases to 95% when having the number of samples as denominator. Other studies have reported a prevalence of positive samples from healthy controls between 4 to 16% **(179, 186, 190)**, showing that in our study, the LB assay had a good performance for discriminating healthy individuals from patients with myositis. However, some experts have suggested that the accuracy of the LB test might improve if only sera with moderate to strong reactivities are taken as positive **(180, 181, 195)**. Indeed, we re-analyzed our results after excluding patients with low reactivity (DU < 25). However, the agreement between the LB and IP did not change significantly.

For the rest of the autoantibodies, the agreement between both assays varied for the less common specificities such as anti-PL-7, -PL-12, or -MDA5. Indeed, we consider the relatively low number of patients in **Paper I** was the most important limitation. Another important limitation is the absence of a control group including patients with other rheumatic diseases. However, the aim of **Paper I** was to investigate the reliability of the LB assay to detect different subtypes of patients

with already diagnosed myositis, and if these LB seropositive patients were concordant with the historically considered gold standard (IP assay). All these considerations support that the usefulness of the LB assay clearly depends on the context of where and when the autoantibodies are tested.

Autoantibodies and their role as predictors of response to treatment

There is currently no consensus on valid and reliable markers for response to treatment for patients with myositis. Therefore, in **Paper II**, we aimed to explore the usefulness of autoantibodies as predictors of clinical improvement after conventional treatment.

Several serum molecules such as IL-6, type 1 interferon (IFN), B-cell activating factor (BAFF), and TNFα have shown good correlation with disease activity in patients with myositis, and have demonstrated to be sensitive to change, therefore they have been proved to be useful as biomarkers of disease activity **(196, 197)**. However, these molecules are not easily tested in the daily clinical setting. Autoantibodies, on the other hand, are known to serve as predictors of both disease activity and clinical improvement in patients with established disease or refractory to initial therapy **(198)**. In line with these observations, we found in **Paper II** that patients with anti-synthetase autoantibodies had better muscle strength, even after having adjusted for initial MMT8 score at index date, after one year of treatment. We also found that the group of DMSA autoantibodies had a strong association with moderate response in the multivariate analyses, but not associated with minor or major response. Our findings in **Paper II**, agree with previous reports that have demonstrated that patients with DMSA have a better prognosis **(74, 120, 198, 199)**. Reed et al. **(196)** found that anti-Mi-2 positive patients showed higher improvement in muscle strength compared with anti-synthetase positive patients, especially in patients with higher serum IFN scores at baseline (i.e., before the infusion of RTX). An important difference in our study is that the DMSA group was mainly represented by anti-MDA5 and anti-TIF1γ positive patients and only 14% of patients (4/28) were positive for anti-Mi-2. As discussed above, patients living in Northern Europe seem to have less prevalence of anti-Mi-2 autoantibodies, while other DMSA are more frequent. In the same study by Reed et al. **(196)**, anti-TIF1γ patients showed an improvement in the Th1 score compared with the other autoantibody-groups. With respect to anti-MDA5 patients, Allenbach *et al*. **(200)** hypothesized that the high expression of nitric oxide synthase 2, a molecule characterized in macrophage activation in a Th1 milieu, found on the sarcoplasm of muscle fibers of anti-MDA5 patients might have a protective and homeostatic effects. Interestingly, Allenbach *et al*. also observed in the same study that anti-MDA5 positive patients showed lower levels of the IFN signature score measured in muscle tissue compared with classical DM. One can speculate that, besides the association with good response treatment, intrinsic factors to patients with DMSA may confer lower risk for muscle injury and muscle damage, as we observed in **Paper III**.

As commented above, anti-MDA5 positive patients were one of the most common DMSA in the cohort in **Paper II**. It is reported that anti-MDA5 positive patients have a particularly strong association with RP-ILD that correlates with high mortality**:** a Chinese study **(201)** found that the five-year survival rates were significantly lower in MDA5+ patients (50.2%) compared to patients with ASyS (97.7%) (P<0.001). In addition, studies on Asian populations suggest that patients with MDA5 associated RP-ILD may be unresponsive to intensive treatment **(202)**, but Ceribelli et al. **(203)** found that this life-threatening condition seems to be uncommon in European anti-MDA5+ patients. One explanation might be that the notion of the aggressiveness of the phenotype associated with the disease has made clinicians more aware of this severe disease and therefore, patients are screened in an earlier phase than in previous years. This claim may to some extent explain the better long-term prognosis of DMSA patients, as found in **Paper II-III**.

In **Paper II**, nonetheless, we did not focus only on the effect of biologic treatment. Rather, we decided to include other common immunosuppressors used as standard of care as well as the use of glucocorticoids to reflect a usual clinical setting. We found that a high initial dose of GC at index date, but no other treatments, was associated with all levels of response, indicating that more intensive treatment at an early stage of the disease correlates with better odds to respond to medical therapy. Another important prognostic factor was the lag time from symptom onset of myositis to diagnosis, that is, a shorter lag time is associated with better odds of achieving response to treatment, which is consistent with Joffe et al. **(120)** A novelty of our study, nonetheless, was the inclusion of patients with a disease duration less than one year and IBM were excluded, as this group of patients is usually non-responsive to pharmacological treatment. This phenomenon, i.e., better prognosis associated with shorter lag time from first symptoms to diagnosis, has also been described in patients with RA and psoriatic arthritis **(204, 205)**, and represents a window of opportunity because a shorter span from symptoms to diagnosis and onset of treatment might minimize the risk for permanent damage. Our findings in **Paper II** have also implications in the interpretation of findings in **Paper III** where we also observed that the disease duration is associated with more accrued damage.

The use of the ACR/EULAR criteria for clinical response are recommended to evaluate treatment interventions in patients with myositis as they may capture the whole spectrum of disease activity, not only muscle disease. However, a caveat of using these criteria is that a significant part of the total improvement score is composed by subjective measures. It is known that an important number of DMSA patients may experience a significant improvement due to high prevalence of extramuscular activity, especially cutaneous disease, compared with other forms of myositis **(206)**, as shown in the ProDERM study **(145)**. Unfortunately, we did not analyze each domain in the extramuscular activity, and thus we cannot draw conclusions, only speculate. Nonetheless, we did not find differences in the delta of each CSM between the autoantibody groups.

Autoantibodies and organ damage

In **Paper III**, we described the degree of damage measured by the MDI score and the patterns of organ damage depending on autoantibody status. At index date, most patients in our study had muscle damage (represented by muscle atrophy, muscle weakness not attributed to active disease, and muscle dysfunction) followed by damage in the pulmonary, and gastrointestinal systems, similar to the one-center study conducted by Rider et al. **(162)** Our study, however, included more adult than juvenile patients, thus the implications of the autoantibody status should be taken with caution in children with myositis. Another difference in our study is that patients had a mean disease duration of about 20 months (1.7 years) when the first MDI score was evaluated compared with 60 months in the study carried out by Rider et al.

Many differences in the pattern of damage were observed between the autoantibody-defined groups at index date. For example, muscle atrophy and persistent muscle weakness was less common in ASyS patients than the other groups. Lung fibrosis was less common among patients with DMSA and patients with IMNM autoantibodies than the other groups. Patients with DMSA had less damage at first MDI assessment and accumulated damage, measured by the MDI extent score, at a lower rate than seronegative patients. This is consistent with the findings in **Paper II**. Another important finding was the high prevalence of muscle atrophy, persistent muscle weakness and muscle dysfunction in patients with IMNM autoantibodies compared to the other groups, similar to prior reports. For instance, Pinal-Fernandez et al. **(103)** reported that only 50% of anti-SRP recovered full muscle strength after 4 years of follow-up, even after intensive treatment. Similarly, a Japanese study **(207)** showed that patients with anti-SRP patients and anti-HMGCR autoantibodies had more severe limb weakness, neck weakness, dysphagia, and muscle atrophy, although more pronounced in anti-SRP patients.

An unanticipated finding was that anti-PM/Scl patients accumulated organ damage at a higher rate per year compared with the seronegative group, even after excluding IBM cases and after restricting the observations to less than ten years of follow-up. It is difficult to explain this result, but one possibility is that the extensive extra-muscular involvement (joint contractures, skin thickening, anemia and pulmonary hypertension, and ILD) may contribute to the limited muscle function, and thus, it might potentially be taken as organ damage as reported in patients with SSc **(208)**. Another possible explanation might be related to a significant fibrotic component of these patients that may contribute to the higher organ damage compared than patients with other MSA. In patients with SSc, for example, muscle atrophy and muscle weakness are more frequent and more pronounced in patients with diffuse SSc than in limited SSc **(209)**, suggesting a fibrotic component to the low muscle function. Our findings might be consistent with this idea. The present study raises the possibility that genetic and molecular mechanisms specific for anti-PM/Scl patients may be implicated in the development of organ damage. These novel results of **Paper III** support that autoantibodies may represent a useful marker of organ damage, like they are for response to medical therapy as discussed for **Paper II**.

One of the issues that emerges from our findings is that certain items of organ damage may be more frequent in a late stage of the disease than at early stage. Due to this it might not be possible to ascribe the scored damage to the disease or a particular cause **(152, 210)**, there might be several explanations for the differences between organ damage at early and late stage. First, older age is an important risk factor for organ damage and comorbidities, both in children and adult with myositis **(163, 211, 212)**. However, adult patients seem to accumulate damage more frequently than children **(162)**. Next, severe disease activity at diagnosis and persistent active disease is associated with accrued organ damage **(213)**. Another important contributing factor might be the differences in ethnicity, although they have not been explored in adult myositis as in children **(213).** This ethnical diversity may explain different levels of damage similar to patients with SLE **(214)**. Indeed, different genetic haplotypes that are associated with the certain specific autoantibodies **(16, 189)** may contribute to the development of damage.

A very likely effect of treatment on the development of damage, especially GC, cannot be neglected. It is possible that GC therapy may have a significant impact on the development of damage, similar to what is described in patients with SLE **(214, 215)**. Some items of the MDI are in general more likely to be associated with the use of GC, for instance, cataract, diabetes mellitus, osteoporosis, and avascular necrosis. In **Paper III**, the prevalence of cataract, diabetes mellitus and osteoporosis with fracture were among the 10 most common items at the 5-year follow-up. This is consistent with other studies showing an association of low bone mass density, low lean muscle mass, osteoporosis, and vertebral fractures with the use of GC **(216, 217, 218)**. GC have known deleterious effects on skeletal muscle. For example, they may induce mitochondrial dysfunction and muscle wasting leading to muscle atrophy **(219, 220)**. We can speculate that GC therapy might have an important role in the chronic damage in the skeletal muscle of patients with myositis independently of the autoantibody status. Our results support this hypothesis due to the high prevalence of muscle damage both at index date and at 5-year follow-up. Most significant was the correlation between severity of muscle damage with functional disability that became stronger in late stage (i.e., at 5-year of follow-up). Nonetheless, a possible confounding effect of treatment was not analyzed.

Inflammatory markers and Patient Global assessment

In **Paper IV** we found that inflammatory markers were associated with the Patient Global Assessment (PatGA) over time. We found, nevertheless, important differences between women and men in how the functional measures mediated the relationship between CRP and PatGA. In women, for example, we found that inflammatory markers and CK levels were associated with PatGA even after adjusting for functional measures, whereas in men, the association between inflammatory markers and PatGA was partially mediated by some functional measures such as muscle strength and overall disease activity. In women, the association between PatGA and inflammatory markers was via a direct effect but also mediated by functional measures. Additionally, a longitudinal association of all inflammatory markers with PatGA was observed

in both women and men, whereas CK levels did not have a longitudinal association with PatGA in men.

Prior studies have noted differences in the levels of subjective health perception between women and men. For example, symptoms related to the sickness behavior, such as malaise, diffuse muscle pain, arthralgia and numbness are explained in healthy women by circulating inflammatory markers such as IL-1ra and TNF-α **(64, 221)**, but also in women with rheumatic diseases compared with men **(222)**. Interestingly, some evidence indicates that testosterone levels have an inverse correlation with levels of circulating IL-6 receptor and IL-1β suggesting a protecting effect of male hormones against inflammation **(223, 224)**. Nonetheless, psychosocial factors seem to take part in how women and men rate self-perceived health. Indeed, we found that PatGA was higher in women than in men during the first three years of follow-up supporting the idea that an important component of health perception is explained by sex differences.

Muscle mass and muscle strength is on average substantially higher in men than in women. Moreover, the effect of age in losing muscle strength is more abrupt in healthy women than in healthy men **(225)**. We cannot rule out a possible psychosocial effect of the loss of muscle mass in our population that could explain the mediation between inflammatory markers and PatGA via muscle strength. Other factors concerning the differences in the muscle strength between men and women may apply specifically to patients with myositis. For instance, in patients with IBM no association between CK levels and PatGA was found, neither for women nor for men. This was not unexpected as patients with this type of myositis usually do not have high levels of muscle enzymes **(226)**. Also, as this type of myositis is overrepresented in men one can speculate that this could possibly explain a certain mediation of the effect of muscle strength with PatGA. However, the number of patients with this diagnosis was rather low (about 3% of the cohort) making it less likely as a relevant factor in the mediation analysis. On the other hand, patients with IMNM tend to have the highest levels of CK compared to other types of myositis, and usually have a course of chronic severe muscle weakness **(102)**. This feature may partially explain an association between high levels of CK with PatGA with a variable degree of mediation through muscle strength measured by the MMT8. Unfortunately, we did not include IMNM in the regression analyses and thus we can only speculate.

Very little is found in the literature on the question concerning relationship between CRP as well as other inflammatory markers and self-reported health status in patients with myositis. Levels of CRP and ESR are usually not elevated in patients with myositis, except when pulmonary disease or malignancy are present **(86, 227, 228)**. In **Paper IV** we found that CRP levels, as well as ESR, were generally mildly elevated on average across the five years of follow-up. However, despite mild elevations of these markers, a significant association with PatGA over time was observed. There are several possible explanations for this. In rheumatic autoimmune conditions characterized by the type I IFN gene signature, such as dermatomyositis and SLE, CRP is usually not elevated even in the presence of confirmed inflammation as evidenced by increased

circulating levels of ferritin and IL-6 **(58, 229, 230)**. Other pathogenic pathways, more specific to patients with myositis such as high levels of circulating IL-1ra, may interfere with the production of an acute-phase response **(231)**. Thus, other components of the inflammatory response *per se*, not necessarily CRP, may intervene in how patients with myositis rate their health status.

One can argue that low levels of CRP are not likely to significantly contribute to the perception of health status in patients with myositis. However, several other studies firmly support that even low-grade inflammation, i.e., slight elevations of CRP among other elevated molecules, has been shown to be associated with self-reported health in healthy individuals **(232, 233)**. Moreover, the association between low-grade inflammation with different conditions such as cardiovascular disease and chronic pain is well-recognized **(234, 235)**. On the other hand, we cannot rule out some psychosocial factors such as economic status and social support **(236)** or sleep quality **(237)**, well-known determinants of self-reported health that may explain the variability of the PatGA. Unfortunately, we were not able to analyze these factors in **Paper IV** because they are usually not components of the PatGA and they are not regularly asked in another context when meeting patient with myositis. Our findings in **Paper IV** support the idea that systemic inflammation is associated with subjective health perception in patients with myositis and thus it is important not to neglect that even low-degree inflammation may affect the self-perceived health status in these patients.

Limitations

When I carried out the studies included in this thesis, I faced several challenges that deserve to be mentioned. The main limitation in **Paper I** was the low number of patients with rare specificities e.g., anti-SAE and anti-PL12 autoantibodies that precluded from drawing reliable conclusions concerning these rare autoantibodies. On the other hand, a similar limitation was discussed by Lundberg *et al.* when the EULAR/ACR classification criteria were published, even after having included more than 900 cases. In **Paper II**, the main concern was that we conducted our study based on data from one center, thus caution must be taken if one is considering extrapolating our results to other populations. Due to a relative limited number of patients in **Paper II**, the lack of clear differences in the levels of response, i.e., TIS, might be attributed to low power. We cannot rule out confounding by indication when we conducted the regression analyses because disease activity was not included in the regression models. However, no difference in the proportion of patients who were given cyclophosphamide or biologic drugs was found. A limitation common to **Paper II-III** was that we did not analyze individual specificities; rather, we subgrouped the patients using a clinical-serologic approach as previously suggested by Mariampillai *et al*. **(114)**. Another important concern in **Paper II-III** was the seronegative group. In **Paper II**, we actively excluded IBM patients from our cohort and therefore we were confident that these patients were not part of the antibody-negative group; however, other autoantibodies not tested such as anti-FHL1 or anti-cN1a autoantibodies might have been missed and therefore patients have been misclassified as seronegative. In **Paper III**, we fitted a sensitivity analysis by excluding IBM

patients and the results regarding change of the MDI score were similar. Finally, in **Paper IV**, as in **Paper III**, a main concern was the missing data. Indeed, due to the nature of our study (observational and registry-based), missing data is always a challenge. However, we minimized this by conducting a bootstrapping in **Paper IV** making our results more robust.

7 Conclusions

This thesis contributes with new knowledge on the role of autoantibodies and inflammatory markers in the prognosis of patients with myositis.

In **Paper I**, we found that the line blot assay has a moderate agreement with the immunoprecipitation assay in patients classified as myositis according to the EULAR/ACR classification criteria for patients with the most common specificities. Also, the line blot assay seems be useful to identify subgroups of patients with specific clinical manifestations comparable to the immunoprecipitation assay. A special consideration for the anti-TIF1γ sensitivity should be mentioned as this autoantibody is associated with cancer-associated myositis.

In **Paper II**, patients with DMSA were associated with moderate level of response to conventional treatment compared with patients without these autoantibodies. We also found that the initial dose of glucocorticoids as well as shorter time lag from first symptoms to diagnosis were highly associated with better odds to achieve response to treatment.

In **Paper III**, we found that the autoantibody status predicts patterns of damage and trajectories of change in damage over time. Patients with anti-PM/Scl autoantibodies develop more damage than seronegative patients, and patients with dermatomyositis-specific autoantibodies develop less damage than seronegative patients. Additionally, severity of muscle damage was more accentuated in patients with autoantibodies associated with IMNM, i.e., anti-SRP and anti-HMGCR autoantibodies, and this severity correlated strongly with functional disability measured by the HAQ score.

Finally, in **Paper IV**, the levels of inflammatory markers, CRP and ESR but also CK levels as a surrogate of inflammation, correlated longitudinally with the levels of Patient Global Assessment scale. Differences between men and women, however, were noticed. For instance, in men, this association was explained by functional disability and extra-muscular activity. In women, on the other hand, the inflammatory markers had both direct and indirect effects on Patient Global Assessment and the association was not only explained by functional measures.

8 Clinical implications

Detecting autoantibodies in patients with myositis has enormous clinical and prognostic implications. The validation of the line blot assay, which is used on the daily basis in our clinic, may help clinicians to feel more confident when meeting a patient with myositis because the results of this now can be regarded as reliable in the proper clinical context. Our findings come

from patients with a diagnosis of myositis, and we recommend applying the antibody test when the intention of the test is to stratify patients with prognostic purposes, not for diagnosis. In this sense, our findings support the relevance of identifying autoantibody-defined subgroups of patients with myositis early on, and of initiating intensive glucocorticoid treatment as soon as possible after diagnosis.

We can expect a certain pattern of damage after having stratified a specific patient within a certain autoantibody-defined group. This may help the health-care team to identify patients who are at risk of developing certain features of damage. The findings of this thesis suggest that the anti-PM/Scl autoantibody could potentially define an often-overlooked group of patients at risk of developing accumulated damage, suggesting that these patients require close monitoring in the clinic. Our findings also contribute to the knowledge of a potential deleterious effect of treatment in patients with myositis. Therefore, we suggest that strategies to detect earlier therapy-related organ damage should be discussed, standardized and implemented in the myositis clinic.

In recent years, the implementation of patient reported outcomes in clinical trials and in the quality of care on the daily basis is highly recommended. It is important that the patients' perspective should be considered to find out if the therapeutic decisions have an impact on the functional ability and in the quality of life of our patients. More important, our results highlight that certain factors such as inflammatory markers, that previously had been neglected in patients with myositis, can impact on the patient subjective health perception status. We recommend though that these markers should be included in the work-up during the follow-up of patients with myositis.

9 Future perspectives

The results of this thesis concerning the line blot test can be applied only to patients with a diagnosed myositis. However, if this test is considered to be used as a diagnostic tool, a different scenario should be set up to test its potential value. We believe that a larger sample of patients with myositis, including additional clinical subgroups such as IMNM could enrich future studies. Also, I suggest exploring different cut-off levels of the line blot assay for each autoantibody also in a larger cohort with more patients including less frequent autoantibodies. Because there might be some differences between the individual specificities and their level of response to treatment, we believe that further research is needed to assess the prognostic value of each autoantibody using larger datasets with a higher number of rare autoantibodies and in large multiethnic cohorts. Also, future studies exploring the interaction of individual specificities with individual treatments (e.g., methotrexate or azathioprine) should be conducted based on longitudinal registries such as the SweMyoNet.

One of the important questions that resulted from this thesis concerns the seronegative group. Because the presence of specific autoantibodies may help to differentiate a patient with myositis from a patient with a muscular dystrophy or other myopathies, it is critical to characterize this group of patients who are negative to known autoantibodies but have a myositis diagnosis. However, to date this group of seronegative patients has been overlooked. Further research to describe the clinical features and long-term prognosis of these patients is required.

Research focused on organ damage is a new line of research that is still unexplored. This thesis adds new knowledge on some factors that contribute to the accrual of accumulated damage in patients with myositis. Nevertheless, many other factors were not assessed. For example, whether early damage in patients with myositis may confer higher risk for further damage needs to be explored. Also, the strong association of some items of damage with pharmacological therapy should be explored in detail using for instance large datasets like the MyoNet. Because the finding of high rate of accumulated damage in patients with anti-PM/Scl autoantibody was somehow unexpected, we plan to conduct a more detailed study to investigate the factors that might be associated with this pronounced rate of accumulated damage in such patients.

Including PROM in clinical research is highly encouraged because considering the perspectives of patients gives a clinical meaningfulness to science. Because it is difficult to capture all the factors that contribute to the health status of a given patient, we believe that our study on PRO should be regarded as a first approximation to the determinants of the self-reported health state, not a full explanation. Therefore, future research is planned to include other factors, such as pain and fatigue, among other features, as potential predictors of sickness behavior in patients with myositis.

10 POPULÄRVETENSKAPLIG SAMMANFATTNING

Myosit, eller reumatisk muskelinflammation, är en ovanlig grupp sjukdomar som kännetecknas av förhöjd inflammatorisk aktivitet i muskulaturen. Många patienter upplever muskelsvaghet och nedsatt uthållighet i musklerna. Utöver inflammation i musklerna drabbas en del patienter av inflammation i andra delar av kroppen som tex i leder, lungorna eller i huden. Myosit brukar indelas (eller klassificeras) i grupperna polymyosit (PM), dermatomyosit (DM) och inklusionskropp myosit (IBM) utifrån symtom och kliniska fynd.

Under de senaste åren har kunskapen ökat om hur sjukdomen kan utveckla sig men orsaken till utvecklande av myositsjukdom är fortfarande okänd. Nuförtiden vet vi lite mer om vilka faktorer som ökar risken för sjukdomen. Att bilda antikroppar är en normal process i kroppen som ska ge ett skydd mot infektioner. Ibland bildar dock kroppen antikroppar mot kroppens egna strukturer (autoimmunitet). Bildningen av sådana onormala antikroppar är en riskfaktor för att utveckla myosit. Inflammationen som dessa onormala antikroppar skapar kan resultera i flera olika symtom. Var och en av dessa antikroppar ger upphov till en viss sjukdomsbild (fenotyp): anti-Jo1 antikroppar ger lunginflammation och artrit medan anti-SRP antikroppar brukar ge uttalad muskelsvaghet. I modern tid finns flera metoder för att upptäcka dessa antikroppar och nya metoder lanseras då och då. Tyvärr vet vi inte om det går att lita på dessa nya metoder eftersom de inte har validerats mot kända pålitliga metoder. Dessutom är inte antikropparnas betydelse för patientens framtid (prognos) helt kartlagd ännu. Det är heller inte klarlagt om själva inflammationen (mätt med inflammationsmarkörer som sänka och snabbsänka) påverkar hur patienten skattar sin hälsa.

Syftet med denna avhandling var dels att validera ett antikroppstest (line blot), dels att ta reda på om antikroppar kan fungera som prediktorer för behandlingssvar och sjukdomsskada (som ger en bestående ändring av anatomin eller funktionen), samt att utforska sambanden mellan inflammatoriska markörer och patientrapporterade utfallsmått (förkortas på engelska till PROM).

I artikel I validerade vi ett lineblot test genom att jämföra det med ett immunoprecipitation test hos patienter med välkarakteriserad myosit. Patienter som var antikroppspositiva på linblot jämfördes med patienter som var antikroppspositiva vid immunoprecipitation avseende relevanta kliniska manifestationer. Samstämmigheten mellan de två testen var måttlig. De kliniska symtomen hos patienter som var positiva på lineblot överensstämde med tidigare kända kliniska manifestationer för dessa antikroppar.

I artikel II undersöktes sambandet mellan olika antikroppar och behandlingssvar genom att analysera en grupp av patienter med myosit från det svenska myositregistret (SweMyoNet). Andra potentiella faktorer som skulle kunna ha ett samband med behandlingssvar undersöktes också. Resultaten visade att patienter med antikroppar specifika för DM hade en stark association med måttlig grad av behandlingssvar. Det fanns också ett samband mellan tiden från första symtom innan diagnos, initial kortisondos och behandlingssvar.

I artikel III undersöktes sambandet mellan sjukdomsskada och antikroppstatus genom att analysera en grupp av patienter med myosit från det elektroniska internationella myositregistret (MyoNet). Resultaten visade att patienter med anti-PM/Scl antikropp utvecklade sjukdomsskada i högre takt jämfört med patienter utan någon antikropp. Patienter med DM-specifika antikroppar utvecklade sjukdomsskada i lägre takt jämfört med patienter utan någon antikropp. Ett starkt samband mellan muskelskada och funktionshinder hittades, särskilt hos patienter med nekrotiserande myopatier.

I artikel IV undersöktes det långsiktiga sambandet mellan inflammatoriska markörer (sänka [SR] och snabb sänka [CRP]) och självskattad hälsa. Resultaten visade att förhöjda inflammatoriska markörer associerades med sämre självskattad hälsa hos patienter med myosit, särskilt bland kvinnor.

Sammanfattningsvis har denna avhandling gett ny kunskap om antikroppar och inflammationsmarkörers betydelse för den långsiktiga prognosen hos patienter med myosit. I avhandlingsarbetet ingick att validera ett kommersiellt antikroppstest som används på klinik. Antikroppstatus har ett samband med behandlingssvar och sjukdomsskada. Inflammationsmarkörer har ett samband med självskattad hälsa hos patienter med myosit.

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