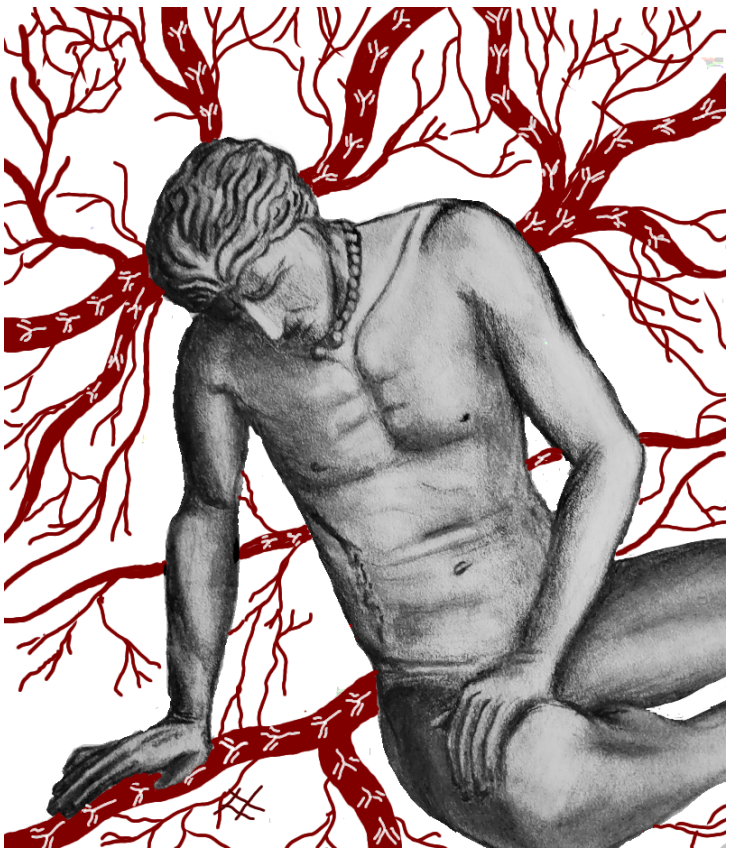
Thesis for doctoral degree (Ph.D.)
2020

Studies on pathogenesis, clinical features and comorbidities of Idiopathic Inflammatory Myopathies



Antonella Notarnicola

Karolinska Institutet

Thesis for doctoral degree (Ph.D.)
2020

Studies on pathogenesis, clinical features and comorbidities of Idiopathic Inflammatory Myopathies

Antonella Notarnicola

Stockholm 2020

KAROLINSKA INSTITUTET * ANNO 1810 *
Karolinska Institutet

Cover image by Aikaterini Chatzidionysiou
All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Universitetsservice US-AB

ISBN 978-91-7831-967-1

Studies on pathogenesis, clinical features and comorbidities of Idiopathic Inflammatory Myopathies

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Antonella Notarnicola

Principal Supervisor:
Professor Ingrid Lundberg
Karolinska Institutet
Department of Medicine, Solna
Division of Rheumatology

Co-supervisor(s):
PhD Catia Fernandes-Cerqueira
Karolinska Institutet
Department of Medicine, Solna
Division of Rheumatology

Professor Per-Johan Jakobsson
Karolinska Institutet
Department of Medicine, Solna
Division of Rheumatology

Opponent:
Professor Emeritus Eric L. Matteson
Mayo Clinic School of Health Sciences, USA
Department of Internal Medicine
Division of Rheumatology

Examination Board:
Associate Professor Ann Knight
Uppsala University
Department of Medical Sciences
Division of Rheumatology

Associate Professor Anna Smed Sörensen
Karolinska Institutet
Department of Medicine, Solna
Division of Immunology and Allergy

Associate Professor Lotta Ljung
Umeå University
Department of Public Health and Clinical Medicine
Division of Rheumatology

Thesis defense will take place on Friday 20th of November 2020, 9:00 am at the Rolf Luft Centrum Lecture hall, L1:00, Anna Steckséns Gata 53, Karolinska University Hospital, Solna, Stockholm

“Per due che come noi non si son persi mai
che se guardi indietro non ci crederai...”

Brunori Sas, from the beautiful song “Per due che come noi”

ABSTRACT

Idiopathic inflammatory myopathies (IIM), a group of rare chronic inflammatory disorders, are characterized by a broad spectrum of clinical manifestations with high morbidity and mortality. The pathogenesis of IIM is largely unknown but accumulating evidence suggests that autoantibodies promote the initiation and perpetuation of the disease. The aims of this thesis were 1) to increase the understanding of the role of anti-Jo1 antibodies and the histidyl-t-RNA synthetase (HisRS) autoantigen in the pathogenesis of the anti-synthetase syndrome (ASS), a distinct subgroup of IIM; 2) to study the incidence and the prevalence of a life-threatening comorbidity of IIM, the arterial and venous thrombosis, and to assess the contribution of traditional risk factors, disease characteristics and biomarkers to its occurrence.

Paper I: A sensitive electro-chemiluminescence immunoassay (ECLIA) was developed to detect HisRs in serum. For the first time, HisRS was found to circulate extracellularly in the serum of healthy individuals and, with higher concentrations, in the serum of anti-Jo1$^-$ patients. Serum levels of HisRS were, instead, undetectable in anti-Jo1$^+$ patients and correlated negatively with anti-HisRS autoantibody levels in serum. A human muscle cell culture was set up showing that primary human myoblasts could release HisRS in the culture medium with increasing amount during differentiation into myotubes and upon stimulation with insulin growth factor 1. The tolerance to endogenous HisRS was efficiently disrupted in different strains of wild-type mice by immunizing mice with murine full-length (FL) HisRS and WHEP. No obvious muscle or lung inflammation was observed in immunized mice compared to control mice. However, upon external induction of tissue-specific damage, the degree of immune engagement with consequent muscle damage and lung injury was significantly exacerbated in immunized mice compared to controls. The administration of HisRS in mice previously lung or muscle-challenged resulted in a significant reduction of the inflammation in both lungs and muscle tissue. Moreover, *in vitro*, HisRS inhibited the activation of T cells isolated from fresh blood of human healthy donors.

Paper II and III: Total IgG were isolated from anti-Jo1$^+$ and anti-Jo1$^-$ patients as well as from healthy controls (HC) and glycans appended to the Fc region of the IgG were explored and compared between the three groups. The Fc-glycan profile of the anti-Jo1 IgG isolated from anti-Jo1$^+$ patients was also investigated. Total IgG and specifically anti-Jo1 IgG from IIM/ASS patients displayed a pro-inflammatory Fc-glycan profile (i.e. agalactosylation) which was overrepresented in patients with interstitial lung disease (ILD). Anti-Jo1 IgG specifically presented lower abundance of bisected and afucosylated forms and Fc-glycan characteristics correlated positively with proteins involved in inflammatory processes.

IgG and IgA were isolated from serum and matching bronchoalveolar lavage fluid (BALF), collected at time of diagnosis and longitudinally, of anti-Jo1$^+$ and anti-Jo1$^-$ patients as well as from HC to determine the reactivity levels against the FL-HisRS protein and its different constructs and splice variants. Reactivity levels of IgG and IgA isolated from BALF and serum of anti-Jo1$^+$ patients were found to be high already at the time of diagnosis and in some cases even before diagnosis, generally decreasing thereafter. Highest reactivity was

registered against the HisRS-FL and the HisRS splice variants. Moreover, IgG against HisRS-FL displayed high affinity already at the time of diagnosis. Patients with high reactivity levels towards HisRS-FL were more likely to have ILD and arthritis, but less likely to have skin rash. Noteworthy, IgG anti-WHEP reactivity in BALF correlated with poor pulmonary function.

In **Paper IV**, the incidence of venous thromboembolic events (VTE) was assessed in patients with IIM in comparison to the general population and patient categories at high risk and the timing of risk in relation to the diagnosis of IIM were identified. In **Paper V**, the prevalence of arterial and venous TE was retrospectively investigated in a large cohort of IIM patients and possible traditional and/or disease-related risk factors and biomarkers linked to arterial and venous TE in patients with IIM were explored.
The incidence rate of VTE was significantly higher in IIM patients than in the general population, especially during the first year after diagnosis, and remained that high even after adjusting for education level, comorbidities, cancer, treatment at baseline and competing risk of death. Among IIM patients, the risk of VTE was even more elevated in those with a history of cancer, in patients with DM, and in those with age ≥72 years. In the retrospectively assessed cohort of IIM patients, one out of 5 patients had presented with an arterial and/or venous TE at the same time of or after the diagnosis of IIM. Even though a higher frequency of male gender and essential hypertension were observed in IIM patients with reported TE and of malignancy in those with history of exclusively venous TE, only older age was an independent risk factor for TE occurrence, while autoantibodies and clinical variables did not contribute. Interestingly, lower levels of e-selectin correlated with higher odds of getting TE in IIM patients.

In conclusion, the discovery of HisRS extracellularly in both healthy individuals and IIM patients supports the hypothesis that HisRS exerts other physiological functions beyond the known intracellular protein synthesis. The inhibition of T cell activation by HisRS and the impact of HisRS in reducing the degree of inflammation in mice previously immunized against HisRS and with previously induced tissue damage suggest a possible immunosuppressive activity of this protein. This could open the path for a potential new therapeutically approach in anti-Jo1 positive patients. The inflammatory Fc-glycan profile as well as the high reactivity and affinity levels in serum and BALF of anti-Jo1 antibodies (conversely undetectable serum HisRS levels) already at the time of and even before diagnosis represent new evidence supporting the role of anti-Jo1 antibodies in the pathogenesis of IIM/ASS. The high incidence and prevalence of arterial and venous thrombotic events in patients with IIM, especially close to diagnosis and in those older, male patients with essential hypertension and history of malignancy, should indicate that a proper screening and preventive measures need to be recommended in this patient population. E-selectin levels could be used as biomarkers to identify IIM patients at higher risk of presenting with TE.

LIST OF SCIENTIFIC PAPERS

I. **Serum-circulating His-tRNA synthetase inhibits organ-targeted immune responses**
R. A. Adams*, C. Fernandes-Cerqueira*, **A. Notarnicola**, E. Mertsching, Z. Xu, W-S. Lo, K. Ogilvie, K. P. Chiang, J. Ampudia, S. Rosengren, A. Cubitt, D. J. King, J. D. Mendlein, X-L. Yang, L. A. Nangle, I.E. Lundberg, P-J. Jakobsson and P. Schimmel *Cellular and Molecular Immunology, 2019, Dec 4*

II. **Patients with anti-Jo1 antibodies display a characteristic IgG Fc-glycan profile which is further enhanced in anti-Jo1 autoantibodies**
C.Fernandes-Cerqueira, N. Renard*, **A. Notarnicola***, E. Wigren, S. Gräslund, R. A. Zubarev, I.E. Lundberg and S.L. Lundström
Scientific reports, 2018, 8:17958

III. **Highly reactive anti-Jo1 autoantibodies to distinct variants and domains of HisRS associate with lung and joint involvement in patients with myositis**
A. Notarnicola*, C. Preger*, S.L. Lundström, N. Renard, E. Wigren, E. Van Gompel, A. Galindo Feria, H. Persson, M. Fathi, J. Grunewald, P-J. Jakobsson, S. Gräslund, I.E. Lundberg*, C. Fernandes-Cerqueira*
Submitted Manuscript

IV. **Venous Thromboembolic Events in Idiopathic Inflammatory Myopathy: Occurrence and Relation to Disease Onset**
A. Antovic*, **A. Notarnicola***, J. Svensson, I.E. Lundberg, M. Holmqvist
Arthritis Care and Research, 2018, Vol.70, pp 1849-1855

V. **Evaluation of risk factors and biomarkers related to arterial and venous thrombotic events in idiopathic inflammatory myopathies**
A. Notarnicola, S. Barsotti, L. Näsman, Q. Tang, M. Holmqvist, I.E. Lundberg, A. Antovic
Submitted Manuscript

*Equal contribution

ADDITIONAL PUBLICATIONS

Pro-inflammatory histidyl- tRNA synthetase- Specific CD 4 + T cells in the blood and lung of patients with idiopathic inflammatory myopathies
A. Galindo-Feria*, I. Albrecht*, C. Fernandes-Cerqueira, **A. Notarnicola**, E.A. James, J. Herrath, M. Dastmalchi, T. Sandalova, L. Rönnblom, P.-J Jakobsson,M. Fathi, A. Achour, J. Grunewald, V. Malmström and I.E. Lundberg
Arthritis Rheumatol., 2020 Jan;72(1):179-191

Performance of the new EULAR/ACR Classification Criteria for Idiopathic Inflammatory Myopathies (IIM) in a Large Monocentric IIM Cohort
S. Barsotti, M. Dastmalchi, **A. Notarnicola**, V. Leclaire, L. Dani, K. Gheorghe, L. Ekholm, M. Bottai, A. Tjärnlund, I.E. Lundberg
Seminars in Arthritis and Rheumatism, 2019, Dec 28

Heterogeneous clinical spectrum of interstitial lung disease in patients with anti-EJ Anti-Synthetase Syndrome: a case series
M. Giannini*, **A. Notarnicola***, M. Tampoia, M. Dastmalchi, I.E.Lundberg, G. Lopalco, F. Iannone
Clin Rheumatol. 2016 Sep;35(9):2363-7

* Equal contribution

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

aaRS	Aminoacyl t-RNA synthetase
AAV	ANCA-associated vasculitis
Ab	Antibody
ABD	Anti-codon binding domain
ACPA	Anti-citrullinated peptides antibodies
ACR	American College of Rheumatology
ACS	Acute coronary syndrome
AIMPs	Aminoacyl tRNA synthetase-interacting multifunctional proteins
AIRE	Autoimmune regulator gene
ALAT	Alanine aminotransferase
ALP	Alkaline-phosphatase
ALT	Alanine aminotransferase
ANCA	Anti-neutrophilic cytoplasmic antibodies
APC	Antigen presenting cells
APL	Antiphospholipid antibodies
ASAT	Aspartate aminotransferase
ASS	Anti-synthetase syndrome
AST	Aspartate aminotransferase
BALF	Bronchoalveolar lavage fluid
BLM	Bleomycin
C	Constant domains
CAC	Coronary artery calcification
CADM	Clinically amyopathic dermatomyositis
CAM	Cancer-associated myositis
CD	Catalytic domain
CI	Confidence interval
CK	Creatine kinase
COP	Cryptogenic organizing pneumonia
CTD	Connective tissue diseases
CTL	Cytotoxic T lymphocyte
CVD	Cardiovascular disease
DAMPS	Damage-associated molecular patterns
DC	Dendritic cell
DLCO	Diffusing capacity for carbon monoxide
DM	Dermatomyositis
DVT	Deep venous thrombosis
DXT	Dexamethasone
ECLIA	Electro-chemiluminescence immunoassay
EJ	Isoleucyl-t-RNA synthetase
ELISA	Enzyme-linked immunoassorbent assay
EM	Electron microscopy
EMG	Electromyography
ER	Endoplasmic reticulum stress

EULAR	European League against Rheumatism
Fab	Fragment antigen-binding
Fc	Fragment crystallizable region
FcR	Fc receptor
FEV1	Forced expiratory volume in 1 second
FHL1	Four-and-a-half LIM protein
FI	Functional index test
FL	Full lenght
FVC	Forced vital capacity
GARS	Glycyl-tRNA synthetase
GC	Germinal center
GrB	Granzyme B
sGOT	Serum glutamic oxaloacetic transaminase (same as ASAT or AST)
sGPT	Serum glutamic pyruvic transaminase (same as ALAT or ALT)
GWAS	Genome-wide association studies
H	Heavy chains
Ha	Tyrosyl-t-RNA synthetase
HARS	Histidyl-t-RNA synthetase
HAQ	Health assessment questionnaire of activities of daily living
HC	Healthy control
H&E	Hematoxylin and eosin
HisRs	Histidyl-t-RNA synthetase
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMGB1	Alarmin high mobility group box protein 1
HMGCR	3-hydroxy-3-methylglutaryl- CoA reductase
HR	Hazard ratio
HRCT	High resolution computer tomography
HTLV-1	Human T-lymphotropic virus-1
IACUCs	Institutional animal care and use committees
sIBM	Sporadic inclusion body myositis
ICAM-1	Intracellular adhesion molecule
ICD-10	International Statistical Classification of Diseases, Tenth Revision
IFN	Interferon
IL	Interleukin
IIM	Idiopathic inflammatory myopathies
ILD	Interstitial lung disease
IMACS	International Myositis Assessment & Clinical Studies group
IMNM	Immune-mediated necrotizing myopathy
IP	Immunoprecipitation
IPEX	X-linked immunodysregulation poly-endocrinopathy enteropathy (IPEX)
IPF	Idiopathic pulomary fibrosis
IQR	Inter-quartile range
JDM	Juvenile dermatomyositis

Jo1	Histidyl-t-RNA synthetase
Jo1FT	Non-Jo1 reactive IgG
KS	Asparaginyl-t-RNA synthetase
Ku	Ku-complex
L	Light chain
LC	Liquid chromatography
LDH	Lactate dehydrogenase
MAAs	Myositis associated autoantibodies
MAC	Membrane attack complex
MARS	Methionyl-tRNA synthetase
MDAAT	Myositis Disease Activity Assessment Tool (
MI	Myocardial infarction
MRI	Magnetic resonance imaging
MDA5	Melanoma differentiation associated gene 5
MHC	Major histocompatibility complex
Mi-2	Nuclear DNA helicase
MMT8	Manual muscle test 8
MS	Mass spectrometry
MSAs	Myositis specific autoantibodies
MRC	Medical research council's scale
NARS	Asparaginyl-tRNA synthetase
NETs	Neutrophil extracellular traps
NHV	Normal healthy volunteers
NSIP	Non-specific interstitial pneumonia
NT-proBNP	N-terminal of prohormone of brain natriuretic peptide
NT5C1a	Cytosolic 5' nucleotidase 1A
NXP2	Nuclear matrix protein 2
OD	Optical density
OJ	Glycyl-t-RNA synthetase
OM	Overlap myositis
OPLS-DA	Orthogonal projections to latent structures discrimination analysis
PBMC	Peripheral blood mononuclear cells
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PE	Pulmonary embolism
PGFT	Protein G flow through
PL7	Threonyl-t-RNA synthetase
PL12	Alanyl-t-RNA synthetase
PM	Polymyositis
Pm-Scl	Nucleolar protein complex
PTM	Post-translational modification
SAE	SUMO activating enzyme
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SPR	Surface plasmon resonance
SPSS	Statistical Package for the Social Sciences
SRP	Signal recognition particle
SS	Sjögren's syndrome
SV	Splice variant
Swemyonet	Swedish Rheumatology Quality Register for IIM
RA	Rheumatoid Arthritis
TCR	T cell receptor
TE	Thrombotic event
TF	Tissue factor
TGF-β	Transforming growth factor
Th	T helper cell
TIF1γ	Trascriptional intermediary factor 1 γ
TLC	Total lung capacity
TNF-α	Tumor necrosis factor-α
TRAIL	TNF-related apoptosis-inducing ligand
UIP	Usual interstitial pneumonia
U1RNP	U1 small nuclear protein
V	Variable domains
VAS	Visual analogue scale
VC	Vital capacity
VCAM-1	Vascular cell adhesion molecule-1
VEH	Vehicle
VTE	Venous thromboembolic event
WB	Western Blot
Zo	Phenylanalanyl-t-RNA synthetase

INTRODUCTION

In 1901, the German scientist and physician Paul Ehrlich speculated on the possibility that the immune system might misdirect its attack by producing toxic antibodies for self-tissues (1). He was indeed reluctant to the idea that this phenomenon, he named “horror autotoxicus”, could actually lead to illness (2). In fact, he hypothesized the presence of protecting mechanisms to avoid that the organism ultimately harms itself (1). After more than one century, we are able to acknowledge his brilliant intuition by describing the sophisticated ability of the immune system to selectively recognize the foreign substances or non-self-antigens while not reacting to native antigens. The so-called breaking of immunological self-tolerance and the consequent initiation of an autoimmune response is considered to be the crucial event leading to the development of an autoimmune disease. Despite major advances in understanding which genetic and environmental factors or infectious pathogens may contribute to the exposure of autoantigens to the immune system and to the start of clonal multiplication of autoreactive T cells and to autoantibody production, the etiology and the pathogenesis of the majority of known autoimmune diseases are still poorly understood (3).

Autoimmune diseases represent a unique medical challenge because of the high complexity and heterogenicity of clinical phenotypes with frequent multiple organ involvement, high morbidity with big impact on quality of life and current lack of tailored target therapy with curing purpose. So far, more than 80 autoimmune diseases have been described, affecting 5% of the world population and being a major burden for the economic and health care system (4, 5). Therefore, a better understanding of the pathogenic mechanisms that govern these conditions is highly relevant to identify preventive and therapeutic approaches.

This PhD thesis focuses on a group of chronic inflammatory systemic disorders, the idiopathic inflammatory myopathies (IIM), where accumulating evidence suggests that an aberrant activation of the immune system plays a central role in the initiation and perpetuation of the systemic inflammation.

1 Immunological tolerance

Immunological tolerance refers to a state of unresponsiveness of the adaptive immune system toward a given specific antigen. The absence of immune reaction towards native or self-antigens is defined as **immunological self-tolerance**. Numerous regulatory pathways are needed to prevent immune responses when immature lymphocytes encounter native antigens in the primary lymphoid organs (central tolerance) or when mature lymphocytes encounter antigens in peripheral or secondary lymphoid tissues under particular conditions (peripheral tolerance) (3, 6-8).

1.1 Mechanisms of central tolerance

Central tolerance for immature B-lymphocytes and T-lymphocytes occurs in the bone marrow and in the thymus, respectively. In the bone marrow, immature B-lymphocytes that encounter a native antigen undergo apoptotic cell death or they change specificity of the surface-bound immunoglobulins which constitute the B cell receptor, a phenomenon known as “receptor

editing". The recognition of any antigen by T cells through the specific T cell receptor (TCR) occurs only if the antigen peptide is presented in the niche formed by class I or class II alleles of the major histocompatibility complex (MHC) on the surface of any nucleated cell type for MHC-I and on professional antigen presenting cells (APC) for MHC-II. In the thymus, immature T cells with TCR showing no affinity for the MHC-peptide complex are neglected and die (positive selection of T lymphocytes with sufficient affinity of the TCR for the MHC-peptide complex). The pool of positive selected T cells contains lymphocytes recognizing both exogenous and self-antigens. T-lymphocytes with high affinity TCR for self-peptides in the context of MHC undergo apoptotic cell death (negative selection) (3, 6-8).

1.2 Mechanisms of peripheral tolerance

Autoreactive mature B and T cells that escaped central tolerance migrate to secondary lymphoid organs. Autoreactive B cells lacking proper activation signals may enter a state of anergy. This happens, for instance, when B cells encounter an antigen in absence of T helper lymphocytes. APC, in particular dendritic cells (DC), are involved in the regulation of peripheral T cell response. Downregulation of co-stimulatory molecules on APC (CD80 and CD86) blocks the activation of autoreactive T cells which become anergic or are deleted.
Regulatory T cells or T-regs, a specific T cell subgroup, are capable to suppress immune responses through secretion of cytokines such as interleukin-10 (IL-10), transforming growth factor (TGF)-β, IL-35 and by altering/limiting DC function (3, 6-8).

2 Definition of autoimmunity and autoimmune disease

Abolition of immunological self-tolerance, due to failure of regulatory checkpoint mechanisms, induces the selection of autoreactive B and T cell clones (9). This does not automatically result in an autoimmune disease. At a certain level, autoimmunity is a physiological event and a pool of autoreactive lymphocytes in the immune cell repertoire is actually maintained since it has been shown to be crucial for immune system development and for prevention of pathogenic autoimmunity (10).
Therefore, an autoimmune disease develops when, in individuals with genetic susceptibility, upon exposure to environmental, infectious or internal triggers, an aberrant response of the adaptive immune system to a self-antigen leads ultimately to tissue injury (9). Widely accepted proofs of pathogenic autoimmunity are 1) the identification of autoreactive T cells and/or autoantibodies in the affected organ or tissue; 2) the induction of the disease after transferring autoreactive T cells and/or autoantibodies in healthy individuals or animals or after immunizing animal models with the autoantigen; 3) the improvement of the clinical manifestations after eliminating or suppressing the autoimmune response (5, 11).

2.1 Genetic susceptibility

A low number of autoimmune diseases are caused by mutations in single genes and are therefore indicated as monogenic autoimmune diseases (12, 13). Examples are the autoimmune poly-endocrinopathy syndrome type 1 induced by a mutation in the autoimmune regulator gene (AIRE) which affects the negative selection in the thymus and thus self-antigen presentation

(12, 13) and the X-linked immunodysregulation poly-endocrinopathy enteropathy (IPEX) syndrome caused by a mutation within the gene FOXP3 involved in the generation of regulatory T cells (14). In the majority of patients, genetic factors, such as rare (<1%) or common (>1%) single nucleotide polymorphisms (SNPs), copy-number variants and epigenetic modifications, are claimed to confer higher susceptibility for development of autoimmune diseases (15). Genome-wide association studies have shown that genetic variants of human leukocyte antigen (HLA) genes within the MHC locus on the chromosome 6 and of genes outside the MHC locus but with crucial roles in pathways of B-cell and T-cell activation or innate receptor signaling (e.g. IRF5, STAT 4, PTPN22, TNFSF4) are strongly associated with several autoimmune diseases (13, 15). Some risk alleles may predispose for more than one autoimmune disease. For instance, the haplotype MHC class II molecule HLADR3 and, in particular, HLA-DRB1*0301 associates with Type I diabetes (16), idiopathic inflammatory myopathies (IIM) (17) and systemic lupus erythematosus (SLE) (18). The haplotype DR4 and its HLADRB1 subtypes (shared epitope) correlate, instead, with Rheumatoid Arthritis (RA) (19).

2.2 Environmental triggers

Specific genetic components do not account alone for risk of being affected by autoimmune diseases. In fact, the concordance rate of autoimmune diseases in monozygotic twins is between 12 and 65% (13). Environmental factors, proposed as triggers in genetic predisposed individuals, include infectious agents, chemicals and pharmaceutical agents, nutrition, the microbiota, hormones, smoking, ultraviolet light, collagen/silicone implants, heavy metals, vaccines (13, 15).

2.3 Autoantigens

An aberrant autoimmune response, with autoreactive T cells and autoantibodies as main actors, is often directed to:

- *tissue-restricted* autoantigens (e.g. beta cells of the pancreatic islets in Type I Diabetes (20) or thyroglobulin/thyroid peroxidase in Hashimoto thyroiditis (21));
- *ubiquitously expressed* autoantigens, albeit resulting in distinct clinical phenotypes (e.g. Sm-ribonucleoproteins and double-strand DNA in SLE (22-25) or anti-topoisomerase-1 in diffuse scleroderma with interstitial lung disease (26)).

The association between the immune reactivity to a certain autoantigen and a particular clinical phenotype is not affected by the frequency of autoantibody reactivity in a specific patient population (27). For instance, antibodies to citrullinated proteins are specifically found in 70-90% of patients with RA (28) as well as anti-alanyl-tRNA synthetase antibodies are detected in less than 5% of patients with unique anti-synthetase syndrome (29).

With some exceptions, as for example in RA with autoantibodies against the Fc portion of IgG in serum (rheumatoid factor) (30), many autoantigens targeted in systemic autoimmune diseases are localized intracellularly, in nuclei, cytoplasm, bound to organelles and cell-membrane and are part of multimolecular machines with important role in DNA replication, DNA repair, RNA processing and splicing, gene expression, protein translation (27).

The events listed below may explain why an intracellular autoantigen becomes suddenly visible to the immune system and elicits autoantibody production (Figure 1):

- *Post-translational modifications (PTMs)*, i.e. alteration of the amino-acid sequence of the protein due to e.g. phosphorylation, deamination, glycosylation, ubiquitination, citrullination, occur physiologically after protein synthesis and impacts protein folding, protein functions and interaction with other proteins (31). Under pro-inflammatory circumstances and cell death, protein structure modifications may reveal new or cryptic epitopes otherwise not presented to T cells ensuing an autoimmune process (32, 33). Antibodies targeting citrullinated peptides in RA represent an example of strong association between PTMs and an autoimmune disease (34).
- During cell death induced by *granule-mediated cytotoxic T lymphocytes (CTL)*, autoantigens may undergo *proteolytic modifications.* Unique autoantigen protein fragments are generated, for instance, by Granzyme B (GrB) cleavage, a serine protease contained in CTL granules (35). Immune epitopes have been found close to cleavage GrB sites, suggesting that the GrB cleavage may mediate novel antigen presentation (36). Higher expression of cleaved forms of autoantigens has been observed in target tissues of autoimmune diseases (36), e.g. the GrB-cleaved histidyl-t-RNA synthetase (HisRs/Jo-1) autoantigen is almost exclusively found in the lungs of patients with anti-synthetase syndrome (37) or the GrB cleaved form of the nucleolar autoantigen nucleophosmin in differentiated smooth cells of patients with systemic sclerosis and pulmonary hypertension (38).
- In case of *impaired clearance* of apoptotic cells and *defective degradation* of neutrophil extracellular traps, *NETs* during NETosis, intracellular components such as DNA, histones, ribonucleoproteins are enriched at the surface of the cells in apoptotic bodies and become immunogenic, triggering autoantibody production. This is known to contribute, for instance, to the pathogenesis of SLE (39, 40).
- the immune system can cross-react to self-molecules which present a significant homology with the structure of a microbial agent, a phenomenon known as *molecular mimicry (41, 42)*.

Figure 1

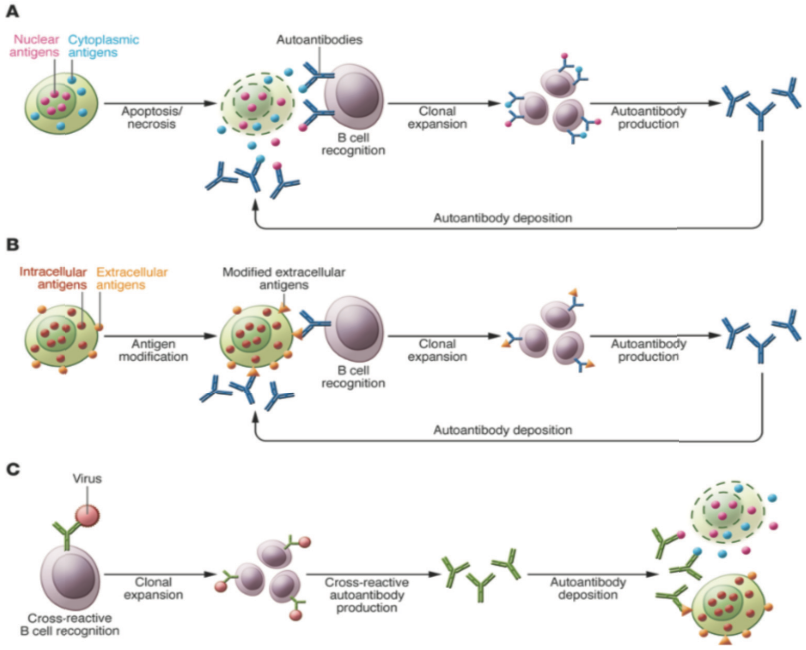


Figure 1. Three possible events for recognition of intracellular antigens by autoantibodies. (A) Cell death through apoptosis or necrosis and release in the extracellular milieu of intracellular antigens. (B) Modification of antigen structures and exposure of neo-epitopes. (C) Cross-reactivity due to molecular mimicry. *Reprinted by permission from Journal of Clinical Investigations, Jolien Suurmond and Betty Diamond, Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity, J Clin Invest. 2015;125(6):2194–2202*

2.4 Autoantibodies

Autoantibodies, immunoglobulins binding constituents of the body itself and secreted by plasma cells differentiated from auto-reactive B cell clones, represent the hallmark of several autoimmune diseases (43). The tests used for their detection in serum, albeit with different sensitivity and specificity, comprise fundamental **diagnostic tools (44)**. For example, an individual testing negative for anti-nuclear antibodies by indirect immunofluorescence on Hep-2 cells is extremely unlikely to be affected by SLE (45). Within a systemic autoimmune disease, the presence of certain autoantibodies can correlate with the development of distinct clinical manifestations, as for anti-P-ribosomal protein and central nervous system involvement in lupus (46). Furthermore, some autoantibodies may confer higher risk for complications during pregnancy, even when the woman does not present with any clinical symptom of an autoimmune disease (e.g. anti Ro and anti-La and risk for neonatal lupus syndrome (47) or anti-cardiolipin and risk for mid-trimester fetal loss (48)). Autoantibody titers may correlate with disease activity and their fluctuation may have a prognostic value, predicting disease flares or

response to treatment (49). In anti-neutrophilic cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), ANCA titers significantly decrease or disappear during remission and, conversely, increase in case of active disease or before a recurrent event (50). In RA, even though the presence of anti-citrullinated peptides antibodies (ACPA) is associated with more aggressive disease and development of bone erosions, studies on the relationship between the variation of ACPA titers and disease progression or response to therapy showed variable results (51-53).

Beyond the role as serological biomarkers, autoantibodies can directly induce systemic inflammation and tissue injury and are, in this case, indicated as **pathogenic**.

To understand how pathogenic autoantibodies are generated and behave, it is important to start from the following general concepts:

- *Antibody structure*: two heavy (H) chains and two light (L) chains held together by disulfide bonds. Both heavy and lights chains contain constant (C) and variable (V) domains. The four constant domains at the C-terminal of the two heavy chains constitute the fragment crystallizable region (Fc) stem of the immunoglobulin which mediate effector functions through binding to Fc receptors (FcR) on other cells of the immune system and via complement activation. The Fc part is also linked to diverse glycans which determine the glycosylation pattern of the antibody (54). The variable arms (fragment antigen-binding, Fab), composed of the two light chains and the N-terminal halves of each heavy chain, bind the antigen molecule (Figure 2) (55, 56).

Figure 2

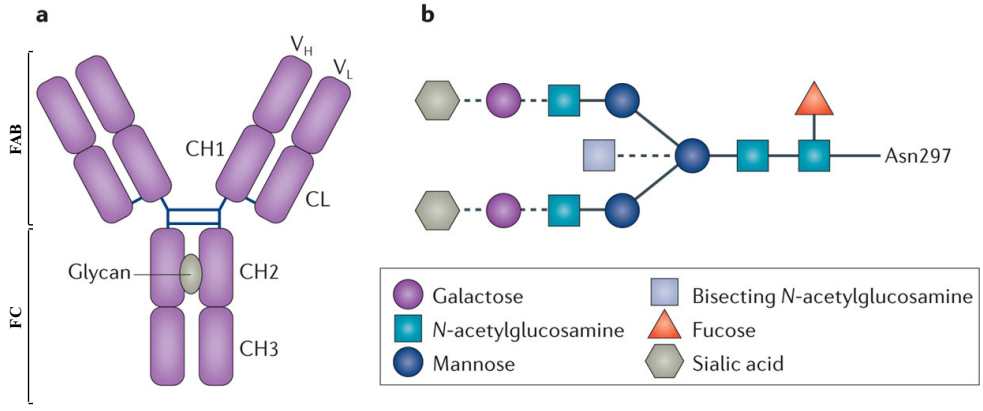


Figure 2. Antibody structure. a) the antibody is basically composed of a light chain with a variable and constant region and a heavy chain with a variable region, a hinge and three constant region domains, each with different functions. The glycan is attached to Asn297 in the CH2 domain. b) the antibody glycan with possible combination of sugars. *Reprinted by permission from Springer Nature, Nikolay Nikolow et. al., An FDA perspective on the assessment of proposed biosimilar therapeutic proteins in rheumatology, Nature Review Rheumatology 13, (123-127), 2017*

- *Antibody diversity*: the constant regions of the heavy and light chains are encoded by genes undergoing rearrangements. This determines the different isotype (IgG, IgA, IgM,

IgE, IgD) and sub-class of the antibody (**immunoglobulin isotype class switch recombination**) as well as the different effector Fc properties. The high variability of the Fab regions is due to point mutations inserted into the genes encoding the variable sequences, a process called **somatic hypermutation.** This allows the binding with a vast number of potential antigens (55).

- *Polyclonal versus monoclonal antibodies*: **polyclonal** antibodies are antibodies showing reactivity to different epitopes of one antigen and produced by plasma cells which originate from multiple B lymphocytes clones. **Monoclonal** antibodies derive from one single B lymphocyte clone and recognize only one epitope of the antigen (57).
- *Antibody **specificity***: the ability to recognize a specific epitope in presence of other epitopes. The higher the specificity, the lower is the cross-reactivity of the antibody with several epitopes of different antigens (57).
- *Antibody **affinity** and **avidity***: the strength of the bond between the antibody and the antigen or, in other words, the capacity of the antibody to dissociate from the antigen, measured with the dissociation constant K, determines the antibody affinity. The higher the K, the lower the affinity and vice versa. Antibodies repeatedly exposed to the same antigen acquire greater affinity for the antigen (**affinity maturation**). Antibodies can bind antigens with multiple binding sites as well as antigens can bind more than one antibody (antibody and antigen valency). The overall strength of a multivalent interaction between the antibody and the antigen is measured by **avidity** (57, 58).

Natural autoantibodies, circulating in healthy individuals at a concentration of 1-2 mg/ml in the blood, are commonly polyclonal IgM with low affinity and minimal somatic hypermutations. They provide the first defense against pathogens and are involved in regulation of B cell development and B cell responses and in protection against cancer (59).

Pathogenic autoantibodies, instead, are somatically mutated and affinity maturated polyclonal and monoclonal immunoglobulins, belonging to any class type, but preferentially IgG and IgA (59). They may present with altered glycosylation pattern (60, 61). Their pathogenetic action can be exploited directly, by stimulating or suppressing the antigen function (e.g. Grave's disease mediated by antibodies against thyroid stimulating hormone receptor (62)), or indirectly through two major pathways:

- *Formation of immune complexes* which precipitate in targeted tissues and activate the complement system leading to direct cell lysis and recruitment of leukocytes to remove the antigen and enhance inflammation.
- *Binding to FcR-carrying effector cells*, such as monocytes, macrophages, dendritic cells, neutrophils, mast cells, platelets and natural killer cells, which determine activation of tyrosine kinases, stimulation of phagocytosis, degranulation and release of cytokines and inflammatory mediators, and antibody-dependent cellular cytotoxicity (63, 64).

Indirect pathogenic effects are claimed, for instance, for ANCA and ACPA in AAV and RA, respectively (63).

At the experimental level, proofs of autoantibody pathogenicity can be obtained *in vitro* by demonstrating autoantibody-related cellular damage or immune activation, or *in vivo,* by

observing the occurrence of an autoimmune disease after passive transfer of autoantibodies in animal models (65, 66). At clinical level, the presence of autoantibodies in serum years before the onset of the disease (67, 68) (e.g. ACPA in RA (69)), with characteristics as epitope spreading, affinity maturation, glycosylation changes, isotype switching, as well as the effectiveness of B-cell target therapies are considered indirect evidences of pathogenicity (64).

2.5 Autoreactive T cell driving autoantibody production and tissue injury

Processes like B cell maturation, B cell clonal expansion, differentiation of B cells into plasma cells, somatic hypermutation, and immunoglobulin class switching take place in germinal centers (GC) of secondary lymphoid organs. These processes are regulated by the follicular T helper cells (Th), a distinct $CD4^+$ T helper cell subset, which originate from naïve $CD4^+$ T cells through antigen stimulation and interactions with APC (70). In autoimmune diseases, targeted tissues, such as the synovia in RA and the kidney in SLE, often present with ectopic GC where cross-talk between T and B cells is crucial for production of high affinity autoantibodies (15, 70). Autoreactive follicular Th cells selectively activate those B cells reactive to the same antigen recognized by T cells. Thus, antigens bound to autoantibodies include the peptide recognized by autoreactive $CD4^+$ T cells. It follows that the specific B cell autoimmune response is generally **antigen-driven and T cell dependent** (64, 70).
Autoreactive $CD4^+$ T cells together with APC are also important to induce the recruitment of autoreactive $CD8^+$ T cells responsible for direct killing of target cells. The activation of autoreactive $CD8^+$ T cells and their transformation into effector cytotoxic T cells require the binding with the complex MHC class I-autoantigen at the surface of the target cell or of the APC. Once activated, $CD8^+$ T cells kill target cells through degranulation of cytolytic enzymes like perforin and granzyme B, induction of apoptosis through Fas-Fas-ligand and release of cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (13, 71, 72).

2.6 Active role of target tissue in autoimmunity

Target tissues of pathological autoimmune responses are not simply passive bystanders. Physiologically, pro-inflammatory and anti-inflammatory pathways within the tissue are finely regulated and balanced. This allows to fight infections or tumors by avoiding excessive tissue damage. In individuals genetically susceptible to autoimmune diseases, tissue intrinsic mechanisms that control or inhibit inflammation can be defective. In presence of pro-inflammatory cytokines, target cells are self-capable of releasing chemokines which induce the migration of activated T and B lymphocytes to the tissue. Once in the tissue, the activated lymphocytes interact directly with target cells which start to up-regulate MHC-class I and co-stimulatory molecules. In this way, chronic inflammation is established and autoimmune tissue destruction is promoted (15, 73).

3 Idiopathic inflammatory myopathies

The term idiopathic inflammatory myopathies (IIM), in short myositis, refers to a group of rare chronic inflammatory disorders. Even though the word myositis literally means muscle inflammation, the burden of inflammation is indeed systemic, affecting in most cases not only skeletal muscle but even the skin, lungs, heart, joints and the gastrointestinal tract. Typical muscular symptoms are muscle weakness and impaired muscle endurance which lead to problems in walking, climbing stairs or lifting objects above the head. Within extra-muscular symptoms, skin rash, dyspnea and dry cough due to interstitial lung disease (ILD), arthritis, Raynaud's phenomenon, mechanic's hands, and fever are widely recognized (74). The heterogenicity of clinical manifestations and their broad range of severity explain why the diagnosis is often a challenge for the clinicians who needs to collaborate with experts in different disciplines such as the rheumatologist, neurologist, pulmonologist, cardiologist, pathologist, and physiotherapist.
By combining different sets of diagnostic and classification criteria based on serological tests including autoantibodies, clinical, histological features and imaging, the following subgroups can be distinguished: polymyositis (PM), dermatomyositis (DM), juvenile dermatomyositis (JDM), clinically amyopathic dermatomyositis (CADM), immune-mediated necrotizing myopathy (IMNM), cancer-associated myositis (CAM), overlap myositis (OM), anti-synthetase syndrome (ASS), and sporadic inclusion body myositis (sIBM) (75).

3.1 Epidemiology

Idiopathic inflammatory myopathies belong to the group of rare diseases (76). Accurate epidemiological data is difficult to provide because of large variation of ethnical, geographical and diagnostic/classification criteria applied in different studies. According to a systematic literature review of 16 surveys published in 2015, the overall IIM incidence was estimated at 7.98 cases/million/year (95% CI 7.38, 8.66) between 1951 and 2010 (76). After analyzing the results of 10 surveys, overall IIM prevalence was estimated at 14 cases/100 000 inhabitants (95% CI 12.84, 15.46) between 1982 and 2010 (76). Exactly the same prevalence of IIM was found in Sweden, as described by a nation-wide population study published by our research group in 2017 (77). The Swedish IIM incidence, instead, was higher with reported 11 new cases per million people per year with age peak at 50-79 years (77). At IIM subgroup level, OM, DM and IMNM account for almost 90% of myositis cases (78). DM was the most common diagnosis among 3067 patients in the Euromyositis registry (79) and a higher frequency of DM cases has been observed closer to the equator (80, 81). The female gender is generally more affected with a female: male ratio of 2:1 (76) with the exception of sIBM where the ratio is inverted (76). Few population-based studies have investigated the incidence and prevalence of sIBM, reporting 1.2-4.9 incident cases per million per years (76) and a prevalence ranging from 14.9 per million in Australia in 2007 (82) up to 35 per million in Norway in 2014 (83). Two peaks of age at onset, the first before the age of 20 and the second between 44-55 years for IIM as a whole and 60-65 years for sIBM have been observed (76).

3.2 Diagnostic and classification criteria

3.2.1 Bohan and Peter criteria

In 1975, Bohan and Peter (84, 85) proposed the diagnostic and classification criteria with a sensitivity of 94-96% and a specificity of 29-55% (86). They have been widely used for more than 40 years. The criteria included 5 variables (Table 1) and were able for the first time to differentiate DM from PM, however not between PM and sIBM.

Table 1
Bohan and Peter's diagnostic criteria for PM and DM (84, 85)

First, rule out all other forms of myopathies
1. Symmetrical weakness, usually progressive, of the limb-girdle muscles with or without dysphagia and respiratory muscle weakness
2. Muscle biopsy evidence of myositis
Necrosis of type I and type II muscle fibers; phagocytosis, degeneration, and regeneration of myofibers with variation in myofiber size; endomysial, perimysial, perivascular, or interstitial mononuclear cells.
3. Elevation of serum levels of muscle-associated enzymes (CK, LDH, transaminases, aldolase)
4. EMG triad of myopathy a. Short, small, low-amplitude polyphasic motor unit potentials b. Fibrillation potentials, even at rest c. Bizarre, high-frequency repetitive discharges
5. Characteristics rashes of dermatomyositis
Definite PM: all first four elements, probable PM: 3 of first 4, possible PM: 2 of first 4.
Definite DM: rash plus 3 others, probable DM: rash plus 2 others, possible DM: rash plus 1 other
Legend: CK, creatine kinase; LDH, lactate dehydrogenase, EMG, electromyography; PM, polymyositis; DM dermatomyositis

3.2.2 Griggs criteria

The more frequently used diagnostic and classification criteria for sIBM were postulated by Griggs et al. in 1995 (Table 2) (87). According to the evaluation of diagnostic criteria for sIBM made by Lloyd et al. (88) the sensitivity of Griggs criteria is 11-29%, while the specificity is 100%.

Table 2
Griggs criteria for diagnosis of sIBM (87)

A. Clinical features
1. Duration > 6 months
2. Age of onset > 30 years
3. Weakness Proximal and distal of arms and legs and must exhibit at least one of: a. Finger flexor weakness; b. Wrist flexor > extensor weakness; c. Quadriceps weakness ≤ Grade 4 MRC
B. Laboratory features
1. Creatine kinase < 129 UI/L normal
2. Muscle biopsy
a. Inflammatory myopathy (with partial invasion)
b. Rimmed vacuoles
c. Either
i. Intracellular amyloid deposits, or
ii. 15- to 18-nm tubulofilaments by EM
Diagnostic criteria: (i) Definite IBM = all muscle biopsy features. None of the clinical/laboratory features are mandatory and (ii) Possible IBM = partial invasion with- out other pathological features + characteristic clinical and other laboratory features.
Legend: IBM, inclusion body myositis; EM, electron microscopy; MRC, medical research council's scale

3.2.3 EULAR/ACR criteria

The EULAR/ACR (European League against Rheumatism/American College of

Rheumatology) criteria (89), published in 2017, are data driven and developed by a multi-disciplinary team, aimed to identify IIM patients with a broad spectrum of phenotypes and to capture those with minimal or absent muscle involvement. Sixteen variables are weighted differently on the basis of their ability to contribute to the diagnosis. Compared to several of previous classification criteria a myositis-specific autoantibody, anti-Jo-1 is included. The sum of each score provides the probability of having IIM. A probability of 55% had the best sensitivity of 87-93% and specificity of 82-88%. The criteria can be applied even when muscle biopsy is not available if typical skin rash is present. The included items and corresponding scores are listed in Table 3.

Table 3
EULAR/ACR criteria for diagnosis of IIM (85)

When no better explanation for the symptoms and signs exists, this classification can be used		
Variable	Score	
	No muscle biopsy	Muscle biopsy
Age of onset of first symptom assumed to be related to disease ≥ 18 and < 40 years	1.3	1.5
Age of onset of first symptom assumed to be related to 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 manifestation		
Heliotrope rash	3.1	3.2
Gottron's papules	2.1	2.7
Gottron's sign	3.3	3.7
Other clinical manifestation		
Dysphagia or esophageal dysmotility	0.7	0.6
Laboratory measurements		
Anti-Jo1 autoantibody present	3.9	3.8
Elevated serum levels of CK or LDH or ASAT/AST/SGOT or ALAT/ALT/SGPT**	1.3	1.4
Muscle biopsy features - presence of		
Endomysial infiltration of mononuclear cells surrounding but not invading myofibers		1.7
Perimysial and/or perivascular infiltration of mononuclear cells		1.2
Perifascicular atrophy		1.9
Rimmed vacuoles		3.1

Anti-Jo1, anti-histidyl-tRNA synthetase; CK creatine kinase; LDK, lactate, dehydrogenase; ASAT/AST/SGOT, aspartate aminotransferase; ALAL/ALT/SGPT, alanine aminotransferase

3.3 Myositis specific and associated autoantibodies

Myositis specific autoantibodies (MSAs) represent specific diagnostic markers for IIM and are commonly mutually exclusive (90, 91). Exact overall prevalence of MSAs is difficult to determine due to the availability of different tools for detection and lack of standardized guidelines (92). However, based on large cohort studies including children and adults with IIM, MSAs may be detected in approximately 60-70% of patients (93-95). MSAs target intracellular self-antigens with role in DNA transcription, chromatin remodeling, epigenetic modifications, protein synthesis or translocation, muscle cell metabolism and differentiation, innate immune response (96, 97). MSAs are associated with specific phenotypes, disease course and response to treatment (96-100). Name of known MSAs, target autoantigens, association to clinical subgroup and frequency in IIM are summarized in Table 4A.

Myositis associated autoantibodies (MAAs) occur in more than 50% of patients with IIM (95, 101), but they are not disease-specific, also being found in other connective tissue diseases. Common MAAs are anti-Ro/SSA, anti-Ku, anti-PM/Scl and anti-U1

ribonucleoprotein antibodies (U1RNP) (96) (Table 4B).

Table 4

A. Myositis specific autoantibodies (74, 96, 101-104)

Autoantibody	Autoantigen	Associated clinical subgroup	Frequency in adult IIM (%)
anti-aaRS	Aminoacyl-t-RNA synthetases	ASS	30
anti-Jo1	Histidyl-t-RNA synthetase	ASS	15-25
anti-PL7	Threonyl-t-RNA synthetase	ASS	3-5
anti-PL12	Alanyl-t-RNA synthetase	ASS	3-5
anti-OJ	Isoleucyl-t-RNA synthetase	ASS	<5
anti-EJ	Glycyl-t-RNA synthetase	ASS	<5
anti-KS	Asparaginyl-t-RNA synthetase	ASS	<2
anti-Zo	Phenylanalanyl-t-RNA synthetase	ASS	<2
anti-Ha (YRS)	Tyrosyl-t-RNA synthetase	ASS	<2
anti-Mi-2	Nuclear DNA helicase	DM, JDM	2-45 (4-10 in JDM)
anti-TIF1γ	Trascriptional intermediaryfactor 1 γ	DM, CAM, JDM	5-20 (20-30 in JDM)
anti NXP2	Nuclear matrix protein 2	DM, CAM, JDM	10-15 (20-25 in JDM)
anti-MDA5	Melanoma differentiation associated gene 5	DM, CADM, JDM	15-30
anti-SAE	SUMO activating enzyme	Severe DM	2-8 (<2 in JDM)
anti-SRP	Signal recognition particle	INMN	5 (<2 in JDM)
anti-HMGCR	3-hydroxy-3-methylglutaryl- CoA reductase	INMN	5-8 (1 in JDM)
anti NT5C1a	Cytosolic 5'-nucleotidase 1A	sIBM	30-76 in sIBM
anti-FHL1	Four-and-a-half LIM protein	Severe myopathy	25

B. Myositis associate autoantibodies (96, 104)

Autoantibody	Autoantigen	Associated clinical subgroup	Frequency in adult IIM (%)
anti-U1RNP	U1 small nuclear RNP	OM	10 (5 in JDM)
anti-Ro52	Ro52/TRIM21	ASS, OM	20-30 (6 in JDM)
anti-Ku	Ku complex	OM	< 1
anti-Pm-Scl	Nucleolar protein complex	OM	8-10 (5 in JDM)

3.4 Serological, histopathological and clinical profile of the myositis subsets

3.4.1 Polymyositis (PM)

Polymyositis commonly affects adults in their middle-age presenting with proximal and symmetrical muscle weakness, creatine kinase (CK) elevation, myopathic pattern on electromyography (EMG), and muscle edema on magnetic resonance imaging (MRI) in absence of skin manifestations. The muscle biopsy typically reveals spread MHC-I upregulation and cytotoxic $CD8^+$ T cells invading non-necrotic muscle fibers, even though these findings are not specific for PM and may be observed in sIBM, but less frequently in DM and ASS (Figure 4a-b-c). PM is rarely an isolated pathological condition but it is frequently part of the overlap myositis or ASS subgroups. Moreover, PM could be misdiagnosed with sIBM or muscular dystrophies due to similar clinical presentation in the early phase.

None of the known MSAs are exclusively associated to PM (74, 75, 102).

3.4.2 Dermatomyositis (DM, JDM, CADM)

Dermatomyositis occurs in both children (juvenile DM, JDM) and adults with peak of incidence between 50-60 years old, more frequently in women and with higher prevalence in geographical areas close to the equator (75, 81).

A violaceous, periorbital and edematous rash known as heliotrope rash (Figure 3d), erythematous scaly bumps over joints, often the MCP joints -so called Gottron's papules (Figure 3f), erythematous flat rash on elbows and knees (Gottron's sign, Figure 3a-b-c), on anterior of the chest (V-sign) or on shoulders and back (Shawl sign), or over the hips (Holster sign) represent pathognomonic skin features. Active skin lesions may resolve leaving areas of hyperpigmentation, telangiectasias and atrophy (poikiloderma, Figure 3e). Periungual erythema, Raynaud's phenomenon and pruritus are quite common among patients with DM. Skin biopsies reveal an interface dermatitis characterized by lichenoid inflammation of the dermal-epidermal junction, vacuolar changes in the epidermidis and perivascular inflammation, although not specific for DM (Figure 3g).

Symmetrical muscle weakness can precede or accompany the skin rash or may be absent as in the CADM (74, 75, 102). CK may raise up to 50 times the upper limit of normal range or may be normal. Usually, a myopathic pattern is observed at EMG. MRI shows muscle edema in the muscle fascicles or around them as result of fascial involvement. The muscle edema reflects the presence of fluid due to inflammatory cell infiltrates composed of plasmocytoid dendritic cells, T ($CD4^+$) and B lymphocytes, and macrophages which typically infiltrate the perimysium or surround medium-size blood vessels and capillaries. The latter are also site of membrane attack complex (MAC) deposition which is, together with the perifascicular atrophy, specific histopathological findings of DM. As in other subgroups, MHC-I is often up-regulated on the sarcolemma of muscle fibers (Figure 4c, 4e-f).

DM patients with circulating antibodies (Ab) towards Mi-2 nuclear antigen often present with classical DM skin features and mild muscle inflammation with a favorable disease course and good response to treatment. Anti-TIF-1γ and anti-NXP2 Ab are common in JDM and, in case of anti-NXP2, skin lesions are often complicated by calcinosis. Only in adults, anti-TIF1γ and NXP2 Ab are strongly associated with malignancy within 3 years from DM diagnosis (103). Severe and aggressive skin involvement with development of ulcers is expected in presence of Ab recognizing SAE or MDA5. Anti-MDA5 Ab may occur in DM patients with mild or absent muscle inflammation (CADM) and rapidly progressive ILD, especially in the Asian population (74, 75, 96, 102, 104-106).

Figure 3

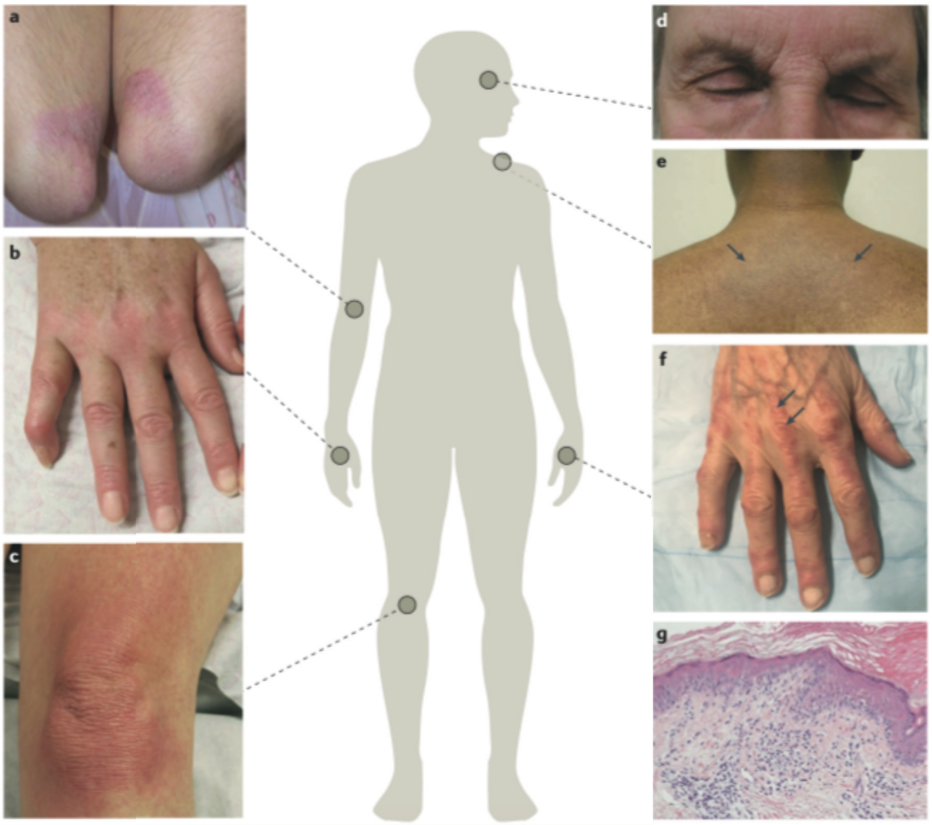


Figure 3. Cutaneous clinical and histological manifestations of dermatomyositis. Erythema over areas that experience stretching (Gottrons´s sign), including the elbows (a), the metacarpophalangeal, proximal and distal interphalangeal joints (b) and the knees (c); the presence of heliotrope erythema on the eyelids (d); poikiloderma (e, indicated by arrows); and Gottron papules, presenting as erythematous papules over joints on the hands (f, indicated by arrows). Interface dermatitis of the skin (g). *Reprinted by permission from Springer Nature, Lundberg I. et al., Classification of Myositis, Nat Rev Rheumatol 14, 269–278 (2018)*

3.4.3 Sporadic Inclusion Body Myositis

Sporadic Inclusion Body Myositis (sIBM) usually has onset after the age of 50 years. The disease is more common among men with a male-female ratio of 2:1-3:1 (107). The typical clinical manifestation of sIBM is a progressive and slowly advancing loss of muscle strength and development of muscle atrophy accompanied by mild CK elevation. In contrast with the other IIM subgroups, sIBM muscle pattern may be asymmetrical and it may involve both proximal and distal muscles, characteristically the quadriceps muscles leading to high propensity to fall and flexor muscles of the fingers. Dysphagia afflicts up to 50% of the patients, sometimes being severe and causing bronchoaspiration. Extra-muscular manifestations are rare. Muscle biopsies typically show cytotoxic $CD8^+$ T cells invading non-necrotic muscle fibers, so called rimmed vacuoles, tubofilamentous inclusions and cytoplasmatic amyloid deposits (Figure 4g-h).

Anti-cytosolic 5 nucleotidase 1A (NT5C1a) Ab can be found in 30-76% of sIBM patients,

particularly in those with more severe clinical picture and higher mortality rate. Indeed, anti-NT5C1a Ab are not specific for sIBM, occurring even in 5–10% of patients with PM, 15–20% of patients with DM, 10% of patients with SLE and 12% with Sjögren's syndrome (SS). sIBM overlaps with SS in approximately 15% of cases, displaying in circulation anti-Ro and anti-La antibodies (74, 75, 102, 106, 108).

3.4.4 Immune-mediated necrotizing myopathy (IMNM)

Immune-mediated necrotizing myopathy is a distinct subgroup characterized, clinically, by severe and rapidly progressive proximal muscle weakness of the arms and legs, very high CK elevation up to 20-50 folds the upper normal limit, myopathic EMG changes, muscle edema at MRI and, histologically, by necrosis of muscle fibers, minimal lymphocytic infiltration, macrophage infiltrates, MHC-I upregulation and MAC deposition on non-necrotic fibers (Figure 4d). In children, although vary rare, the clinical picture could be extremely difficult to differentiate from dystrophies. Extra-muscular manifestations are rare. Two MSAs are associated with IMNM, anti-SRP and anti-HMGCR antibodies. Anti-SRP positive patients are usually younger, weaker, with higher degree of muscle necrosis, atrophy and fatty replacement and higher likelihood to present with extra-muscular features such as ILD and cardiomyopathy compared to those with circulating anti-HMGCR Ab. Up to 60% of anti-HMGCR positive patients have been exposed to prior intake of statins, HMGCR inhibitors and lipid-lowering medications, which do not associate with presence of anti-SRP antibodies. Autoantibody-negative patients with IMNM have higher risk of malignancy compared to those with anti-SRP and anti-HMGCR antibodies. Prognosis of IMNM is worse than in the other IIM subgroups due to high rate of persistent muscle weakness despite treatment (102, 106, 109-111).

3.4.5 Overlap myositis (OM)

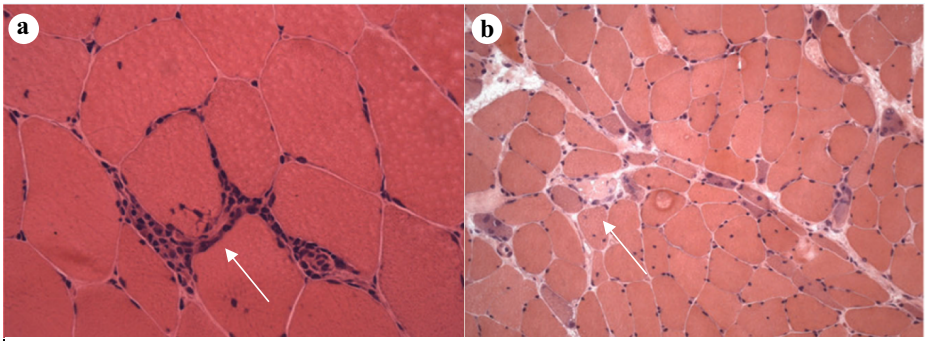
Overlap myositis (OM) is a clinical entity where myositis accompanies clinical features of other connective tissue diseases (CTD), such as SLE, systemic sclerosis or Sjögren's syndrome. Overlap of myositis and systemic sclerosis, also recognized as a distinct entity called scleromyositis (112), accounts for the majority of cases. Some authors classify a patient as affected by OM if criteria for both IIM and CTD are fulfilled; others require only the presence of myositis plus one of the overlap manifestations even without meeting CTD criteria. MAAs, particularly anti-Ro, anti-U1RNP, anti-PmScl, anti-Ku, are frequently found in OM, alone or together with autoantibodies specifically found in the associated CTD.
Muscle involvement is generally mild and muscle enzymes are slightly elevated. Arthritis, esophageal dysmotility, Raynaud's phenomenon and ILD are the most common extra-muscular manifestations. There are few studies looking at the histopathological findings in OM, showing a biopsy pattern closer to the one observed in DM than in other subgroups (113-115).

3.4.6 Cancer-associated myositis (CAM)

The diagnosis of cancer-associated myositis can be made when a patient presents with cancer within three years from the diagnosis of myositis (116). Malignancy may occur before,

concurrent with or after the myositis diagnosis. The risk of developing cancer is highest during the first year after diagnosis (117). Although cancer has been observed in all IIM subgroups (116), DM is the most common. Data on type of cancer vary depending on the population included in the study. According to a pooled analysis from Sweden, Denmark and Norway published in 2001 (118), adenocarcinomas of the lungs, ovaries, and breasts were frequently found in DM patients, while hematological malignancies such as non-Hodgkin lymphoma in PM patients. Ovarian cancer is the most frequent malignancy in CAM diagnosed in European and North American cohorts, but not in the Asiatic cohorts where nasopharyngeal and lung cancers are the most frequent (116). Among clinical and laboratory parameters, older age, male gender, skin ulcerations and skin necrosis, elevated CK and inflammatory markers (C-reactive protein, erythrocyte sedimentation rate), HLA-A28 type have been associated with higher risk of malignancy in IIM patients (116). On the contrary, ILD, anti-Jo1 antibodies, and arthritis/arthralgia have been found to protect from cancer in some IIM cohorts (116). In CAM, the clinical manifestations of the IIM subgroup are considered to be a paraneoplastic phenomenon as supported by several observations of their improvement or worsening in case of cancer remission or recurrence, respectively (119-121). Significant higher prevalence of malignancy has been observed in IIM patients testing positive for anti-TIF1γ or anti-NXP2 autoantibodies. A meta-analysis of six studies including 312 patients with IIM showed that presence of anti-TIF1γ antibodies has a 70% sensitivity and 89% specificity for diagnosing cancer-associated myositis, with positive predictive value of 58% and negative of 93% (122, 123). Moreover, a study conducted by our research group demonstrated that serum levels of anti-TIF1γ antibodies may be detected before the onset of cancer and may disappear in case of cancer remission after successful treatment (124). These findings support the important role of anti-TIF1γ antibodies in identifying IIM patients at high risk for malignancy who should carefully be screened and monitored. In three independent studies, anti-NXP2 antibodies have been associated with cancer in male patients above the age of 54 years with DM, mostly within one year from DM diagnosis (125-128). Both anti-TIF1γ and anti-NXP2 antibodies do not associate with cancer in children (125, 129).

Figure 4



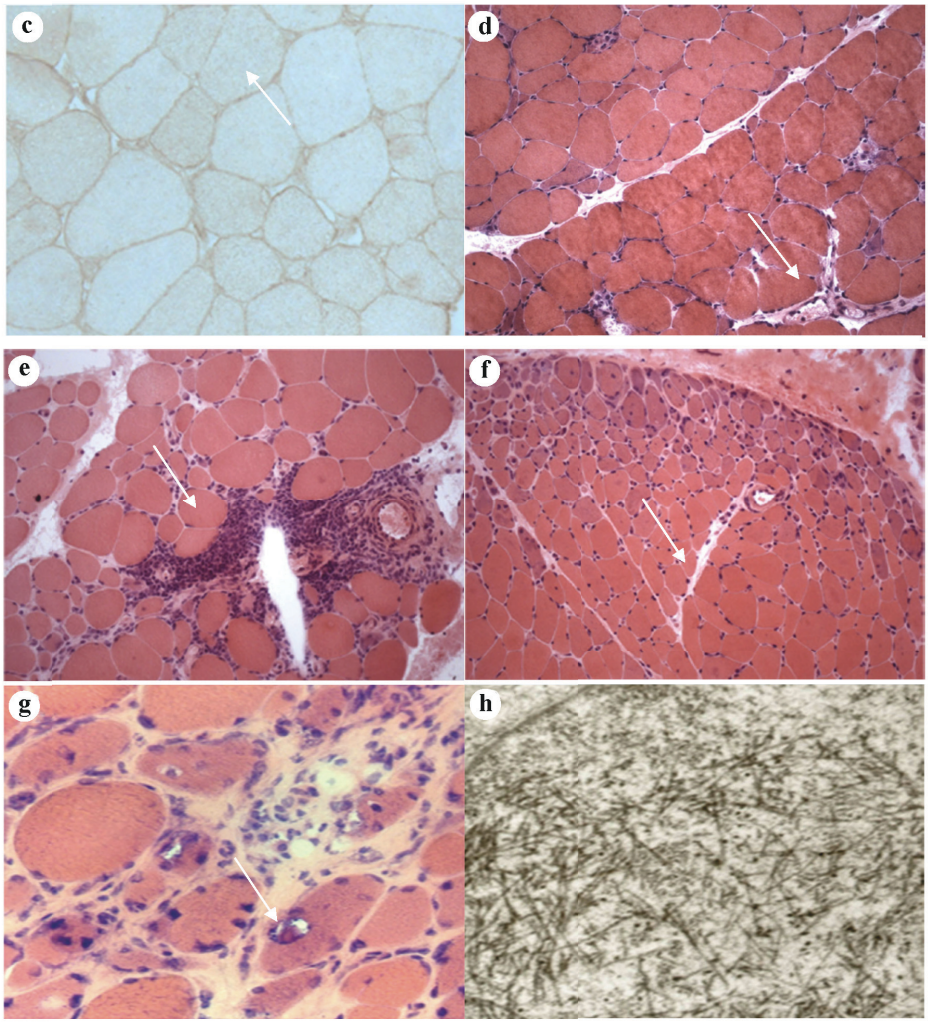


Figure 4. Histopathological features of the different subgroups of IIM. (a-b-c) Muscle biopsy sample from a patient with PM, (hematoxylin and eosin staining). (a) A small inflammatory infiltrate surrounding and invading a healthy muscle fiber. (b) Necrotic and regenerating muscle fibers. (c) Diffuse expression of MHC class I antigen on the sarcolemma of muscle fibers. **(d)** Muscle biopsy sample from a patient with IMNM (hematoxylin and eosin staining). Few necrotic fibers invaded by macrophages. **(e-f)** Muscle biopsy sample from a patient with DM (hematoxylin and eosin staining). (e) Large perivascular inflammatory infiltrate. (f) Perifascicular atrophy. **(g-h)** Muscle biopsy sample from a patient with sIBM. (g) Rimmed vacuoles in the cytoplasm of some muscle fibers (hematoxylin and eosin staining). (h) Several filamentous inclusions in the nucleus of muscle fibers (electron microscopy). *Reprinted after permission from Autoimmunity Highlights, Vattemi et al., Muscle biopsy features of idiopathic inflammatory myopathies and differential diagnosis, Autoimmun Highlights (2014) 5:77–85*

3.4.7 Anti-synthetase syndrome (ASS)

Antisynthetase syndrome (ASS) is characterized by the occurrence of antibodies against aminoacyl-tRNA-synthetases (aaRS) and by different clinical manifestations, encompassing inflammatory myopathies, ILD, arthritis, Raynaud's phenomenon, mechanic's hands and fever (130, 131). Positive testing for one of the eight known aaRS (anti-Jo-1, PL-12, PL-7,

EJ, OJ, KS, Ha and Zo, Table 4A) is mandatory to make the diagnosis of ASS, while the clinical spectrum is often heterogeneous, whereby isolated or combined features are possible. Anti-Jo-1 antibodies are found in 15-30% of patients with IIM and in up to 90% of those with ILD. Autoantibodies targeting other aaRS are less common, each with prevalence below 5% (93).

Anti Jo-1 positive patients usually present with myositis, arthritis or ILD, while anti-PL7 and anti-PL12 are strongly associated with isolated severe ILD. Clinically, muscle involvement may be consistent with both PM and DM, while histologically, perifascicular atrophy similar to the pattern in DM seems to be predominant. In addition, electron microscopy-based nuclear actin aggregation has been seen only in ASS muscle biopsies but not in others IIM. Severity range of ILD is broad, going from asymptomatic cases to acute distress respiratory syndrome. Within ILD patterns, non-specific interstitial pneumonia (NSIP) is the most frequent, followed by usual interstitial pneumonia (UIP) and cryptogenic organizing pneumonia (COP). Cumulative survival seems to be higher in aaRS positive patients with ILD compared to those with idiopathic pulmonary fibrosis (IPF). Arthritis usually occurs at the onset of ASS, being rheumatoid arthritis-like especially in ASS patients testing positive for ACPA antibodies. Mechanic's hands, described as erythematous and fissured hyperkeratosis of the palmar or lateral edges of the fingers, highly correlate with ASS diagnosis, although also reported in others overlap myositis, especially in presence of anti Pm-Scl antibodies. Raynaud's phenomenon is not a specific feature of ASS and it has more frequently been observed in patients with anti-PL12 and anti-PL7 than in patients with others aaRS. Relapsing-remitting fever is one of the symptoms in nearly 20% of ASS patients (79, 102, 106, 132-135).

3.5 Diagnostic workup and assessment of disease activity and damage

An extensive diagnostic work-up is needed to characterize which tissues/organs are involved by the inflammatory process and to choose the most appropriate treatment.

After collection of a precise medical history including information on smoking, history of neuro-muscular and/or connective diseases in the family, exposure to myopathic drugs and toxins, following investigations are usually performed:

- Evaluation of muscle strength by manual muscle test 8 (MMT8) and of muscle endurance by functional index test (FI-2 or FI-3) (136);
- Serum levels of muscle enzymes (CK, lactate dehydrogenase (LDH), myoglobin, aspartate-amino transferase (AST) and alanine aminotransferase (ALT));
- Analysis of MSAs and MAAs;
- Electromyography to look for a typical myopatic pattern with small amplitude, brief, polyfasic action potentials;
- MRI of the muscles to look for fascial and/or muscle oedema, muscle atrophy and fatty replacement of the muscles;
- Muscle biopsy;
- Inspection of the skin and possible skin biopsy;
- Capillaromicroscopy in case of severe Raynaud's phenomenon;
- Peripheral joint status, ultrasound and x-Ray of the affected joints;
- Evaluation of lung involvement by high-resolution computer tomography (HRCT) and

pulmonary function tests;

- Evaluation of cardiac involvement by serum levels of NT pro-BNP (N-terminal of prohormone of brain natriuretic peptide), electrocardiography, ecocardiography and MRI of the heart;
- Evaluation of gastro-intestinal tract by esophagus x-ray with barium or videofluoroscopy in case of swallowing difficulties;
- Screening for malignancy.

The evaluation of disease activity is based on the following disease activity core set measures identified by the International Myositis Assessment & Clinical Studies group (IMACS) (137):

1. Physician's global disease activity measured by visual analogue scale (VAS);
2. Patient's global disease activity measured by VAS;
3. Manual muscle test (MMT)-8 to evaluate the muscle strength;
4. Health assessment questionnaire of activities of daily living (HAQ);
5. Serum levels of muscle enzymes (at least two of the following: CK, LDH, AST, ALT);
6. Extra muscular activity score: evaluation of constitutional, cutaneous, gastrointestinal, articular, cardiac, and pulmonary activity by using Myositis Disease Activity Assessment Tool (MDAAT)

Improvement is defined as 3 of 6 core set measures improved ≥ 20%, with no more than 2 (not including MMT-8) worsening by ≥ 25% (137). The Total Improvement Score (range 0-100) is the sum of the improvement scores assigned to the 6 IMACS core set measures. Total improvement scores ≥20, ≥40, ≥60 represent minimal, moderate and major improvement, respectively, as defined by ACR/EULAR response criteria (138).

Evaluation of damage is based on the IMACS Myositis Damage index scoring (MDI) (139) which assesses the persistent changes in anatomy, physiology, pathology or function present for at least six months in eleven organ systems (muscle, skeletal, cutaneous, gastrointestinal, pulmonary, cardiovascular, peripheral vascular, endocrine, ocular, infection, malignancy).

3.6 Treatment of IIM

Despite the progress in identifying and sub-classifying IIM patients with different characteristics, the treatment approach is still far away to be tailored to the single patient and treatment recommendation is mainly based on open studies and case reports rather than randomized-controlled trials. High dose glucocorticoids represent the first choice to reduce muscle inflammation. Moreover, extra muscular manifestations, especially skin rash, arthritis and ILD, in general also respond well to steroids. To straighten steroid efficacy or as steroid-sparing agents, conventional disease modifying drugs (azathioprine, methotrexate, calcineurin inhibitors, mycophenolate mofetil, cyclophosphamide), biologic and immunomodulatory agents (rituximab, abatacept, anakinra, tocilizumab, JAK-inhibitors, intravenous immunoglobulins) are frequently added in the early phase of the disease and their choice is often guided by the main organ involvement. Combination of multiple immunosuppressive drugs and biologic/immunomodulatory agents or step-up approach are preferred in case of severe and rapidly progressive weakness, dysphagia or ILD. As a

complementary treatment, physical exercise programs should be strongly recommended at any phase to improve muscle strength and prevent disability. Among IIM subsets, sIBM is known to be resistant to treatment with immunosuppressive agents with a relentless course leading to progressive loss of function in daily activities (140-144). Several trials investigating new pathways possibly involved in the pathogenesis of IIM are now ongoing (www.myositis.org; https://clinicaltrials.gov).

3.7 Morbidity and mortality in IIM

Despite the advances in treatment strategies, IIM are still characterized by high morbidity and mortality impact (145).

Morbidity is related to muscle symptoms, extra-muscular manifestations, especially those associated with lung, heart and esophageal involvement, infectious complications, steroid and immunosuppressive drug toxicity, and malignancies (145). According to short- and long-term outcome studies, remission rate varies from 20 to 70%, indicating that a consistent group of patients will instead experience relapsing-remitting or chronic symptoms (145-148). Studies assessing functional disability status and quality of life showed persistent mild to moderate disability and decreased quality of life in more than 50% of patients with IIM (147, 149, 150).

Even though the 5-year survival rate has improved with the availability of glucocorticoids and immunosuppressive drugs, IIM patients have a significant higher risk to die early compared to the general population. Main causes of death are cardiovascular disease, respiratory failure due to ILD or infections, and cancer. The ranking order of causes of death differs between studies. The most important predictors of mortality are older age, male gender, non-Caucasian ethnicity, smoking, cardiovascular diseases, ILD, cancer, skin ulcers, dysphagia and longer duration of symptoms (145, 151).

The cardiovascular comorbidities in IIM patients will be discussed in detail in Chapter 5.

3.8 Disease mechanisms in IIM

Interactions between specific genetic and environmental factors are thought to be the triggers of both immune and non-immune mediated mechanisms involved in the pathogenesis of IIM. Activation of different pathways in genetically predisposed individuals upon exposure to certain environmental components could explain the occurrence of specific symptom constellations which characterize the various IIM subsets (Figure 5) (152, 153).

3.8.1 Genetic risk factors

Genome-wide association studies (GWAS) have shown that the strongest disease association lies within the MHC region on chromosome 6 (154, 155). *HLA-DRB1*03:01* strongly correlates with PM and sIBM, while HLA-B*08:01 with DM. *HLA-DRB1*01:01* and *HLA-DRB1*13:01* have been specifically found in patients with sIBM. Moreover, different HLA alleles and certain amino-acids positions in the HLA region may differentiate between adults and children, among ethnic groups and associate with distinct MSAs. For instance, in anti-HMGCR positive patients with IMNM, HLA-DRB1*11:01 and HLA-DRB1*07:01 associate with adult and juvenile patients, respectively (153, 156-158). Non-HLA loci, including

PTPN22, TRAF6, BLK, STAT4, PLCL1, CCR5, have been linked to IIM. The association with the mentioned genes supports the implication of both innate and adaptive immune responses in the pathogenesis of IIM (153).

3.8.2 Environmental risk factors

Genetic variants identified from GWAS and, in particular, Immunochip studies cannot explain alone the heterogenicity of the IIM clinical picture (159). Several epidemiological studies, case series, and animal models have investigated the possible association between infectious agents, drugs or environmental components and IIM. Examples of microorganisms suspected to be inducers of IIM are the human immunodeficiency virus (HIV), the human T-lymphotropic virus-1 (HTLV-1), Hepatitis B, Toxoplasma, Borrelia, and group A Streptococcus (153). Infections of the respiratory tract have been associated with increased likelihood of presenting with IIM (160). Beyond the known correlation between treatment with statins and occurrence of IMNM, even drugs as D-penicillamine, colchicine, chloroquine, interferons, anti-TNF-alpha agents have been claimed to act as potential triggers of IIM (153). Among environmental factors, probably the most relevant observation is that smokers who are carriers of *HLA-DRB1*03:01* have increased probability to develop anti-Jo1 antibodies (161). In addition, UV-radiation has been reported to correlate with DM and anti-Mi-2 antibodies (162, 163).

3.8.3 Immune mediated mechanisms

The muscle microenvironments in IIM is enriched with myeloid and plasmocytoid dendritic cells, macrophages, T and B cells supporting the role of both innate and adaptive immune system in the pathogenesis of IIM (152, 153).
$CD8^+$ T effector T cells can be seen invading non-necrotic muscle fibers of PM-sIBM patients by releasing perforin and granzyme-B (152, 153, 164, 165). $CD4^+$ T cells mainly constitute the perivascular inflammatory infiltrate in the muscle of DM patients (166). Affected muscles may also present infiltrates of pro-inflammatory T cells, $CD8^+$ or $CD4^+$ lacking the co-stimulatory molecule CD28 ($CD8^+$ or $CD4^+$ $CD28^{null}$), with cytotoxic properties and apoptosis resistant, which have been correlated with poor response to treatment (167-170). Studies on JDM have also suggested possible impairment of the Tregs subset (171, 172). The presence of B cells, plasma cells, immunoglobulins transcripts in the muscle of IIM patients as well as the strong association between MSAs and specific IIM subsets strongly suggest that the humoral component of the immune system is as well implicated (152, 153). Direct proofs for the pathogenicity of the MSAs are still lacking but supporting evidence is growing for anti-Jo1, anti-HMGCR, anti-Mi-2 and anti-c1A antibodies (173-175). The state of the art on the possible role of anti-Jo1 antibodies in the pathogenesis of IIM/ASS will be discussed in detail in chapter 4.
Muscle cells and immune-infiltrating cells highly express innate immune receptors as Toll-like receptors which, in turn, activate NF-KB signaling, cytokine and chemokine secretion. This leads to further recruitment of immune cells which will interact with antigen presenting dendritic cells. The latter are the major source of type I interferons (IFNs), key cytokines in IIM and in particular in DM. Overexpression of the genes induced by type I IFN has been observed in muscle, skin, blood of DM patients with levels of type I IFNs correlating with

disease activity (176-179). IFNα and IFNβ were shown to have toxic effects on muscle tissue and to induce up-regulation of MHC-I on muscle cells, increasing the susceptibility of the muscle fibers to the attack from the immune system (180, 181). MAC deposition in the wall of intermediate-size vessels of muscle and skin of DM patients reveals that the complement cascade is also activated leading to endothelial cell swelling, vacuolization, expression of vascular adhesion molecules and recruitment of T cells to the site of inflammation in perimysial and endomysial spaces (152).
So far, the muscle has been the major source of information on immune-mediated mechanisms in IIM. However, there is growing evidence supporting the lung as site of possible immune activation in IIM/ASS. This will be explored in more detail in chapter 4.

3.8.4 Non-immune mediated mechanisms

In some patients with IIM, the severity of the clinical symptoms does not correlate with the degree of inflammation in muscle biopsy or there is a progressive worsening despite active immunosuppressive treatment. These observations motivated to investigate whether non-immune mediated mechanisms play a role in the pathogenesis of IIM. The best studied non-immune pathways are 1) hypoxia; 2) the endoplasmic reticulum stress (ER) with consequent accumulation of unfolded proteins, release of free radicals, up-regulation of TNF-related apoptosis-inducing ligand (TRAIL), expression of heat-shock proteins HSP70 and HSP90 and the alarmin high mobility group box protein 1 (HMGB1); 3) impairment of autophagy; 4) mitochondrial dysfunction (152, 153). Major details are beyond the purpose of this summary and will not be discussed.

Figure 5

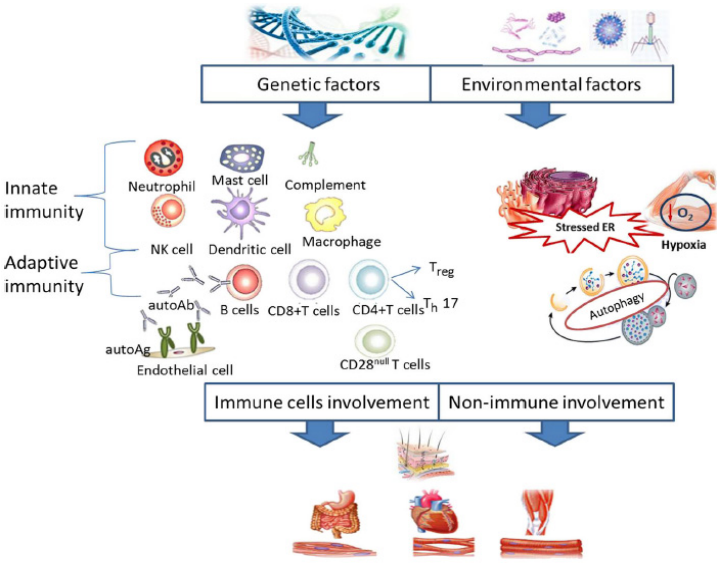


Figure 5. Immune and non-immune mechanisms involved in the pathogenesis of IIM. Genetic and environmental factors influence the adaptive and innate immune response, and this leads to the activation of several cellular components (neutrophils, NK cells, macrophages, mast cells, dendritic cells, B cells, and T cells such as $CD8^+$, $CD4^+$, Treg, Th17, $CD28^{null}$) involved in the inflammatory response. However, other mechanisms are involved in this complex scenario, including non-immune mechanisms such as autophagy, endoplasmic reticulum stress, and hypoxia.
Reprinted after permission from Springer Nature, Ceribelli A. et al., The Immune Response and the Pathogenesis of Idiopathic Inflammatory Myositis: a Critical Review, Clinic Rev Allerg Immunol (2017) 52:58–70

4 Specific background of Papers I-II-III

The pathogenesis of anti-Jo1 positive anti-synthetase syndrome has not yet been fully elucidated despite advances in the past years in the understanding of the role of autoimmunity against the histidyl-tRNA synthetase (HisRS or HARS). Here below I summarize the evidence published so far.

4.1 Protein structure, canonical and non-canonical functions of HisRS

HisRS belongs to the family of aminoacyl-transfer (t)RNA synthetases (aaRS), a group of essential ubiquitous intracellular enzymes which catalyze the binding of the amino-acids to their cognate tRNAs during protein synthesis (182). To date, 36 aaRS have been described in human cells, 16 aaRS exerting their role exclusively in the cytoplasm, 17 in the mitochondria and 3 in both compartments (183). The enzymatic activity exerted during protein synthesis constitutes the physiological function of aaRS and the catalytic domain is well conserved through species from Escherichia Coli to humans (182, 184). New additional domains of aaRS, have been added to the catalytic domain throughout evolution, demonstrating that aaRS interact with other proteins and have acquired other non-canonical functions (182). In fact, eukaryotic aaRS are part of multimolecular complexes with aminoacyl tRNA synthetase-interacting multifunctional proteins (AIMPs) (185). New variants of aaRS are generated through alternative splicing, post-transcriptional and post-translational modifications and proteolytic cleavage, expanding the functional repertoire (182, 184). Emerging evidence suggests that HisRs and the other aaRS are actively interacting with innate and adaptive immune system (186) and they take part both inside and outside the cell in the regulation of processes such as immune cell development, angiogenesis, inflammatory response, and mTOR signaling (187-189). Some aaRS have been shown to contribute to bacterial metabolism and virulence, others to have anti-bacterial, viral, fungal properties (182, 190). There are also reports of aaRS with potential role against tumorigenesis and as inducers of inflammatory response in cancer models (191, 192).

HisRS is a homo-dimeric protein composed of 509 amino-acids, three domains, the WHEP domain located in the N-terminus of HisRS, an internal catalytic domain (CD), and the anti-codon binding domain (ABD) in the C-terminal side (193). In 2012, a monomeric HisRS splice variant (SV) comprising the WHEP domain and the ABD (lacking the CD) was discovered (193, 194). In 2014, an additional HisRS splice variant composed of the first 60 amino acids (WHEP domain itself) was described (195, 196) (Figure 6). Autosomal dominant mutations in the WHEP, CD and ABD domains have been found in patients with Charcot-Marie Tooth disease and Usher B syndrome (197, 198).

Figure 6. Graphical representation of HisRS antigens. HisRS full-length (HisRS/HisRS-FL), WHEP splice variant, catalytic and anti-codon binding domains (CD and ABD, respectively), and WHEP+ABD splice variant (SV). Supplementary Figure 1, Manuscript III

4.2 Autoimmunity against HisRS autoantigen in the pathogenesis of IIM/ASS

In vitro experiments demonstrated that HisRS can be secreted from cells (195), but *in vivo* secretion and presence of HisRS in extracellular compartments have not been reported so far. How and where HisRS becomes the target autoantigen of a specific immune response in IIM/ASS is still unknown. Notably, the pathogenic role of antibodies against HisRS (anti-Jo1 Ab) is suggested by clinical and epidemiological observations: 1) prominent association between anti-Jo1 Ab and distinct clinical entity such as ASS (135); 2) anti-Jo1 Ab titers correlate with disease activity and may be found before the disease onset (199, 200); 3) smoking appears to be associated with an increased risk of possession of anti-Jo1 Ab in HLA-DRB1*03-positive IIM cases (161); 4) anti-Jo1 Ab are mutually exclusive, generally not occurring with other myositis specific autoantibodies (201).
The HisRS antigen is expressed at low levels in all cells including healthy muscle fibers and at high levels in inflamed muscles and regenerating fibers. This suggests that it is unlikely that the immune response towards HisRS starts in unperturbed muscles. HisRS is also expressed by bronchial epithelial lung cells from healthy individuals and with a higher expression in the bronchial epithelial cells compared to other cells (37, 202). Proteins may become immunogenic when cleaved in aberrant ways (203) and Levine et al. showed that HisRS can be cleaved by granzyme B (GrB) in the lung, potentially revealing neo-epitopes. HisRS seems to be more susceptible to the GrB cleavage in the lung than in other tissues, including muscle and the cleaved conformation of HisRS is recognized by anti-Jo1 Ab (37). The main anti-Jo1 Ab epitope of HisRS has been suggested to be localized within the first 60 amino acids near the N-terminal (WHEP domain) (204, 205) even if anti-Jo1 Ab may recognize different HisRS epitopes (epitope spreading) (206, 207), disfavoring the hypothesis of anti-Jo1 Ab production as result from a cross-reactive antigen (mimicry theory). So far, it is not known how the reactivity to different domains/variants of HisRS behaves through the disease course and whether Ab against different domains/variants of HisRS are associated with specific clinical features of IIM/ASS. Furthermore, it has been shown that anti-Jo1 Ab, mainly IgG1 isotype, undergo class-switching and affinity maturation, supporting that HisRS-driven specific T cells directly contribute to the development of anti-Jo1 Ab (199, 206). Ascherman et al. demonstrated HisRS-induced T-cell proliferation in peripheral blood and T-cell response was enhanced if HisRS fragments were presented by dendritic cells (203). Our research group has found activated $CD4^+$ T cells with pronounced Th1 phenotype in bronchiolar alveolar lavage (BAL) fluid and peripheral blood in patients with anti-Jo1 Ab and HLA-DRB1*03 genotype, by stimulating T cells with a candidate T cell epitope of HisRS, or with the full-length protein. The activation of T cells was abrogated by antibodies against DR but not DQ, underlying that an MHC-II restricted context is required. Lung derived T cells from BAL fluid compared to blood produced high amounts of the pro-inflammatory cytokine IFN-gamma in response to stimulation with HisRS protein and one of the HisRS peptides (aminoacids residues 11-23 in the N-terminus of the HisRS protein). In addition, anti-Jo1 Ab were found in the BAL fluid and T cells surrounding plasma cells were observed in germinal-like structures in lung tissues of anti-$Jo1^+$ patients but not anti-$Jo1^-$ or patients with chronic obstructive lung disease (208). In another study aiming at comparing the T cell-receptor (TCR) expression in BAL fluid, muscle tissue, and peripheral blood obtained from patients with recently diagnosed myositis with and without lung involvement, T cell expansions in $CD4^+$ and $CD8^+$ cells were found in

BAL fluid but not in peripheral blood. Notably, the same TCR expression was observed in BAL and muscles of patients with the HLA–DRB1*03 allele and anti-Jo1 antibodies, suggesting that the inflammatory process may be driven by T cells with identical antigen specificity in lungs and muscles (209).

All these findings are contributing to the hypothesis of the lung as the site of disease initiation, where the break of tolerance towards HisRS may occur for the first time. How the immune response perpetuates and involves other tissues, e.g. muscles, needs to be addressed. One interesting pathway may involve type 1 IFN which is a known inducer of MHC-I (210). Our research group found that anti-Jo1^{+} sera induced type 1 IFN production in human blood cells (211). Given that both MHC-I and type I INF-induced genes expressions are high in muscle tissue and peripheral blood of myositis patients (212, 213), one possibility is that anti-Jo1 Ab mediate pathogenesis via IFN. In 2012 Howard et al. also showed *in vitro* that HisRS possess chemokine-like properties capable of stimulating lymphocytes, activated monocytes, and immature dendritic cells (214). Moreover, enhanced formation and/ or decreased degradation of neutrophil extracellular traps (NETs) has been found in the plasma of anti-Jo1+ patients. NETs are capable to damage endothelial and epithelial components of various tissues. (215). So far, no functional studies on the effects of anti-Jo1 Ab on lung or muscle cells have been published.

In other autoimmune diseases, variations of glycans appended to the IgG Fc-region have been suggested to play a role in the pathogenesis, by modulating the immune response. The glycosylation status of the Fc-region affects the affinity of antibodies and their capacity to interact with Fcγ receptors and induce complement activation and cytokine secretion (60). In RA, the IgG-Fc glycosylation pattern differs between ACPAs and non-ACPA-IgG and changes in the glycans have been reported prior to RA onset (216-218). Low levels of IgG-Fc galactosylation are known to be associated with high pro-inflammatory status, disease activity and severity. Interestingly, agalactosylated ACPAs correlate with response to methotrexate in early RA (219). Fc-agalactosylated IgG have been also found in pathologies involving the respiratory tract such as sarcoidosis and chronic asthma (220). In patients with IIM, more agalactosylated forms of IgG have been found compared to healthy siblings and age/sex matched controls (221). The glycosylation status of anti-Jo1 Ab and total IgG present in serum of anti-Jo1$^{+/-}$ patients has not yet been explored.

4.3 Animal models of anti-Jo1 positive ASS

Spontaneous, induced or transgenic animal models of human myositis have failed to fully reproduce all the clinical features of human myopathies and in particular the coexistence of lung and muscle pathology as in ASS (222, 223). Inoculation of human HisRS protein in mice resulted in the production of low levels of anti-Jo1 Ab and presence of inflammatory infiltrates only in the muscle which was the site of injection (224). The lack of development of specific clinical manifestation in these mice were probably due to production of Ab against the human HisRS protein and not to the native murine one. In fact, the attempt of immunization of mice strains with the amino-terminal portion of murine HisRS was instead followed by the development of high titers of anti-Jo1 Ab and pronounced interstitial lung disease, demonstrating that the break of tolerance towards HisRS occurs in a species-specific manner (225). In mice immunized with the native HisRS protein, muscle inflammation was observed although less prominent than the inflammation in the lungs (225). More recently,

MRI was used to study the pattern of muscle inflammation 14 days after immunization with the amino-terminal portion of murine HisRS (226). Circulating anti-Jo1 Ab were detectable already seven days after immunization and Ab titers correlated with raising CK levels. In control mice which were injected only with the adjuvant, CK levels remained at background levels, discharging the hypothesis of CK elevation due to the intramuscular injection. Inflammatory infiltrates in the perimysial connective tissue, which is a common histological finding in DM/ASS, were found in immunized mice in contrast with the pattern of muscle inflammation observed in the mouse model of acute muscle necrosis induced by inoculation of cardiotoxin (226). Although these animal models give some information that can be relevant to understand the pathophysiology of human ASS, we still need detailed molecular studies of patients with ASS followed over time to understand the development of immune reactivity towards HisRS and the possible role of anti-Jo1 autoantibodies in the pathogenesis.

5 Specific background of Papers IV-V

5.1 Cardiovascular comorbidities in IIM

According to a recent Swedish, nationwide, population-based study on mortality in patients with IIM, IIM patients had > 3-fold higher risk to die than the general population. The three major causes of death, malignancies, pulmonary and cardiovascular diseases (CVD), were similar between the two groups but, contrary to the general population, the highest risk of death in IIM patients was seen within the first year after IIM diagnosis and then the mortality rate decreased over time (227). This suggests that other mechanisms rather than traditional risk factors may contribute to the development of the leading causes to death.

In other autoimmune diseases like RA and SLE (228-231), the association between inflammation and CVD, especially at disease onset, has been largely studied, while limited data are available on IIM. One large Canadian cohort of incident PM/DM patients and a cohort of gender and age matched adults without PM and DM randomly selected from general population were retrospectively followed for 15 years to compare the incidence rate of myocardial infarction (MI) and ischemic stroke. The incidence rate of MI and ischemic stroke per 1000 person-years was 22,52 and 10,15, respectively, for PM patients versus 5,50 and 5,58, respectively, for general population. In DM patients the risk of MI was near 3-fold higher than in general population while the risk of ischemic stroke was not statistically different. Only in PM patients, the risk of both MI and ischemic stroke was highest in the first year after diagnosis (232). The risk of acute coronary syndrome (ACS) was also found higher, particularly within the first year after diagnosis, in a large Swedish cohort of IIM patients (in particular in those with PM and sIBM but not DM), compared to age and sex-matched general population comparators. While older age accounted for risk factor in both patients and comparators, male gender was associated with increased risk of ACS only in the general population (233). Focusing on venous thromboembolic events (VTE), deep venous thrombosis (DVT) and pulmonary embolism (PE), two population-based cohort studies have estimated the risk of VTE in IIM patients compared to the general population, both indicating an increased risk shortly after start of follow up from IIM diagnosis. In a Canadian cohort, after adjusting for relevant risk factors, the hazard ratio (HR) (95% confidence interval-CI) for occurrence of VTE was 7 for PM and 8,39 for DM (234, 235). However, in both studies, the incidence rate was not stratified by demographic and clinical characteristics, not helping clinicians to identify which patients are at risk and should be carefully screened for VTE risk factors. Higher prevalence of smoking, hypertension, diabetes, obesity, dyslipidemia has been reported in IIM patients compared to general population (236-240), but it has not yet been elucidated whether these CV risk factors are differently distributed in IIM patients with or without history of arterial and/or venous TE.

On a pathophysiologic level, systemic inflammation is thought to promote accelerated atherosclerosis (241, 242), one of the possible causes of arterial TE. Diederichsen et al. found in patients with PM/DM severe coronary artery calcification (CAC), even though in this study CAC correlated with age and smoking rather than myositis (236). With attention on VTE, the three components of Virchow triad, which are venous stasis, hypercoagulability, and endothelial damage, may be directly affected by IIM. Muscle weakness may contribute to venous stasis by reducing mobility. Mechanisms of hypercoagulability and endothelial damage are discussed in the next paragraph.

5.2 Cross-talk between inflammation and coagulation

Highly regulated biological systems mediate the interaction between inflammatory response to injury or pathogens and coagulation pathways. Dysregulation of any of the actors maintaining this delicate balance may lead to exacerbation of inflammation and thrombosis. In inflammatory conditions, damage-associated molecular patterns (DAMPS), cytokines and chemokines determine up-regulation of pro-coagulants such as tissue factor (TF) and TF-factor VII complex, downregulation of natural anticoagulants, such as proteins C and S, moreover platelet activation and suppression of fibrinolysis leading to a hypercoagulable state (243). Endothelial cells are activated by pro-inflammatory cytokines with consequent up-regulation of adhesion molecules implicated in leukocyte transmigration. Migration of leukocytes through endothelial junctions to the site of inflammation is strictly regulated by the selectin-dependent activation of β2 integrins on leukocytes, which is responsible of tethering and rolling of leukocytes on the vessel wall. Then, the interaction of leukocyte β1 and β2 integrins with their receptors on endothelial surface (vascular cell adhesion molecule (VCAM)-1 and intracellular adhesion molecule (ICAM)-1) allows a firm adhesion of leukocytes before their transmigration (244). Increased serum levels of adhesion molecules are found in case of endothelial dysfunction which plays a central role in the cascade of events leading both to arterial and venous thrombotic events. Circulating VCAM-1 predicted cardiovascular death in patients with acute coronary disease independently of other risk factors (245, 246). Soluble ICAM-1 was indicated as predictor of myocardial infarction (247) and peripheral artery disease (248) in healthy men. High levels of e-selectin correlated with myocardial infarction and carotid artery atherosclerosis (249, 250). On the contrary, low e-selectin levels have been associated with recurrent venous thromboembolism in the general population (251, 252).

Since 1990s, increased expression of e-selectin, VCAM-1 and ICAM-1 on endothelial cells, infiltrating cells in the endomysium and perimysium and sarcolemma of non-necrotic fibers has been observed in muscle specimens of patients with PM, DM, sIBM and JDM, although with some differences among subgroups (253-256). In a rat model of human polymyositis strong expression of ICAM-1 on the sarcolemma of non-necrotic muscle fibers and in the endothelium of vessels in the endomysium and perimysium was found to correlate with the degree of inflammation in muscle biopsies (257). With respect to myositis disease duration, upregulation of VCAM-1 and ICAM-1 has been found both in the untreated early phase and despite immunosuppressive treatment, suggesting either premature involvement of adhesion molecules in the pathogenesis of IIM or role in perpetuating inflammation (258). Higher soluble levels of adhesion molecules have been reported in PM, DM and sIBM patients compared to controls (254, 259-261). However, it has not been investigated in IIM patients if serum levels of adhesion molecules are different in case of arterial and/or venous TE and in relation to patient characteristics, autoimmune profile and traditional CVD risk factors.

Another factor with an association to TE is the anti-phospholipid antibody (APL), but limited information is available on APL in patients with IIM. An association between history of TE and APL in patients with IIM has been described in few cases with IIM (262-264). The exact prevalence of APL in IIM is not known.

AIMS

The overall aims of this thesis were 1) to increase the understanding of the role of anti-Jo1 antibodies and HisRS autoantigen in the pathogenesis of the anti-synthetase syndrome (ASS), a distinct subgroup of idiopathic inflammatory myopathies (IIM) and 2) to study the impact of and the risk factors associated with a life-threatening comorbidity of IIM, the arterial and venous thrombosis.

Specific aims

Paper I: To investigate if HisRS is detectable in extracellular compartments (serum) and whether levels correlate with anti-Jo1 autoantibodies. To verify if the sequestration of HisRS is an epiphenomenon or plays a causal role in the disease.

Paper II: To investigate if the glycosylation profile of total IgG from anti-Jo1 positive and anti-Jo1 negative patients is different and if particular Fc-glycan features are associated with specific clinical manifestations.

Paper III: To address the reactivity and affinity of anti-Jo1 autoantibodies from serum and bronchoalveolar lavage fluid (BALF) and associations with clinical data in longitudinally followed patients with IIM/ASS.

Paper IV: To assess the incidence of venous thromboembolic events in patients with IIM in comparison to the general population and to identify patient categories at high risk and the timing of risk in relation to the diagnosis of IIM.

Paper V: To assess the contribution of traditional and/or disease-related risk factors and biomarkers linked to arterial and venous thrombotic events in patients with IIM.

EXPERIMENTAL PROCEDURES

1 Patient cohorts, data sources, clinical information

With the exception of Paper IV (paragraph 1.4), patients included in this thesis were regularly followed at the Rheumatology clinic, Karolinska University Hospital, Stockholm, Sweden. Classification of IIM was made according to the Bohan and Peter criteria (84, 85) for patients included in Paper I-II-III and according to the EULAR/ACR classification criteria for IIM (probability threshold of 55%) (89) for patients included in Paper V. Griggs criteria (87) were applied for sIBM. The diagnosis of ASS was based on the presence of anti-aaRS autoantibodies, plus one of the following features: ILD, myositis, arthritis, Raynaud´s phenomenon, fever, or mechanic´s hands (265). Diagnosis of ILD was based on the American Thoracic society criteria (266, 267). Imaging (lung x-ray and/or HRCT) and lung function tests consistent with the diagnosis of ILD were verified for all patients. Clinical information was collected by review of patient medical charts and by extracting data from the Swedish Rheumatology Quality Register for IIM (Swemyonet) (268), and the Euromyositis register (79). All patients and controls gave their written informed consent to participate in the studies.
Patient cohorts included in each paper are described below.

1.1 Paper I

First available serum samples from 357 patients with IIM were selected for the purpose of this study. By performing a validate test (immunoprecipitation (IP), Line Blot, Enzyme-linked immunosorbent assay (ELISA)) for the presence of anti-Jo1 antibodies at least once during the disease course, 61 anti-Jo1 positive and 286 anti-Jo1 negative patients were identified. Seventy-one out of 357 patients fulfilled the criteria for ASS and 94 out of 357 presented with ILD. Age at serum sampling, gender and IIM subgroups distribution are presented in table 1 of Paper I. Serum samples from 115 age- and gender-matched healthy individuals (obtained from the Rheumatology biobank) were also included.

1.2 Paper II

First available serum samples from 44 patients with IIM, 19 anti-Jo1 positive and 25 anti Jo1 negative, and 24 age- and gender-matched healthy controls were included. Patients were defined as anti-Jo1 positive if they had ever tested positive for anti-Jo1 antibodies by standardized immunoassays (IP, Line Blot, ELISA). Information on age, gender, IIM subgroups, disease duration between time of diagnosis and time of sampling, extra-muscular manifestations, presence of MSAs and/or MAAs, disease activity data (physician and patient VAS, MMT-8, MDAAT, CK levels, HAQ), immunosuppressive treatment at time of first available serum sample is summarized in Table 2 of Paper II. Sera at different longitudinal time points were retrieved from 11 out of 19 anti-Jo1 positive patients and 7 out 25 anti-Jo1 negative patients (Supplementary Table 1, Paper II). Clinical data collected at time of longitudinal serum sampling are presented in Supplementary Table 2, Paper II.

1.3 Paper III

Two cohorts of patients were selected for this study. Cohort 1 is the same as in Paper II. Cohort 2 is composed of 10 patients with IIM/ASS, 6 anti-Jo1 positive and 4 anti-Jo1 negative patients. The definition of anti-Jo1 positivity is the same as applied in Paper I and II. First available serum samples in relation to IIM/ASS diagnosis as well as longitudinal serum samples up to 24 years after diagnosis were retrieved from Cohort 1. Matching BALF and serum collected at time of diagnosis were available in the biobank from Cohort 2. Serum samples from 24 age-and gender-matched healthy controls were also analyzed. More than 60 variables representing clinical features, laboratory parameters, disease activity and outcome measures were collected at time of each sampling. Demographic data of cohort 1 and 2 are summarized in Table 1 and Supplementary Table 1, respectively, of Paper III.

1.4 Paper IV

A cohort of patients with IIM was identified by using the Nationwide Patient Register, which includes non-general practitioner outpatient visits in Sweden since 2001 and inpatient care since 1987. To be included in the IIM cohort, each individual had to be age ≥18 years, have had the first ever visit between 2002 and 2011, and a follow-up visit within 1–12 months, coded to indicate IIM according to International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes. The ICD-10 codes used are shown in Supplementary Table 1 of Paper IV. The second registered visit was used as the index date which corresponds to the date of start of follow up for the patients included in the analysis. Because of overlapping ICD-codes between different IIM subgroups, patients were classified as affected by DM or by other IIMs. Each patient with IIM was matched with 10 randomly selected individuals from the Swedish Population Register (which includes all Swedish residents). Matching variables were sex, residential area, and birth year. Each population-based comparator was required to be alive and living in Sweden at the index date of the corresponding patient with IIM. Clinical information on comorbidities (hypertension, inflammatory bowel disease, interstitial lung disease, or ischemic heart disease) ever before the index date for patients and comparators was retrieved from the Nationwide Patient Register. The Prescribed Drug Register was used to capture the occurrence of relevant therapies (anticoagulants, hormone replacement therapy, oral contraceptives, antidiabetic drugs, methotrexate, glucocorticoids, antihypertensive drugs, and statins 365 days prior to and up to 15 days after the index date) in patients and comparators. Since data on dispensed drugs were available from 2005 and later, only patients with IIM and general population comparators identified from 2005 to 2011 were included in this study. The Cancer register was checked to detect all cancers that had occurred in patients and comparators before the index date. Baseline characteristics, comorbidities and treatment of patients with IIM and the general population cohort are summarized in Table 1 of Paper IV. Each IIM patient or comparator was defined as a case if during the follow-up period, starting at the index time, the occurrence of a venous thromboembolic event (primary outcome) and in particular of deep venous thrombosis and pulmonary embolism (secondary outcome) was verified by a visit with corresponding ICD-10 codes and by one prescription for relevant anticoagulants within 30 days. The follow-up period ended for patients and comparators at outcome, first emigration, death, or December 31, 2013, whichever came first (Figure 7).

Figure 7

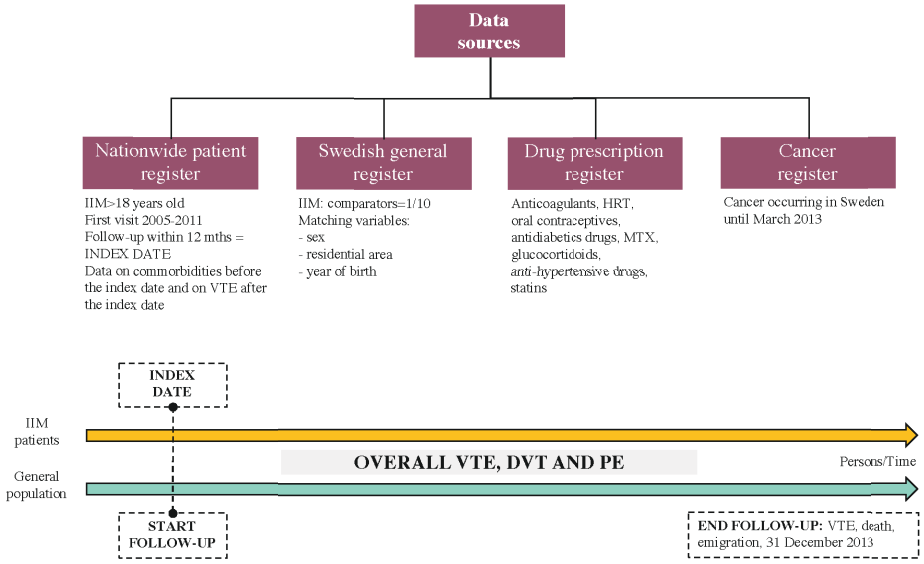


Figure 7. Graphical representation of data sources used to identify IIM patients and comparators from general population in Paper IV and of follow up period to verify the occurrence of the primary and secondary outcomes. IIM, idiopathic inflammatory myopathies. Comorbidities: hypertension, inflammatory bowel disease, interstitial lung disease, or ischemic heart disease ever before the index date. HRT, hormone replacement therapy. MTX, methotrexate. Overall VTE= incidence rate of venous thromboembolic event per 1000 persons/time. DVT, deep venous thrombosis. PE, pulmonary embolism.

1.5 Paper V

All patients with IIM who were followed at the Rheumatology Clinic, Karolinska University Hospital, Stockholm, Sweden, between January 1, 1993, and December 31, 2014 (n=316) were identified from a local registry. Fulfilment of the EULAR/ACR classification criteria for IIM (probability threshold of 55%) (89) and availability of clinically relevant variables for the purpose of the study were used as entry parameters to select the patients to include, resulting in a study population of 253 individuals. Occurrence of arterial (myocardial infarction, transitory ischemic attack, stroke and peripheral arterial thrombosis) and/or venous (DVT and PE) thrombotic events (TE) was checked retrospectively before and after IIM diagnosis by looking at corresponding ICD-10 codes and by reading individual medical charts. Then, the 253 patients with IIM were divided into two groups: 58 with verified arterial and/or venous TE (cases), and 195 without history of TE (comparators). Seven patients out of 58 cases had reported TE only before the time of IIM diagnosis and were excluded from the analysis. The comparison analysis was then performed between 51 cases with history of TE that had occurred at the time or after the diagnosis of IIM and 195 comparators without history of TE, resulting in a final study population of 246 patients with IIM (Figure 8).
Serum samples taken at the time of IIM diagnosis and stored in -80 °C were retrieved from the 246 patients with IIM and from 40 age- and gender-matched healthy controls.

Figure 8

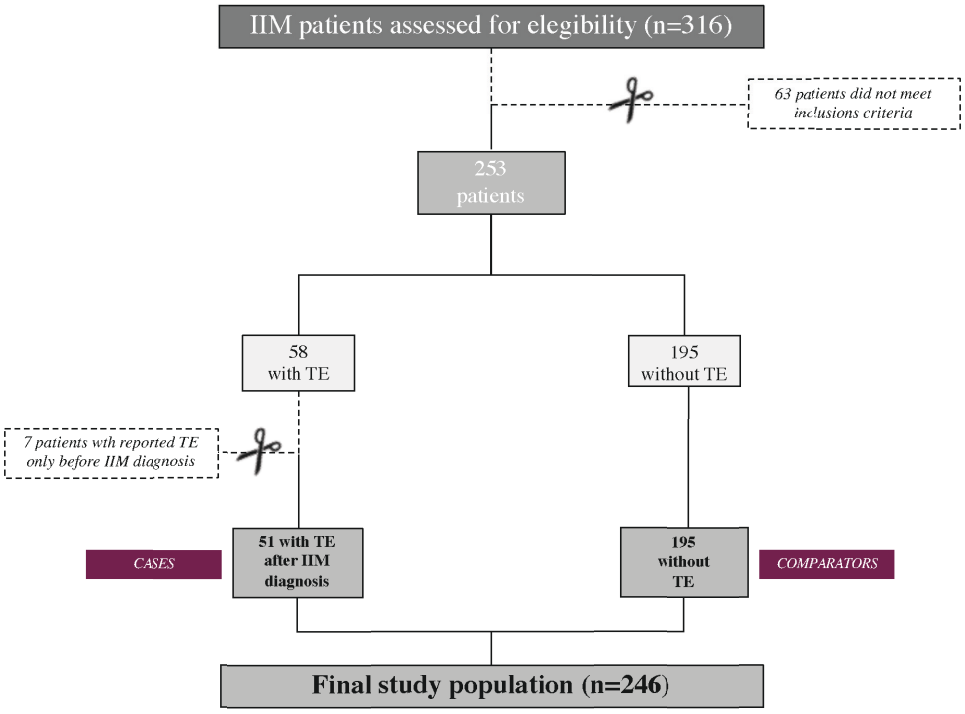


Figure 8. Flow chart of patient inclusion in Paper V. IIM, idiopathic inflammatory myopathies. TE, thrombotic events.

2 In vitro methodology

2.1 Preparation of recombinant HisRS (Paper I, Paper II, Paper III)

In Paper I, recombinant HisRS (amino acids 1–506) was expressed as a soluble protein in *E. coli* without tags. HisRS was purified from lysed *E. coli* cells by anion exchange chromatography (Q-Sepharose HP) at pH 7.4. The protein was further purified using ceramic hydroxyapatite chromatography at pH 7.0. A purity > 95% of the protein was demonstrated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and size exclusion chromatography. In Paper II and III, biotinylated full-length HisRS and HisRS variants (Table 5) and control proteins utilized for enzyme-linked immunosorbent assay (ELISA), western blot and affinity measurements were generated by sub-cloning PCR amplified DNA fragments into the expression vector pNIC-Bio3 (Genbank acc. no code JN792439). Recombinant plasmids were transformed into *E. coli* expression strain BL21(DE3) R3 pRARE2 carrying a plasmid for co-expression of BirA ligase. Protein expression and purification were performed as described in (269). Information on ID, molecular weight, amino acids coverage of the proteins utilized in Paper III is summarized in Table 5.

Table 5
Amino acid (aa) coverage and molecular weight of HisRS antigens and control proteins utilized in ELISA, western blot, and affinity measurements (Paper III)

ID	Antigen aa	Molecular weight (Da)
Biotinylated antigens containing his tag		
HisRS full-length (HisRS-FL, P12081*)	M1-C509	62485.0
WHEP	M1-K60	11687.3
Catalytic domain (CD)	K53-E402	43676.0
Anti-codon binding domain (ABD)	T406-C509	16906.3
Splice variant (WHEP+ABD, SV)	Δ K61-A398	24341.0
Interleukin 8 (IL-8, P10145*)	E21-S99	14183.1
Sacsin(Q9NZJ4*)	M1-K339	43348.8
IRF4 (Q15306*)	M1-E451	56715.8
Antigens with biotin but no His Tag		
HisRS full-length (HisRS-FL)	M1-C509	60019.4
WHEP	M1-K60	9221.6
Anti-codon binding domain (ABD)	T406-C509	14440.7

*Uniprot. ID

2.2 ELISA (Paper I, Paper II, Paper III, Paper V)

The enzyme-linked immunosorbent assay (ELISA) is a largely used type of enzyme immunoassay (EIA) to detect and quantify the presence of proteins in a liquid sample (serum, plasma) by using antibodies directed against the protein to be measured. ELISA is based on the interaction between antigen and antibody. Samples containing the target protein are added on a solid support and the target protein is captured by a specific antibody (Sandwich ELISA). Then, the detection antibody is added forming a complex with the antigen. The detection antibody can be linked itself to an enzyme or is detected by a secondary antibody conjugated with the enzyme. Between each step, a mild detergent solution is used to wash the plate in order to remove non-specifically bound proteins or antibodies. Finally, the enzymatic substrate is added to produce a visible signal which corresponds to the quantity of the antigen. If the target protein in the sample is an antibody, the recombinant antigen for the antibody of interest is immobilized on a capture plate via surface adsorption or via capturing antibody and incubated with the samples. A secondary antibody (anti-human IgG) is used to detect the presence of antibody-antigen complexes. The ELISA plate allows to detect only one protein of interest in each well. Analysis of multiple antigens coated in a single assay well is possible by using the electro-chemiluminescence immunoassays (ECLIA). ECLIA follow the same principles of ELISA: each well is coated with up to ten antigens which are biotinylated to attach to specific regions of the well; the samples are added and antibody binding to the plate antigens is detected by secondary antibodies which are coupled with a Sulfo-tag molecule; the substrate for the Sulfo-tag is added and its conversion, induced by an electric pulse, generates chemiluminescence. A high-resolution camera quantifies the ECLIA signal in the various sectors of the well and reports the luminescence signal in each well sector corresponding to a specific antigen.

2.2.1 Measurement of endogenous human HisRS and HisRS-specific (Jo1) antibodies by ECLIA (Paper I)

Levels of human HisRS were measured in serum samples of IIM/ASS patients by using a home-made developed ECLIA assay following the Meso Scale Diagnostic protocol. Both capture and detection antibodies targeted the N-terminal of HisRS (approximately amino acids 1–60 of HisRS) and HisRS quantification was calibrated with highly purified recombinant HisRS. An ECLIA was also developed to detect HisRS-specific (Jo1) antibodies. The HisRS capture plate was coated with recombinant HisRS and incubated with serum samples. Sulfo-tagged goat anti-human IgG (MSD Cat. #R32AJ-1) were used as detection antibodies. Antibody levels were compared to standard curves of a mouse monoclonal antibody (clone 1C8). Levels were expressed in pM (upper limit of quantitation: ULOQ = 2333 pM, lower limit of quantitation: LLOQ = 3.2 pM).

2.2.2 Detection of HisRS, anti-Jo1 IgG and IgA by ELISA (Paper I, Paper II, Paper III)

HisRS ELISA was used to quantify HisRS protein in the supernatant obtained from human muscle cell culture (Paper I). In paper II and paper III, detection in serum and/or in BALF of total IgG, anti-Jo1 IgG, total IgA, anti-Jo1 IgA was performed by adding recombinant biotinylated full length, domains and variants of HisRS (HisRS-full length (FL), WHEP domain, CD, ABD, and SV) at 10 µg/mL to streptavidin-coated 384 well plates. Alkaline-phosphatase (ALP) monoclonal antibody MT78 (Mabtech) and ALP monoclonal antibody MT20 (Mabtech) were used as secondary antibody for IgG detection and for IgA detection, respectively. For anti-Jo1 reactivity analysis of total IgG the peroxidase F(ab'2) fragment goat anti-Human IgG (Jackson Laboratories) was employed. After the last washing step, pNPP substrate for ALP antibody detection or TMB for peroxidase antibody detection (Mabtech and Sigma) were added. The reaction with TMB was stopped after 15 minutes by adding 1M H_2SO_4. When using pNPP substrate, the OD (optical density) was measured every 15 minutes at 405 nm and, when using TMB substrate, the OD was measured one time at 450 nm (SoftMax Pro version 4.8, Molecular Devices). Anti-Jo1 IgG purified from total IgG isolated from a sera pool of IIM/ASS patients was employed as a standard curve in order to quantify the antibody levels.

2.2.3 Detection of adhesion molecules and anti-phospholipid antibodies by ELISA (Paper V)

Soluble adhesion molecules were analysed in serum samples of IIM patients and HC using Human ICAM-1/CD54 Allele-specific Immunoassay, Human sVCAM-1/CD106 Immunoassay and Human sE-Selectin/CD62E Immunoassay (all from R&D Systems Inc. USA) based on a quantitative sandwich enzyme immunoassay technique. The results were expressed in ng/mL. The anticardiolipin antibodies (IgA, IgG, IgM) and anti-β_2-glycoprotein I antibodies (IgA, IgG, IgM) were measured in IIM patients by the multiplex immunoassay Bioplex 2200 system in accordance with the manufacturer's instructions (Bio-Rad, Hercules, CA, USA) and the routine protocol in the clinical immunology laboratory. They were considered positive when ≥20 U/ml. The term any antiphospholipid antibodies included positive anticardiolipin antibodies or anti-β2-glycoprotein I antibodies.

2.3 Isolation of total IgG and anti-Jo1 IgG by development of in-house prepared affinity columns

Serum samples of IIM/ASS patients and HC were centrifugated and diluted 1:5 (vol/vol) in PBS. Total IgGs from serum were purified on HiTrap Protein G HP columns (GE Healthcare, Stockholm, Sweden). IgG was eluted with 0.1 M glycine–HCl buffer (pH 2.7) and the pH immediately neutralized with 1M Tris (pH 9). The buffer of eluted IgG was exchanged to PBS by dialysis and concentrations measured by nanodrop (ThermoFisher Scientific). To prepare the Jo1 affinity column, 1 mg of recombinant HisRS (non-biotinylated) was coupled to N-hydroxysuccinimide activated pre-packed sepharose column (size 1 mL, Ref 17071601 GE Healthcare) according to manufacturer instructions (Isolation protocol of total IgG and anti-Jo1 IgG used in Paper II is visualized in Figure 9A).

2.4 Western blot and dot blot (Paper I, Paper II, Paper III)

Western blot (WB) is an analytical technique which is widely used to detect and semi-quantify specific proteins in complex protein mixtures. Proteins contained in a sample are first denatured and then separated by gel electrophoresis. The electrophoresis membrane is incubated with a primary antibody which recognizes and binds the target protein. A secondary antibody is added to bind the primary antibody and the targeted protein is visualized by various techniques such as chemiluminescence staining, immunofluorescence and radioactivity. Dot blot is a simplification of WB method. Proteins to be identified are not separated by gel electrophoresis, while the sample is directly applied on the membrane in a single spot. Dot Blot is a faster method but it does not provide information on the size on the target protein. In Paper I, WB was applied to analyze HisRS, methionyl-tRNA synthetase (MARS) and tubulin proteins in cell lysates (intracellular) and in concentrated medium obtained from human muscle cell culture by using specific antibodies. Dot blot was used to detect HisRS protein as follows. Serum (1µL) was applied to nitrocellulose membranes. Blots were probed with monoclonal mouse anti- human HisRS N-terminal (Abcam) and developed with a polyclonal goat anti-mouse IgG HRP conjugate (Dako) followed by enhanced chemiluminescent detection. In Paper II, samples derived from the IgG purification protocol (serum, serum-depleted IgG=protein G flow through (PGFT), total IgG, non-Jo1 reactive IgG (Jo1FT) and anti-Jo1 IgG) underwent WB and dot blot to confirm the efficiency of the purification protocol (Figure 9 C-D). In Paper III, one microgram of each HisRS protein variant (HisRS-FL, WHEP, CD, ABD, and SV) underwent WB. The different membranes, each containing a different HisRS protein variant, were individually incubated with anti-Jo1 IgG from 19 anti-Jo1^{+} patients, 2 anti-Jo1^{-} patients and 3 healthy controls. Secondary antibody rabbit anti-human IgG HRP labelled (sc-2769 Santa-Cruz Biotechnology) was employed. Images were acquired and bands were quantified on a ChemiDoc XRS^{+} System using Image Lab™ Software (Bio-Rad).

Figure 9

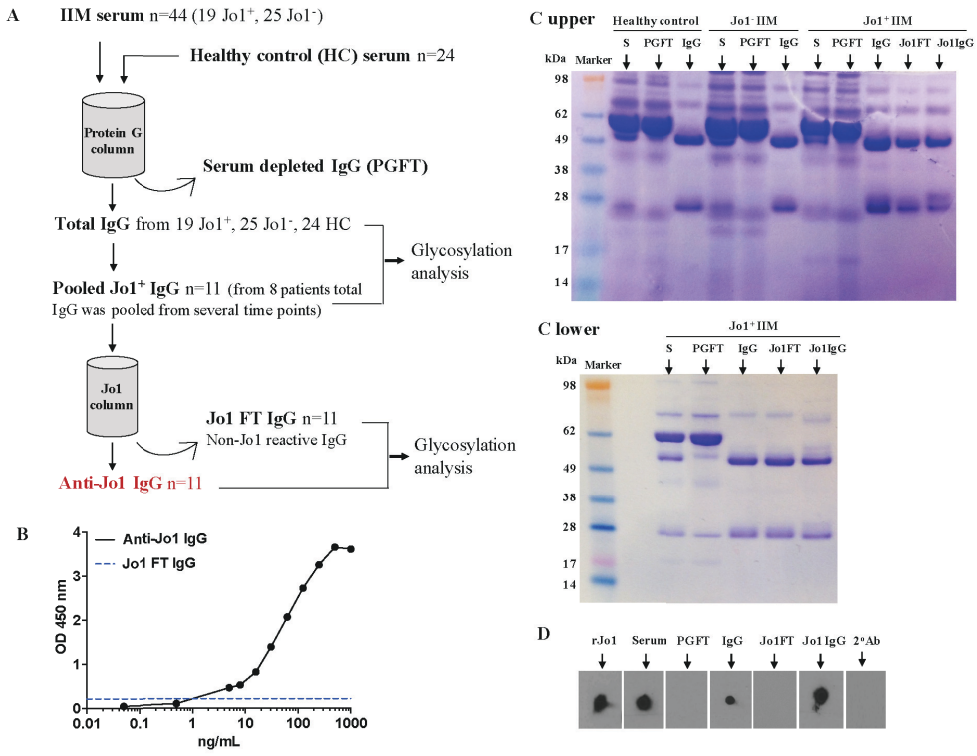


Figure 9. (A) Diagram of the different IgG subtypes purified from IIM (Jo1$^+$ and Jo1$^-$) and HC sera in Paper II (Adapted from Ossipova *et al* 2014 (270) **(B)**. Anti-Jo1-IgG ELISA to confirm reactivity of anti-Jo1 IgG eluted from the Jo1-affinity column (average of duplicates from 3 different experiments). Dash line represents OD displayed by non-Jo1 reactive IgG (Jo1FT). (**C upper and lower panels**) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) image of protein fractions collected throughout anti-Jo1 IgG purification. Protein bands are visualized by Coomassie brilliant blue staining. S, serum from HC, Jo1$^-$ IIM and Jo1$^+$ IIM; PGFT, protein G flow through = serum-depleted IgG. IgG, total IgG eluted from the protein G column; Jo1FT, non-Jo1 reactive IIM IgG collected from the Jo1 affinity column. Jo1IgG, anti-Jo1 reactive IgG eluted from the Jo1 affinity column. Because additional bands were detected in the molecular weight corresponding to the light and heavy chain of anti-Jo1 IgG one extra gel was loaded with fractions from anti-Jo1 IgG purification of another Jo1$^+$ IIM patient (**C lower panel**). **(D)** Dot-blot image illustrating reactivity to the recombinant (r)Jo1/HisRS limited to IIM Jo1$^+$ serum (second square), total Jo1$^+$ IIM IgG (forth square), and anti-Jo1 IgG fraction (sixth square). rJo1, nitrocellulose membrane containing recombinant (r)Jo1/HisRS incubated with commercial anti-Jo1 antibody. 2°Ab, nitrocellulose membrane-containing rJo1/HisRS uniquely incubated with the secondary anti-mouse antibody. Full-length gels are displayed. Acquisition of gels and dot-blot images was performed in a standard scanner.

2.5 Human muscle cell culture (Paper I)

Adult human skeletal muscle cells (HSkMC, Cell Applications) were seeded at 40,000/cm^2 on collagen-coated plates and grown in growth medium for 24h before changing to differentiation medium. The first day in differentiation medium was regarded as Diff Day 0.

Medium was renewed every 2 days, and media was harvested as 0–2, 2–4, and 4–6 samples, with three biological replicates per condition. Cells were fixed on differentiation days 2, 4, or 6 and stained for myotubes using anti-human myosin antibody; nuclei were stained with Hoechst 33342 nuclear stain. The myotube area was analyzed by determining the fusion index (calculated as the number of nuclei within the myotube-stained area over the total number of nuclei) and the nuclear number per imaging field, with a total of 20 images from two biological replicates.

2.6 T cell activation protocol (Paper I)

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of healthy donors by density-gradient centrifugation. T cells were purified using a negative selection magnetic kit (StemCell Technologies) following the manufacturer's instructions. The purity of the T cells was > 95%. Activation of T cells was induced on a ninety-six-well flat-bottomed plates with anti-CD3 antibody (BioLegend, clone UCHT-1) and anti-CD28 antibody (BD Biosciences, clone CD28.2). T cells were treated with HisRS (0.1 nM). After 24 h of incubation at 37 °C, the supernatants were collected for ELISA analysis of interleukin (IL)-2, IL-10, IL-13, IL-17A, TNF- α, IFNγ, and IL-9 and Granzyme B levels. For flow cytometry, T cells were incubated on ice with an FcR Blocking Reagent (Miltenyi Biotec) for 10 min and then stained with anti-CD4 BV510, anti-CD8 APC/ Cy7, anti-CD69-PE/Cy7, and anti-CD40L-BV421 (all from BioLegend). Propidium iodide (Miltenyi Biotec) was added as a viability marker. Samples were acquired on a MACSquant flow cytometer (Miltenyi Biotec) and analyzed with FlowJo software (Tree Star). T-cell checkpoint receptor expression experiments were performed with Primity Bio's surface profiling platform (Fremont, CA).

2.7 Liquid chromatography/mass spectrometry (LC-MS/MS) (Paper II)

Liquid chromatography/mass spectrometry (LC-MS) is an analytical chemistry technique that allows to separate multiple components in a mixture with LC and to provide structural identity of the individual components with high molecular specificity (MS). Variations of the glycans appended to the different isotypes of IgG obtained from HC, anti-Jo1 positive and negative serum samples and from samples enriched for anti-Jo1 antibodies were analyzed by LC-MS/MS. Samples were digested with trypsin in duplicates according to previously described protocols (216, 271). A in house-developed software (Quanti) (272) was set to determine the Fc-glycopeptide profiling for each sample. In addition to Fc-glycan profiling, samples were simultaneously subjected to regular peptide sequencing of MS/MS spectra (216, 271).

2.8 Surface plasmon resonance (Paper III)

Affinity measurements of serum-derived IgG to HisRS-FL from anti- Jo1 positive patients were performed using surface plasmon resonance (SPR). The measurements were carried out using the Biacore T200 biosensor instrument (GE healthcare) and single cycle kinetics mode. The Biacore T200 evaluation 3.1 software (GE healthcare) was used for analysis.

3 In vivo methodology

3.1 Animal studies

Studies conducted in animals were performed to verify if anti-Jo1 antibodies are responsible of muscle and lung injury *in vivo* after inducing only break of tolerance against HisRS or after efficient immunization and tissue challenge. HisRs protein was administered to test if it has an immunosuppressive effect.

3.1.1 Immunization against HisRS

Either HisRS antigen (200 μg in a 100 μL vehicle volume) or vehicle alone (Sham Vax) were injected subcutaneously at two different sites (e.g., flank, base of tail; 100μL per site) in female SJL/J mice (The Jackson Laboratory, 8 weeks of age). In the treated group, after 2-week intervals, 200 μg of antigen in 100 μL vehicle was injected. In independent experiments, 7 weeks after the initial immunization, animals were challenged by oropharyngeal administration of bleomycin (2 U/kg, Hospira, Lot: B051485AA, lung challenge) or intramuscular administration of cardiotoxin (skeletal muscle challenge) (10 μg per injection; 50 μL of 0.2 mg/mL solution) in the quadriceps, gastrocnemius and tibialis anterior muscles of one side (Sigma Cat. #C9759). Corresponding controls received vehicle treatments in the same manner. One week after challenge, mice were placed in a chamber containing 2% isoflurane. The latency of anesthetic was monitored and a longer time of latency indicated an impaired gas exchange and thus impaired lung function. Muscle function was assessed 6 days after challenge by evaluating the degree to which mice splayed the rear legs when picked up by the tail. In healthy animals, rear legs were extended far from the body (score = 0), whereas in animals with impaired muscle function, the foot remained close to the body (score = 2). Twelve days after lung challenge or 7 days after muscle challenge, serum and BALF were harvested for measuring HisRS and antibody titers; tissues were collected, fixed, processed to produce slides, stained with hematoxylin and eosin (H&E) and scored by a pathologist. Mediastinal lymph nodes were collected for immunophenotyping.

3.1.2 Bleomycin-induced lung injury

Starting from 8 days after intratracheally administered bleomycin in PBS saline at a dose of 3mg/kg in a volume of 50 μL per animal using a Microsprayer®, female C57bl/J mice received intravenously mouse-HisRS at a dose of 3 mg/kg once daily from day 8 to day 21. Dexamethasone was orally administered to the mice at doses of 0.25mg/kg once daily from day 0 to 21. Four or 5 randomly selected mice underwent HRCT scans and images were analyzed by Onis Viewer (DigitalCore, Japan). Lung density was determined in two sections and eight regions of interest. BALF was collected and centrifuged, and the supernatant was collected and stored until biomarker analysis. Lungs were harvested, processed to produce slides and stained with Masson's trichrome. The degree of pulmonary fibrosis was evaluated using the Ashcroft score (273) for the quantitative histological analysis (Figure 10).

Figure 10

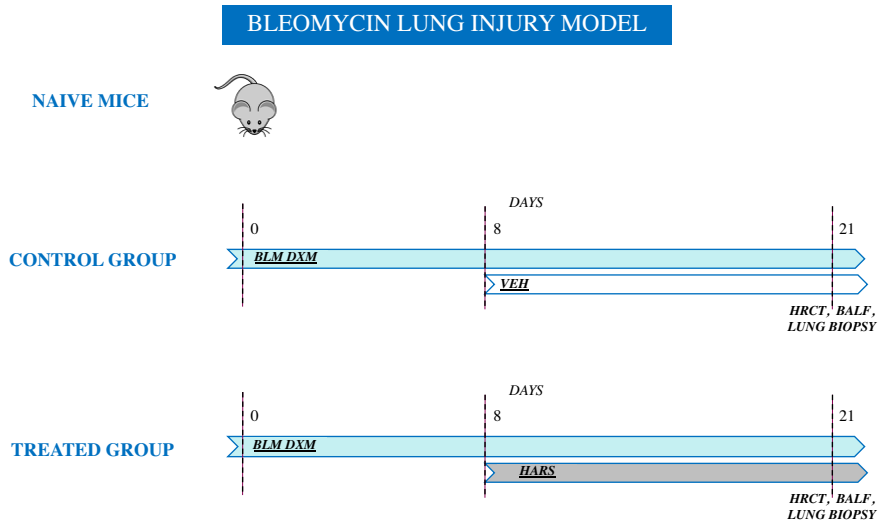


Figure 10 Schematic representation of bleomycin lung injury model. BLM, bleomycin. DXM, dexamethasone. VEH, vehicle. HRCT, high resolution computer tomography. BAL, bronchoalveolar lavage fluid

3.1.3 Cardiotoxin-induced myositis

Cardiotoxin extracted from *Naja mossambica mossambica* was injected into the tibialis anterior, quadriceps and gastrocnemius muscles of one mouse hind limb (Sigma Cat. #C9759; 10 µg/injection). The contralateral limb was injected with saline and served as a control. Tissues were harvested 7 days after cardiotoxin administration and were then stained with hematoxylin and eosin.

3.1.4 Statin-induced muscle injury

Ten-week-old female Sprague-Dawley rats (n = 8 per group) were treated with cerivastatin for 7 days and then every other day. On days 6–14, HisRS was administered intravenously at doses of 0.3, 1, or 3mg/kg. In parallel, negative controls received the vehicle. All rats were euthanized on day 15, and hamstring muscles were harvested, fixed, processed to produce slides, and stained with H&E. A pathologist blinded to the treatment groups assessed myofiber degeneration/ necrosis. A subjective, semiquantitative scoring system was utilized by the pathologist: 0 = no significant lesion; minimal change = 1; mild change = 2; moderate change = 3; and marked change = 4. Subsequently, the sections were quantified for necrotic myofibers and the number of nuclei per 20x field using imaging software at aTyr Pharma by a scientist blind to treatments. The number of nuclei was measured as an indication of inflammatory cells infiltrating the muscle (Figure 11).

Figure 11

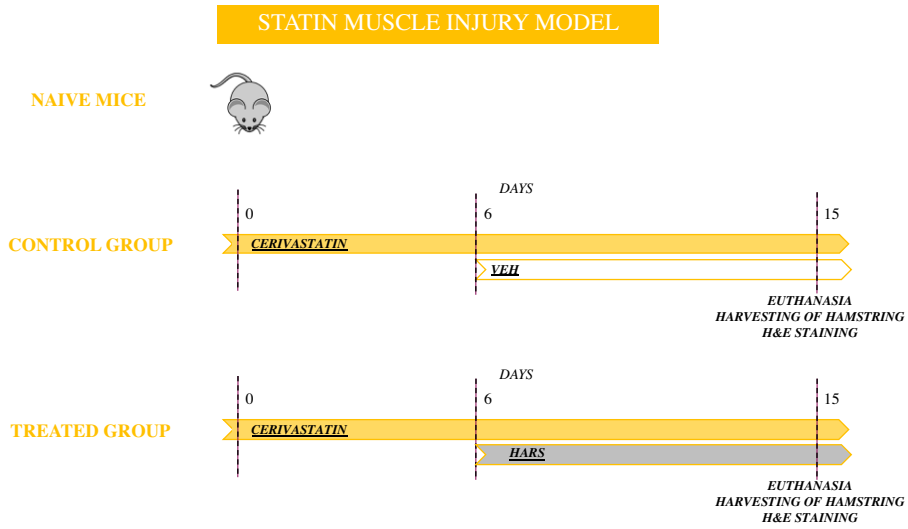


Figure 11. Schematic representation of statin muscle injury model. VEH, vehicle. H&E, hematoxylin and eosin staining

3.1.5 Transcriptional profiling of muscle tissue

Hamstring tissue was excised from treated and control rats. Transcriptional profiling of muscle tissue was performed by quantitative polymerase chain reaction (qPCR). Expression of muscle-associated *(Acta1, Actb, Acvr2b, Adrb2, Agrn, Akt1, Akt2, Atp2a1, B2m,Bcl2, Bhlhe41, Bmp4, Camk2g, Capn2, Capn3, Casp3, Cast, Cav1, Cav3, CD138, CD8b, Cryab, associated Cs, CTGF, Ctla4, Ctnnb1, CYCS, Dag1, Dars, Des, DLEC, Dmd, Dysf, Emd, Fbxo32, FOXO1, Foxo3, FSP1, Gapdh, GARP, Gars, GITR, Gpr44, Gusb,, Hars, Hars2, Hk2, Hprt1, Hsp90ab1, Igf1, Igf2, Igfbp3, Igfbp5, Ikbkb, Il17ra, Il17rb, Il17rc, Il17re, Ldha, Lep, Lmna, LTA, Mapk1, Mb, Mbnl1, Mef2c, Mstn, Musk, Myf5, Myf6, Myh1, Myh2, Myod1, Myog, Myot, Neb, Nos2, Pax3, PAX6, PAX7, Pdk4, Ppp3ca, PRF1, Ptprc, Qars, Rhoa, Rora, Rplp1, Rps6kb1, Runx3, Sgca, TRIM63, Utrn)* and immune-associated genes (*B2m, Ccl20, Ccl5, Ccr4, CCR5,Ccr6, CD117, CD11A, CD11b, CD127, CD14, CD18, CD19, CD20, CD25, CD28, CD29, CD3, CD4, CD45R, CD49D, CD8a, associated CD8b1, COL1A2, COL3A1, CXCR3, EBI3, F4/80, Fasl, FGF1, Fgf2, Foxp3, FZD7, Gapdh, Gata3, Gata4, Hars, Hsp90ab1, ICAM1, ICOS, Ifgr, IFNa1, IFNg, IKZF2, IL10, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL15, IL15RA, IL17a, Il17c, Il17d, Il17f, IL18RA, Il1b, IL1r, Il2, Il21, Il22, Il23a, Il23r, Il25, Il27, Il2ra, IL33r, Il4, Il4ra, IL5, Il6, Il6ra, Itgax, MAF, MCP1, MIF, MMP1, MMP3, Mmp9, NCAM, Nfkb1, NK1.1, Rig1, RORC, Stat1, Stat3, Stat4, Stat5a, Stat6, TBX21, Tgfb1, Tgfb3, Tnf, VCAM1, WNT7a)* was analyzed and compared between groups.

4 Statistical analysis

Continuous variables with normal distribution were presented as means with standard deviations (SD), while variables that violated normality were presented as medians with 25-75th percentiles [25-75th] or as medians with inter-quartile range (IQR). Normal distributed variables between two distinct groups were compared using the two independent sample t test while variables collected at different time points from the same group were compared by performing the paired t-test or the Wilcoxon rank sum test. Comparison of categorical variables was performed using Fisher's exact test or Chi-square test, when appropriate.

Kruskal-Wallis (correction for multiple comparisons by Dunn's test) or Mann-Whitney tests were employed when quantitative variables were compared among all groups or between two groups, respectively. In Paper I, Spearman correlation was applied between serum levels of circulating HisRS and anti-HisRS antibodies. $P<0.05$ denoted significant difference. In Paper II and in Paper III, multivariate modelling using Principal Component Analysis (PCA) and Orthogonal projections to latent structures discrimination analysis (OPLS-DA) was performed using SIMCA 15.0 (Umetrics, Sweden) following mean centering, log transformation and UV scaling. Model performance was reported as the cumulative correlation R^2X[cum], and predictive performance – as Q^2[cum] based on seven-fold cross validation. In Paper IV crude incidence rates were calculated by dividing the number of events during follow-up by the corresponding person-years at risk. All incidence rates are given as the number of events per 1,000 person-years. The incidence rates were calculated in men and women separately, as well as based on subgroups of IIM, age group at diagnosis, stratified by cancer status at the index date. The overall rate difference and 95% confidence intervals (95% CIs), adjusted for age and sex, were also calculated using an additive Poisson model. Cox proportional hazards models were constructed to estimate hazard ratios (HRs) to compare the incidence rate of VTE, DVT and PE in patients with IIM and the general population. All models were adjusted for age at the index date as a continuous variable, birth year, year of IIM diagnosis (corresponding in matched general population comparators), sex, and residential area. Cumulative incidences of VTE at 1, 5, and 8 years after start of follow-up were estimated and plotted for IIM and the general population, taking the competing risk of death into account, after performing a competing risk analysis using the Fine and Grey method. A sensitivity analysis with start of follow up 30 days after the index date was conducted to decrease the risk of having hospitalization as detection bias. In Paper V logistic regression models were fitted with TE as the dependent variable. Covariates of interest were assessed in univariate regression models, and covariates that were significant were entered as independent variables in multivariable logistic regression models. All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS, version 22.0, IBM software, USA), or GraphPad Prism version 8 (La Jolla, USA), or SAS software, version 9.4, R software, version 3.3.3, and STATA IC software, version 11.2.

5 Ethical approval

All studies in this thesis were approved by the Regional Ethical Committee of Stockholm and performed according to the Declaration of Helsinki guidelines on studies with human subjects. Subjects provided written informed consent. Studies conducted in animals were performed following prior approval of local institutional animal care and use committees (IACUCs) at aTYR Pharma, San Diego, U.S. and were in accordance with accepted standards to ensure animal welfare.

RESULTS AND DISCUSSION

1 HisRS is present in circulation in healthy individuals and its sequestration may contribute to the development of ASS (Paper I)

Extracellular HisRs or HARS circulates in healthy individuals and levels are reduced in presence of anti-HisRS antibodies

Secretion of HisRS from cells (195) has been reported only *in vitro* experiments. In study I we developed a sensitive ECLIA, calibrated with recombinant HisRS, to detect levels of HisRS *in vivo*, i.e. in serum of patients with IIM with (n=61) or without anti-Jo1 antibodies (n=286) and healthy individuals (NHV, normal human volunteers, n=115). Free HisRS was found in 98% of NHV and, in contrast, was undetectable in 84% of 61 patients with anti-Jo1 antibodies ($p<0.0001$). In the group of anti-Jo1 negative patients (n=286), levels of free HisRS were significantly higher than in NHV. Levels of circulating HisRS did not differ between distinct IIM subgroups (PM, DM, JDM, sIBM). Instead, when stratifying the patient according to the presence or not of ILD, levels of HisRS were significantly lower in patients with ILD (n=94) compared to those without (n=209, $p<0.0001$). Presence of HisRS in circulation was also confirmed by Dot Blot. Levels of two other aaRS, asparaginyl-tRNA synthetase (NARS, the autoantigen target KS) and glycyl-tRNA synthetase (GARS, the autoantigen target EJ) were also detected by ECLIA in circulation of both NHV and IIM patients. Interestingly, no differences in levels of NARS and GARS were observed between NHV and patients and among IIM subgroups with or without anti-Jo1 antibodies or ILD. A quantitative ECLIA, calibrated with a commercially available N-terminal HisRS monoclonal antibody, was developed to measure the concentration of anti-HisRS antibodies and the results were compared with the commercially available assay used for analysis of anti-Jo1 status in the clinic. The ECLIA confirmed the presence of anti-HisRS antibodies in 51/61 anti-Jo1 positive patients and 8/286 anti-Jo1 negative patients. A negative correlation was observed between anti-HisRS autoantibody levels in serum with circulating levels of free HisRS (Spearman correlation $r = -0.6244$; $p<0.0001$).
The presence of HisRS in healthy individuals raises the question whether HisRS is secreted from cells to exert a physiological function extracellularly beyond the known intracellularly role of protein synthesis. Higher levels of free HisRS in anti-Jo1 negative patients may suggest that the physiological secretion in circulation is increased in inflammatory conditions, but the cellular source is unclear. Instead, in anti-Jo1 positive patients HisRS is likely bound to anti-Jo1 antibodies explaining why levels are undetectable.

Human muscle cells secrete HisRS

Muscle inflammation is one of the clinical manifestations characterizing the anti-Jo1$^+$ ASS. We observed that primary human skeletal muscle myoblasts could release HisRS in the culture medium with increased amounts during differentiation into myotubes. Levels of HisRS in culture medium were detected by ELISA and confirmed by WB. The release of HisRS was specific since the control proteins, MARS and tubulin, were not found in culture medium, but only in cell lysates. Thus, cell lysis seems not to be responsible for HisRS secretion from muscle cells. IGF-1 was used a known modulator of muscle cell growth and regeneration. The release of HisRS in culture medium increased upon stimulation with IGF-

1. This finding is consistent with the previous demonstration of higher expression of HisRS by regenerating muscle fibers and may also justify the high levels detected in serum from anti-Jo1$^-$ patients (202). Possible downstream effects of HisRS secreted in the muscle environment should be addressed in future studies.

Break of tolerance to HisRS is not enough to cause obvious lung or muscle inflammation in mice models

The tolerance to endogenous HisRS was efficiently disrupted in different strains of WT mice by immunizing mice with murine full-length (FL) HisRS and WHEP. High titers of anti-HisRS antibodies and corresponding undetectable levels of free HisRS were found in both BALF and serum of immunized mice. Antibody levels were significantly more elevated in mice immunized with the WHEP domain compared to those immunized with the FL-protein, highlighting the WHEP domain as the major epitope of the antibody reactivity. No obvious muscle or lung inflammation was observed in immunized mice compared to control mice. However, upon external induction of tissue-specific damage, by injecting cardiotoxin in the skeletal muscle or by administering bleomycin in the lungs, the degree of immune engagement with consequent muscle damage and lung injury was significantly exacerbated in immunized mice than controls. Lung function, assessed by measuring the time of latency in response to inhaled anesthetic isoflurane, was more impaired in mice with circulating anti-HisRS than controls. In addition, 12 days after bleomycin challenge, the mediastinal lymph nodes of immunized mice had increased levels of activated CD4$^+$ and CD8$^+$ T cells.

HisRS has in immunomodulatory effect in vitro

In vitro effects of recombinant HisRS were investigated on T cells isolated from human blood of 8 healthy donors. Freshly resting T cells were not affected by HisRS, while activation of CD4$^+$ and CD8$^+$ T cells by anti-CD3 or CD8 antibodies was inhibited by HisRS, as demonstrated by less release of inflammatory cytokines, cell-surface markers and Granzyme B. The inhibition of T cells was robust already at sub-nM concentrations of HisRS, but was abolished upon removal of the N-terminal WHEP domain. In addition, HisRS treatment modulated the expression of immune checkpoint receptors specifically on activated T cells. Our findings are in contrast with the work of Howard *et al.* which showed *in vitro* that HisRS possess chemokine-like properties capable of stimulating lymphocytes, activated monocytes, and immature dendritic cells (214). Moreover, we also demonstrated in a previous published work (208) that lung derived T cells from BAL fluid of anti-Jo1$^+$ patients compared to blood-derived T cells of anti-Jo1$^+$ patients produced high amounts of the pro-inflammatory cytokine IFN-gamma in response to stimulation with HisRS protein, and in particular with one of the HisRS peptides (residues 11-23 within the HisRS protein, located in the WHEP domain). Whether the source of T cells (blood of healthy donors in Paper I and BALF from anti-Jo1$^+$ patients in (208)) accounts for the difference in the results needs to be further elucidated.

HisRS has therapeutic activity in in vivo inflammatory disease models

Administration of murine HisRS 8 days after the induction of lung injury with bleomycin reduced the degree of lung fibrosis as shown by CT scans, and as well of lung immune infiltration and of the release of cytokines in BAL. Daily administration of cerivastatin caused

severe skeletal muscle damage and required euthanasia at day 14. In contrast, rodents treated with HisRS from day 6 showed less morbidity and better survival than controls in a dose-related manner.

To summarize, in Paper I we demonstrated that extracellular HisRS circulates in healthy individuals but is reduced or absent in anti-Jo1 positive patients. Levels of free HisRS were in fact inverse correlated with the levels of anti-HisRS antibodies. Our data showed that HisRS probably has an immunoregulatory function when secreted outside cells. In *vitro*, HisRS inhibited T cell activation and it had, at least in mouse, immunosuppressive effect in bleomycin and statin induced inflammatory models. On the contrary, the degree of inflammation in lungs and muscle was exacerbated in mice with high titers of anti-HisRS antibodies. Therefore, we hypothesize that the sequestration of HisRS and, specifically, of the WHEP domain is a major contributor to the pathogenesis of ASS. However, we cannot rule out that immune complexes of anti-Jo1 antibodies and HisRS have independent pathogenic capacities. So far, no functional studies on anti-Jo1 antibodies have been published.

2 Anti-Jo1 positive patients exhibit a characteristic IgG-Fc and anti-Jo1 IgG glycan profile (Paper II)

Total IgG were isolated from 19 anti-Jo1$^+$ and 25 anti-Jo1$^-$ patients as well as from 24 HC. Anti-Jo1 IgG were isolated from a pool of serum collected at different time points from 11 anti-Jo1$^+$ patients. Total IgG concentration among serum proteins was similar between IIM patients and HC. Half of the glycans appended to the Fc region of IgG_1 antibodies of IIM/ASS patients were significantly different from those found in HC ($p < 0.05$), showing in particular a decrease in galactosylation. No significant differences were observed in the glycan profile of IgG_2 and IgG_3 isotypes between patients and HC. Decrease in galactosylation (major agalactosylated form FA2) is known to be associated to inflammatory conditions and to factors like age, gender and heritability. Therefore, we tested if the unchanged FA2 of IgG_2 (FA2_2) could be used as an intraindividual control of the change in FA2 of IgG_1 (FA2_1). The logarithm (FA2_1/ FA2_2) was used as a factor to distinguish patients from HC. Receiver Operating Characteristic (ROC) curve analysis of log (FA2_1/ FA2_2) generated an area under the curve (AUC) of 79±6% when comparing IIM/ASS patients to HC. The AUC went up to 88±6% for anti-Jo1$^+$ patients and for those with ILD, independently of the anti-Jo1 status. The AUC decreased to 72±8% for anti-Jo1$^-$ patients. By comparing the different glycan features according to the sum of galactosylated Σ[G], sum of agalactosylated Σ[aG], sum of afucosylated Σ[aF], sum of bisected Σ[B], and sum of sialylated Σ[S] forms, IgG_1 and IgG_2 isolated from anti-Jo1$^+$ patients presented lower abundances of bisected and afucosylated glycopeptides compared to IgG from anti-Jo1$^-$ patients. Anti-Jo1 autoimmune reactive IgG (enriched from the IgG from the anti-Jo1 positive patients) was also screened for glycan profile and compared intra-individually to the IgG prior to anti-Jo1 enrichment and to the remaining non-anti-Jo1 specific IgG following enrichment (termed flow through, FT). Anti-Jo1 IgG showed a similar but enhanced profile of the characteristics observed in total IgG from anti-Jo1+ patients. Anti-Jo1 IgG were generally less bisected, less afucosylated and more agalactosylated compared to the total IgG from anti-Jo1+ patients and subsequently compared to the IgG from anti-Jo1- patients and the HC. Multivariate statistical

modelling (MVA) was performed to understand if Fc-glycan features would correlate with patient clinical features and protein data obtained from the IgG enriched samples. Bisected and afucosylated forms negatively correlated with anti-Jo1+ patients while agalactosylation status factor (log[FA2_1/FA2_2]) was associated with anti-Jo1 positive status, confirming the results of the univariate analysis. Among clinical features, ASS, ILD, presence of MSAs and higher MMT8 correlated positively with presence of anti-Jo1 antibodies. Among proteins, lysozyme, thrombospondin, plasminogen, CD5 antigen like protein, IgD, the conserved lambda chain 2, one kappa variable chain, complement factor H related protein and platelet factor 4 correlated with anti-Jo1+ status.

In conclusion, our study showed that both total IgG and specifically anti-Jo1 IgG from IIM/ASS patients display a pro-inflammatory Fc-glycan profile (i.e. agalactosylation) which is overrepresented in patients with ILD. The majority of patients with ILD tested positive for anti-Jo1 antibodies but ten out or 28 with ILD were anti-Jo1$^-$. The hypothesis is that the agalactosylated features of ASS/IIM IgG (and anti-Jo1-IgG) are enhanced due to underlying lung disease related mechanisms in these patients, as suggested by evidence of the possible immunological role of IgG Fc-glycans in the respiratory tract in other lung pathologies such as sarcoidosis, severe asthma and lung cancer (220, 274). Furthermore, anti-Jo1 IgG specifically presented lower abundance of bisected and afucosylated forms as also observed in ACPA (216). These glycopeptides are known to increase IgG affinity to FcγRII and III receptors, potentially resulting in more potent antibody dependent cell-mediated cytotoxicity (ADCC) (275, 276). Fc-glycan characteristics of anti-Jo1 IgG positively correlated with proteins involved in inflammatory processes, including C-reactive protein. This highlights the close link between the glycosylation pattern of IgG and the inflammatory responses and supports the hypothesis that the autoantibodies have a role in the pathogenesis.

3 Anti-Jo1 antibodies with high affinity and reactivity to different HisRS variants and domains appear early in the IIM/ASS disease and correlate with lung and joint involvement (Paper III)

Reactivity profile against HisRS of serum and BALF-derived anti-Jo1 autoantibodies

IgG isolated from first available serum sample in relation to diagnosis of IIM/ASS collected from 19 anti-Jo1$^+$ patients displayed stronger reactivity against the HisRS-FL and the two splice variants (WHEP domain and SV) in comparison with the ABD and CD domains ($p<0.05$). Sixteen of 19 anti-Jo1$^+$ patients showed reactivity to all HisRS antigens, while one patient showed exclusive binding to ABD and SV and two patients presented no reactivity against any of the HisRS antigens. The ELISA and WB results demonstrated that anti-Jo1 antibodies recognize both conformation-dependent (ELISA) and -independent epitopes (WB), with a strong preference for the WHEP and SV regions. Anti-Jo1$^-$ and HC did not show reactivity towards HisRS-FL or any of the HisRS-variants/domains. In matching BALF and serum samples the highest IgG and IgA reactivity was found against HisRS-FL for patients with anti-Jo1$^+$ IIM/ASS. No reactivity was registered in matching BALF and serum collected from HC and clinically diagnosed anti-Jo1$^-$ patients.

Anti-Jo1 IgG reactivity through the disease course of anti-Jo1+ patients

IgG were isolated from longitudinal serum samples collected at different time points from 16/19 anti-Jo1$^+$ IIM/ASS patients up to 24 years after diagnosis. At time of diagnosis, anti-HisRS-FL, anti-WHEP and anti-SV IgG recorded higher reactivity levels in comparison to anti-CD and anti-ABD IgG. Interestingly, similar reactivity levels were found in three anti-Jo1$^+$ serum samples collected before diagnosis. Three years after diagnosis, median anti-HisRS-FL IgG reactivity levels were still as high as at diagnosis, while the reactivity against WHEP and SV registered a ~3-fold decrease. The reactivity levels against CD and ABD decreased between 6 to 3-fold already one year after diagnosis, remaining low thereafter. Through the disease course, median antibody concentrations fluctuated over time and, in general, the reactivity towards the different HisRS antigens changed simultaneously. Administration of Rituximab, a monoclonal antibody directed against CD-20 in B cells, in 9 of 16 patients did not result in homogeneous decrease of reactivity levels against the different HisRS variants and domains.

High levels of anti-HisRS antibodies correlate with lung and joint involvement in anti-Jo1+ patients

On the basis of the autoantibody levels targeting HisRS-FL in the first available sample close to the time of diagnosis, patients were stratified into low to moderate (n=8) or high anti-HisRS-FL reactivity (n=11). Muscle disease activity did not statistically differ between those with low to moderate or high anti-HisRS-FL reactivity. On the contrary, anti-Jo1$^+$ IIM/ASS patients with high anti-HisRS-FL antibody levels were more likely to be diagnosed with ILD, ever through the disease course (100% compared to 63% for the anti-Jo1$^+$ patient group with low to moderate anti-HisRS-FL IgG levels and 36% for anti-Jo1$^-$ group). The pulmonary function (median values of forced vital capacity (FVC), total lung capacity (TLC) in the low to moderate anti-HisRS-FL reactivity group (FVC 51% and TLC 54%) was significantly lower compared to both anti-Jo1$^+$ with high anti-HisRS-FL levels (FVC 67%, TLC 70%) and anti-Jo1$^-$ (FVC 81%, TLC 76%, $p<0.05$). Interestingly, the group with low to moderate anti-HisRS-FL titers had higher percentage of smokers. The group with high anti-HisRS-FL antibody levels presented a higher frequency of arthritis (73%) in comparison to the low to moderate, and negative sub-groups (38% and 28%, respectively). Skin manifestations were recorded more frequently in anti-Jo1$^+$ patients with low to moderate levels and anti-Jo1$^-$ patients (38% and 36%, respectively) compared to the 18% of patients with high anti-HisRS-FL response.
These findings were strenghthened by the results generated by the multivariate analysis which showed that the anti-HisRS reactivity data together with anti-synthetase syndrome, anti-SSA positivity, presence of MSAs and arthritis strongly correlated with both anti-Jo1$^+$ and ILD$^+$ status, beeing ILD$^+$ status independent of anti-Jo1 status.

Inverse correlation between BALF anti-HisRS IgG levels and pulmonary function parameters

A negative correlation was found between several pulmonary function measures (vital capacity (VC), FVC, TLC, and forced expiratory volume in 1 second (FEV1) and both BALF levels of IgG anti-WHEP and anti-CD, $p<0.05$). On the contrary, BALF anti-WHEP and anti-CD IgG as well as anti-HisRS-FL and anti-SV IgA positively correlated with the BALF's cell concentration and the number of eosinophils ($p<0.05$). The multivariate data analysis

showed that both the anti-HisRS reactivity data from IgG and IgA as well as eosinophils and mast cells correlated strongly with anti-Jo1$^+$ and ILD$^+$ status. Inversely, higher levels of VC, FEV1, TLC, FVC, diffusing capacity for carbon monoxide (DLCO), CD4:CD8 and macrophages correlated prominently with anti-Jo1$^-$ and ILD$^-$ patients.

Anti-Jo1+ IgG display high affinity already at diagnosis of IIM/ASS

Surface plasmon resonance (SPR) was applied to measure the binding profiles of serum-derived IgG to HisRS-FL, close to diagnosis, from the 19 anti-Jo1$^+$ patients. Average kinetic constants could be determined for 14 of the patients and in all cases high average affinity profiles were observed (calculated $^{Ave}K_D$ close to 1 nM).

To summarize, in this study, we found that reactivity levels of IgG and IgA isolated from BALF and serum of anti-Jo1$^+$ patients are high already at time of diagnosis and in some cases even before diagnosis, generally decreasing thereafter. Highest reactivity was registered against the full length HisRS and the HisRS splice variants WHEP and SV. Moreover, IgG against HisRS-FL displayed high affinity already at time of diagnosis. Patients with high reactivity levels towards HisRS-FL were more more frequently affected by ILD and arthritis, but less likely to have skin rash. Noteworthy, IgG anti-WHEP reactivity in BALF correlated with poor pulmonary function. Our results add to previous attempts of epitope mapping performed by using linker mutagenesis and restriction enzymes, or linear peptide design (36, 37, 277-281). We have, instead, investigated IgG and IgA reactivity against naturally occurring folded HisRS variants by ELISA and WB and we could demonstrate that anti-Jo1 antibodies recognized both conformational and non-conformational epitopes within HisRS. Highly reactive and high affinity anti-HisRS antibodies, especially towards the WHEP domain, at time of and even before diagnosis, support the hypothesis that autoimmunity against HisRS, driven by exposure to HisRS autoantigen, might be initiated before diagnosis of IIM/ASS. In addition, the positive correlation between high reactivity levels of anti-HisRS antibodies and ILD as well as the significant correlation between anti-WHEP reactivity in BALF and poor pulmonary function suggest the lung as a potential site of immune activation.

4 Patients with IIM are at high risk of arterial and venous thrombosis especially close to diagnosis (Paper IV and Paper V)

Arterial and venous thrombotic events are a leading cause of death in patients with IIM. A better understating of the factors contributing to the development of this life-threatening comorbidity is needed to try to prevent that from happening. In Paper IV, we assessed the incidence of venous thromboembolic events (VTE) in patients with IIM in comparison to the general population and we aimed to identify patient categories at high risk and the timing of risk in relation to the diagnosis of IIM. In Paper V, we investigated whether and which traditional and/or disease-related risk factors and biomarkers are linked to arterial and venous thrombotic events in patients with IIM.

The absolute and relative risk of VTE is significantly higher in IIM patients than in the general population (Paper IV)

After excluding both patients and general population comparators presenting VTE before the index date, 440 patients with IIM and 4,459 comparators were included in the analysis. The proportion of women and men, the mean age at start of follow-up, and the education level were similar in patients with IIM and the general population. Comorbidities prior to the index date were more common in IIM patients than comparators and IIM patients were more often treated with methotrexate, glucocorticoids, antidiabetic drugs, and statins around the period of the index date. Prescription of anti-hypertensive drugs was instead more common in general population comparators. Median (IQR) follow-up in both cohorts was 4.2 (3.2) years. Seventy-nine patients with IIM (18%) and 305 comparators in the general population (7%) died, while none of the patients with IIM and 34 comparators of the general population (0.1%) emigrated from Sweden during follow-up. Twenty-eight patients in the IIM cohort (6.6%) and 48 comparators of the general population (2.0%) experienced a first-time VTE during follow-up; in the IIM cohort, 15 (54% of all events) were PE events and 13 (46%) were DVT, and in the general population, 19 (40%) were PE events and 29 (60%) were DVT. The crude incidence rate of VTE was 16.2 (95% CI 4.3, 28.2) per 1,000 person-years in patients with IIM, and 2.4 (95% CI 0.9, 3.8) per 1,000 person-years in the general population. In IIM patients but not in the general population the incidence rate was higher in women than in men. Among IIM subgroups, DM (diagnosed in 35% of IIM patients) was more associated with VTE than the other subgroups. There was a tendency in both patients and comparators of increasing rate with ageing. In comparators but not in patients a lower level of education was associated with higher incidence rate of the outcome. Diagnosis of cancer before the index date conferred to IIM patients higher likelihood to present with VTE. After adjusting for age, sex, and residential area, the overall HR of VTE comparing the IIM cohort to the general population was 7.81 (95% CI 4.74, 12.85). Other variables including death as competing risk did not alter this result. The increased relative risks could be seen in all subgroups of IIM, in all age groups, in all education levels, and in both women and men with IIM compared to the general population. When we stratified by time since diagnosis, we noticed an increased incidence rate and relative risk of VTE in the first year of diagnosis with an incidence rate of 39.2 (95% CI 20.7, 57.7 per 1,000 person-years) and HR of 26.6 (95% CI 10.4, 68.0). On the contrary, in the general population cohort, the incidence rate was very low within the first year of follow-up (incidence rate 1.8, 95% CI 0.6, 3.1) and increased slowly with time.

To conclude, in this study we observed that the incidence rate of VTE was significantly higher in IIM patients than in the general population, especially during the first year after diagnosis. The HR was almost eight times more elevated in IIM patients than in general population and remained that high even after adjusting for education level, comorbidities, cancer, treatment at baseline and competing risk of death. The risk was even more elevated in patients with a history of cancer, in patients with DM, or in those with age ≥72 years. Our findings were congruent to the results from a Canadian cohort of patients with IIM (234) and with a previous publication from Sweden (235).

Lower e-selectin levels were associated with a higher risk of presenting with arterial and/or /venous thrombotic events (Paper V)

The prevalence of arterial and/or venous TE was retrospectively assessed in a large cohort of patients with IIM (n=246). TE occurred up to 30 years after the year of IIM diagnosis with peak of prevalence within the first 5 years after IIM diagnosis in 21% of IIM patients. PE was the most frequent event followed by DVT and myocardial infarction. Among demographic and clinical variables, only age at the time of IIM diagnosis was significantly different between cases and comparators. Cases were older than comparators and age at diagnosis was an independent risk factor for TE in the investigated IIM cohort. A patient with IIM had a 3.5% increased chance of having TE per each year of age (Exp B 1.04, 95% CI 1.01-1.06, p=0.004). Even if not significant, men were more prevalent in cases than comparators as demonstrated in the general population (282, 283). Patients with reported venous TE were more likely to be affected by dermatomyositis and testing positive for MSAs, while the arterial group was more often diagnosed with polymyositis. Among comorbidities, only essential hypertension was found more often in cases (22/51, 43%) than comparators (49/193, 25%), p=0.01. Diabetes, dyslipidemia, history of smoking and/or malignancy were, instead, equally distributed between the two groups. Malignancy diagnosed before the occurrence of TE was more common in cases with reported only venous events than those with history of exclusively arterial events. CK and CRP levels were used as proxies for disease activity and did not differ neither between cases and comparators nor between the arterial and venous group. IMACS disease activity core set measures were available at the time of 34/69 thrombotic events. The mean MMT-8 value and HAQ score were both low, indicating that reduced physical activity due to inflammatory myopathy together with functional impairment in IIM patients could potentially be a risk factor for developing TE. In addition, at the time of TE the mean dosage of prednisolone was higher than 10 mg/day indirectly suggesting that IIM patients did not have low disease activity. Regarding possible biomarkers predicting TE in patients with IIM, we analyzed serum levels of aPL at the time of IIM diagnosis and we found that only 6% of the IIM cohort tested positive for aPL antibodies, discarding a possible contribution of aPL in the development of TE in IIM patients. We also analyzed at the time of IIM diagnosis and at the time of TE serum levels of VCAM-1, ICAM-1 and e-selectin. Levels of adhesion molecules were also measured in age and gender matched HC. In IIM patients, median levels (IQR) in ng/mL of VCAM-1 and ICAM-1, but not e-selectin were significantly higher than in HC. Median levels (IQR) of ICAM-1 and e-selectin were significantly higher in comparators than cases, whereas levels of VCAM-1 did not differ between cases and comparators. There was no difference observed in the levels of adhesion molecules between the time of IIM diagnosis and the time of TE, neither between patients with arterial TE or venous TE. Lower e-selectin levels were associated with a higher risk of presenting with TE. The odds of getting TE for lower levels of e-selectin were 0.79 times higher than the odds of getting TE for higher e-selectin levels (Exp B 0.78, 95% CI 0.62-0.97, p=0.03). Each additional unit of e-selectin decreased the chance of developing TE by a factor of 0.24. The association between lower levels of e-selectin and the risk of TE was maintained even when adjusted for age at diagnosis. Notably, low e-selectin levels have been found in individuals with recurrent venous thromboembolism in the general population (251, 252). However, high levels of e-selectin have been correlated with myocardial infarction and carotid artery atherosclerosis (249, 250). In mouse models, e-

selectin inhibition decreased venous thrombosis (284). One possible explanation of our finding is that, while e-selectin is activated on an endothelium surface, the release of its soluble form is decreased (251). One other hypothesis is that, beyond endothelium activation, low e-selectin serum levels are related to high turnover due to hypercoagulability (251).

To summarize, this study reported a high prevalence of arterial and venous thrombotic events in patients with IIM, especially within the first five years after diagnosis. Screening for risk factors should be recommended with particular attention to older male patients with IIM and those with essential hypertension. Low serum levels of e-selectin might predict TE in IIM patients but further studies are warranted to confirm this finding.

CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis aimed to add pieces of knowledge in the understanding on the role of the autoimmunity against HisRS in the pathogenesis of anti-synthetase syndrome, a distinct subgroup of idiopathic inflammatory myopathies.

In **paper I**, we demonstrated, for the first time in *vivo,* that HisRS circulates in healthy individuals, strengthening the hypothesis that HisRS exerts physiological functions extracellularly other than the canonical intracellular protein synthesis. We also detected higher levels of free HisRS in serum of anti-Jo1 negative patients compared to HC, suggesting an increased release of the protein in circulation during inflammatory conditions. It would be interesting to measure levels of HisRS in serum of patients affected by other autoimmune/inflammatory diseases like SLE, RA or sarcoidosis to understand if the increased amount of HisRS in serum of anti-Jo1 negative patients is a specific finding in IIM, or whether it is generally associated to the inflammatory milieu. In order to investigate which cells actively release HisRS, one possibility is to verify if HisRS is bound to microparticles and track their origin. In this study we observed that primary human skeletal muscle myoblasts release HisRS in the culture medium with increasing levels during differentiation in myotubes and upon stimulation with IGF-1. It would be intriguing to verify if the release of HisRS from myotubes is affected by other stimuli such as cytokines and if the release would change when setting up a muscle culture from muscle biopsies of anti-Jo1 positive and negative patients. Investigation of protein network interactions in plasma samples and BAL fluid of healthy individuals and IIM/ASS patients would help to identify the molecular pathways in which HisRS is involved both physiologically and in pathological conditions, expanding previous *in vitro* studies showing that HisRS takes part in the regulation of processes such as immune cell development, angiogenesis, inflammatory response, and mTOR signaling (187-189). In addition, our findings promote the hypothesis that sequestration of HisRS and, specifically, of the WHEP domain is a major contributor of the pathogenesis of ASS. We found that free HisRS is reduced or absent in circulation in presence of anti-Jo1 antibodies with inverse correlation between protein and antibody levels. In immunized mice with HisRS, presence of anti-HisRS antibodies was associated to exacerbation of inflammation in challenged tissues while treatment with HisRS resulted in a better control of the inflammatory status. If these observations were replicated, this would open the path for a new possible therapeutic intervention in the clinical setting of anti-Jo1$^+$ ASS. However, in our study, we could not rule out that immune complexes of anti-Jo1 antibodies and HisRS have independent pathogenic capacities. Therefore, functional studies on anti-Jo1 antibodies are warranted.

In **Paper II**, we demonstrated that both total IgG and specifically anti-Jo1 IgG from IIM/ASS patients display a pro-inflammatory Fc-glycan profile (i.e. agalactosylation, lower abundance of bisected and afucosylated forms) which is overrepresented in patients with ILD. In **Paper III**, we showed that anti-HisRS reactivity levels of IgG and IgA isolated from BALF and serum of anti-Jo1$^+$ patients are high already at time of diagnosis and in some cases even before diagnosis as well as the affinity levels of antibodies against HisRS-full-length protein. High reactivity levels of anti-HisRS-FL antibodies correlated with ILD and poor pulmonary function. The findings of both Paper II and III support the hypothesis that the break of

tolerance to HisRS might occur prior to the onset of clinical symptoms of IIM/ASS and likely in the lungs as possible site of immune activation. One possible model of the pathogenesis of anti-Jo1^{+} ASS could imply the following steps: in genetically predisposed individuals, environmental factors such as smoking or recurrent lung infections could induce years or months before the occurrence of symptoms an aberrant cleavage by Granzyme B of HisRS expressed on epithelial bronchial cells or present in BAL. This would reveal new epitopes of HisRS protein, becoming antigenic for the immune system. Then, the antigenic HisRS could be presented by lung APC to T cells, inducing the clonal expansion of auto-reactive HisRS-specific T cells which, in turn, could activate B cells to produce locally in the lungs anti-HisRS antibodies. Upon HisRS exposure, anti-HisRS antibodies would increase their reactivity and affinity and possibly change even the glycan profile, being directly or indirectly, trough HisRS sequestration, responsible for lung inflammation and damage. Anti-HisRS antibodies might then access the systemic bloodstream and reach previously damaged muscles (e.g. by trauma) with high amount of regenerating fibers, expressing HisRS during differentiation. This would result in muscle inflammation as second target organ after the lungs (Figure 12). Similarly, anti-HisRS antibodies might enter the joints causing arthritis. To verify this hypothesis, we plan to identify blood samples of anti-Jo1 positive patients collected in a Swedish nation-wide biobank years before the diagnosis of IIM/ASS. If the presence of anti-Jo1 antibodies before diagnosis is confirmed, it would be interesting to check if these pre-clinical anti-Jo1 antibodies already present with a pro-inflammatory Fc-glycan profile or if they display pro-inflammatory glycopeptides with the appearance of clinical symptoms. In addition, it would be helpful also to study if the glycan profile of anti Jo1 antibodies correlates with disease activity measures and response to treatment, predicting in this case the course of the disease. Finally, if further studies confirmed that the sequestration of HisRS and its subsequently abolishment of physiological functions extracellularly are crucial events in the pathogenesis of ASS, treatment with HisRS protein to restore normal circulating levels in the pre-clinical phase might be used to prevent the development of ASS in anti-Jo1 positive patients.

This thesis also focused on a leading cause of premature death in IIM, the arterial and venous thrombosis.

The results obtained in **Paper IV** confirmed previous published observations of significantly higher incidence of VTE in IIM patients than in the general population, after adjusting for education level, comorbidities, cancer, treatment at baseline and competing risk of death. The risk of being affected by VTE became extremely elevated during the first year after diagnosis of IIM. In patients with IIM, female gender, DM subgroup, older age and diagnosis of cancer were found to confer higher risk of presenting with VTE. In **Paper V,** we showed that one out of five IIM patients manifested arterial or venous thrombosis, in particular during the first five years after IIM diagnosis and preferentially PE, DVT and MI. Male gender and essential hypertension were found to be associated with higher risk of developing TE, while specific IIM features and presence of antibodies including anti-phospholipid antibodies did not seem to contribute. Moreover, one interesting finding was that lower levels of e-selectin correlated with higher odds of getting TE in IIM patients.
Based on the results of our studies, patients receiving the diagnosis of IIM should be directly screened for risk of developing arterial and/or venous thrombosis and accurately monitored

especially within the first five years after IIM diagnosis. Extra warning should be reserved to old patients with dermatomyositis, essential hypertension and cancer. Impact of prophylactic treatment (anti-platelets aggregation drugs and anti-coagulants) should be addressed in appropriate randomized trials. In addition, if the finding of the association between low e-selectin levels and risk of thrombosis was replicated in independent cohorts, e-selectin levels might be used as biomarkers to predict the occurrence of thrombosis in IIM patients. Finally, the latter observation generates more research questions dealing with the underlying biological mechanism behind low e-selectin levels and thrombosis in myositis.

In conclusion, this thesis provides new information supporting the role of autoimmunity against HisRS in the pathogenesis of the anti-synthetase syndrome. The discovery of HisRS extracellularly, in both healthy individuals and IIM patients, strengthens the hypothesis of HisRS exerting other physiological functions beyond the known intracellular protein synthesis. The results obtained from the T cell assay and animal models suggest a possible immunosuppressive activity of this protein. The inflammatory Fc-glycan profile as well as the high reactivity and affinity levels in serum and BALF of anti-Jo1 antibodies (conversely undetectable serum HisRS levels) already at the time of and even before diagnosis advocate a break of tolerance of the immune system against HisRS already in the pre-clinical phase and most likely in the lungs. In fact, high reactive anti-HisRS IgG associate with the presence of ILD and, noteworthy, IgG anti-WHEP reactivity in BALF correlate with poor pulmonary function. It appears then to be stringent to verify for the presence of anti-Jo1 antibodies prior the onset of the disease and to replicate the results generated by the animal models. This could open the path for a new potential preventive and/or therapeutic approach for anti-Jo1 positive patients with anti-synthetase syndrome.
This thesis also confirms that patients with idiopathic inflammatory myopathies present with a high incidence and prevalence of arterial and venous thrombotic events, especially close to diagnosis and in those older, male, with essential hypertension and history of malignancy. This should indicate that a proper screening and preventive measures need to be recommended in this patient population. Moreover, E-selectin levels could be used as biomarkers to identify IIM patients at higher risk of presenting with thrombotic event.

Figure 12

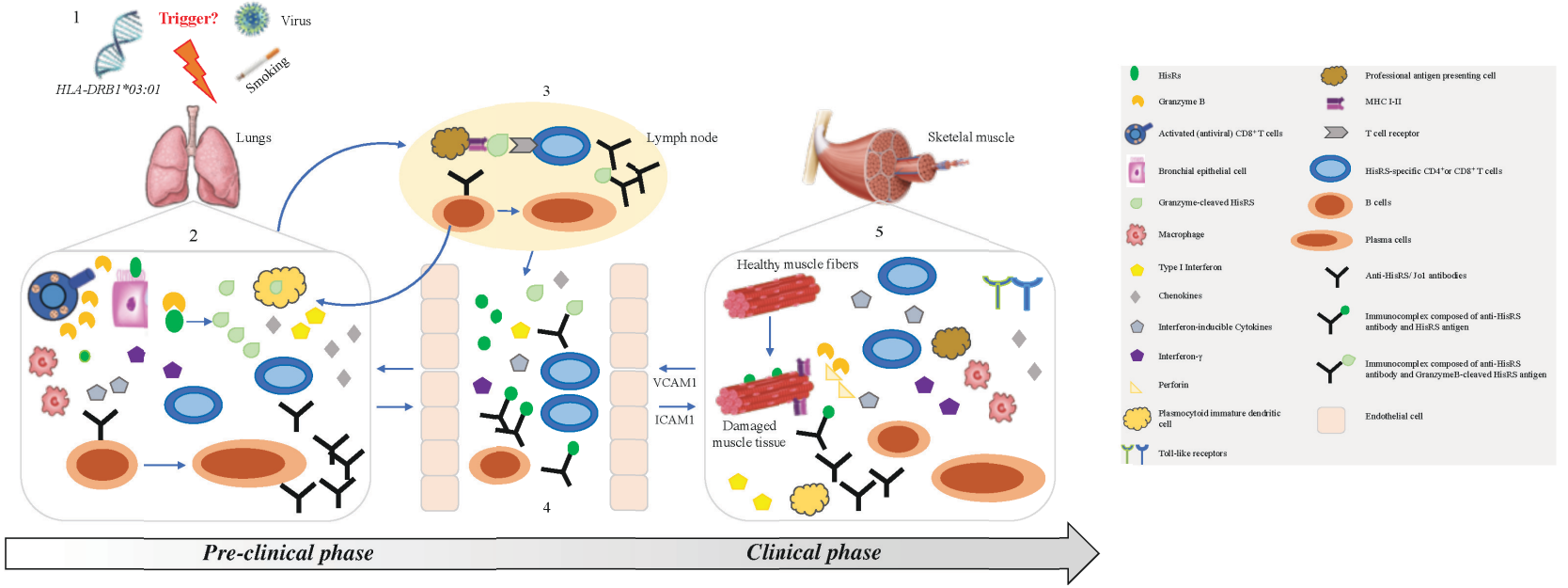


Figure 12. Our hypothetical model for pathogenesis of anti-Jo1 positive patients with anti-synthetase syndrome: (1) in genetically predisposed individuals (e.g. *HLA-DRB1*03:01*) an unknown trigger (e.g. smoking or viral infection) induces years or months before the onset of clinical symptoms (pre-clinical phase) an aberrant cleavage by GrB of HisRS expressed on bronchial epithelial cells of the lungs or present in BAL fluid (2). (2) GrB could be released by anti-viral $CD8^+$ T cells or NK cells. The cleaved HisRS reveals new epitopes becoming immunogenic and being up-taken by plasmocytoid immature DC that start to release type I IFN. Activated DC migrate to a peripheral lymph node (3) and transform in APC. APC present the cleaved HisRS antigen to naïve $CD8^+$ or $CD4^+$ T cells in the context of MHC class I and II, respectively. HisRS-specific T cells activate, clonally expand and reach the bloodstream (3). Chemokines secreted in the lungs by, for instance, macrophages attract T cells to the lungs with locally release of cytokines, e.g. IFN-γ. With the help of $CD4^+$ T cells, B cells activate and, both in the lymph node end locally in the lungs, transform in plasma cells producing anti-HisRS/Jo1 antibodies. Upon HisRS exposure, anti-HisRS antibodies increase their reactivity and affinity and possibly change even the glycan profile, being directly or indirectly, trough HisRS sequestration, responsible for lung inflammation (clinical phase). Anti-HisRS antibodies access the systemic bloodstream and bind free circulating HisRS (4). Anti-Jo1-HisRS immunocomplexes, together with HisRS-specific T/B cells and cytokines (especially type I IFN), may reach previously damaged muscles (e.g. by trauma) with high amount of regenerating fibers, expressing HisRS during differentiation (5). Type I IFN is a potent inducer of the expression of MHC-I on the surface of muscle cells, which in turn become target of activated $CD8^+$ T cells releasing perforin and GrB. This would result in muscle inflammation as second target organ after the lungs (clinical phase).

ACKNOWLEDGEMENTS

I truly believe that the most powerful resource to fight life adversities is the hope that something good can always happen. Research generates knowledge and knowledge can donate the hope of a better life.
I would like to express my gratitude to the people who have contributed to start my career as physician and researcher and have helped me to complete my doctoral studies:

My main supervisor, *Ingrid Lundberg*, thank you for being such an inspiration as a great woman and outstanding scientist. Your enthusiasm, positive energy, intelligence, humbleness, kindness, your being always open for questions and discussions is the key of a successful working group. Thank you for giving me the opportunity to be part of your research team and parallelly work as rheumatologist in the clinic.

My co-supervisor, *Catia Fernandes-Cerqueira*, thank you for supporting and guiding me during the past five years. You are a model for me as a scientist. I really admire your devotion to science. Our scientific brainstorming has been extremely valuable for my growth in this field. Not least, thank you for being such a good friend to me. Looking forward for the next weekend together.

Per-Johan Jakobsson, my co-supervisor thanks for all the inspiring discussions which have increased my passion for translational medical research.

Thank you, *Lars Klareskog*, it is an honor for me to work so close to a scientist of your league. Thank you for creating an environment where people with different cultural and professional background can easily be in contact and collaborate. You are a very knowledgeable and open-minded person.

Anca Catrina, thank you for accepting to be the chair of my PhD thesis defence. I really admire your brilliant scientific work.

My former and current heads of the Rheumatology Unit, *Cecilia Carlens* and *Jon Lampa*, thank you for giving me the possibility to work as rheumatologist and combine the clinical and research work.

My former and current heads in the clinic, *Louise Ekholm* and *Hamed Rezaei*, thank you for building up a respectful and friendly professional relationship.

Iva Gunnarsson, my mentor, thank you for the precious advices as experienced rheumatologist and researcher.

My fantastic myositis research group: *Maryam, Angeles, Valerie, Eveline, Begum, Charlotta, Lara, Karina, Louise, Anna, Anneli, Eva, Lena, Marie, Malin, Mei, Fabricio, Helene A., Kristofer, Helene S., Tang, Ian, Paulius, Karin, Deniz.* It is a pleasure to work with you guys. Thank you for all your help, for the great discussions. Thank you for all the funny moments we have spent together inside and outside the clinic and the lab. Looking forward for more to come.

Thank you to all the co-workers at CMM, Science for life laboratories and Uppsala University. Thank you to all the international and national co-authors of my manuscripts with a special mention to *Charlotta P., Simone B., Marie H., Aleksandra A.*. Thank you to all the patients and healthy individuals who have accepted to be part of my research projects.

A special thanks to my dear *Maryam Dastmalchi.* You are a wonderful person and doctor, *Maryam*, and I am so glad I have met you in my life. You take such good care of me! Your smile, the laughs together, your precious advices are a lighthouse for me. Thank you for welcoming me and Claudio like your family.

My dear friend, *Angeles*, thank you for entering my life with your special light. I am so proud of our friendship, I know we can count on each other, it means a lot to me. Thank you, *Valerie*, for all the

great moments we have spent together, for your contagious laugh, for being a good listener. I can't wait to be again all together, love you girls!

I would like to thank all my colleagues at the Rheumatology clinic in Solna and Huddinge for always being nice, friendly, respectful and willing to share your knowledge. I have learnt a lot from all of you and I believe we all make an excellent rheuma-team. Thank you for all the lunches and after works that help to lower the pressure and clear the mind. A special thanks to my colleagues and friends, Karina G., Marie F., Aleksandra A., Nancy V., Karin G., Eleni T. who are daily by my side with a good word, encouragement, a smile, a warm coffee, a toast together. You have made me really happy with the birthday surprise party, thank you!

My dear Katerina, every single morning when I enter our office you welcome me screaming "Antonellaaa", then you smile or hug me and always ask how I feel or if I need something. Having you sitting beside me makes me happy. Thank you for your sincere friendship, for all the talks and laughs, you are very special and so talented! And thank you for the beautiful cover of my thesis.

Thank you to my lovely Greek friends, *Maria S. and Ioannis K.. Maria,* we have felt from the beginning we would become best friends. You are a wonderful person and I know we will be always there for each other. *Ioannis*, amico mio, thank you for being always so nice, kind, funny. Can't forget the amazing weekend in Rome with the Suzie team! Always excited for the next time together.

Thank you to my Italian family in Stockholm, *Lara and Francesca.* I cannot imagine to be here without you girls. Thank you for taking such good care of me, for checking daily up on me, for making me feel home. *Lara*, thank you for always showing the good side of the coin, for your contagious calmness and joy of living. *Francesca*, thank you for being so extremely emphatic and sensitive, it is a very rare quality. The messages you send when you need to vent are often hilarious thanks to your vivid dark irony. And thank you for sharing your impressive knowledge in so many fields. I love you so much girls!

Thank you to my Italian friends, who are brothers and sisters to me. You know who you are and I miss you all so much. Thank you for always cheering me up. All the beautiful moments of my life would not be the same without sharing with all of you.

I am blessed to have such a beautiful family. Papà Cenzo, mamma Angela, Gigi, Elsa, Maria, Pino, Angela, Marta, Fabrizio, Agnese, Costanza, my grandparents, thank you for loving me unconditionally. I wouldn't be the person I am today without you. You make me happy and strong. With all of you by my side everything is possible.

Last, I want to say thanks to *Claudio*, my husband, my everything, my life. You are my hope, my strength, my happiness. You remind me every day that life is beautiful and worth living every single minute. Everything would be meaningless without you. Ti amo!

REFERENCES

1. Plotz PH. Autoimmunity: the history of an idea. Arthritis Rheumatol. 2014;66(11):2915-20.

2. Silverstein AM. Autoimmunity versus horror autotoxicus: the struggle for recognition. Nat Immunol. 2001;2(4):279-81.

3. Theofilopoulos AN, Kono DH, Baccala R. The multiple pathways to autoimmunity. Nat Immunol. 2017;18(7):716-24.

4. Rose NR. Autoimmune disease 2002: an overview. J Investig Dermatol Symp Proc. 2004;9(1):1-4.

5. Hayter SM, Cook MC. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. Autoimmun Rev. 2012;11(10):754-65.

6. Dighiero G, Rose NR. Critical self-epitopes are key to the understanding of self-tolerance and autoimmunity. Immunol Today. 1999;20(9):423-8.

7. Romagnani S. Immunological tolerance and autoimmunity. Intern Emerg Med. 2006;1(3):187-96.

8. Schwartz RH. Historical overview of immunological tolerance. Cold Spring Harb Perspect Biol. 2012;4(4):a006908.

9. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med. 2001;345(5):340-50.

10. Coutinho A, Hori S, Carvalho T, Caramalho I, Demengeot J. Regulatory T cells: the physiology of autoreactivity in dominant tolerance and "quality control" of immune responses. Immunol Rev. 2001;182:89-98.

11. Damoiseaux JG, Tervaert JW. The definition of autoimmune disease: are Koch's postulates applicable? Neth J Med. 2002;60(7):266-8.

12. Ulmanen I, Halonen M, Ilmarinen T, Peltonen L. Monogenic autoimmune diseases - lessons of self-tolerance. Curr Opin Immunol. 2005;17(6):609-15.

13. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. J Intern Med. 2015;278(4):369-95.

14. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27(1):20-1.

15. Wahren-Herlenius M, Dorner T. Immunopathogenic mechanisms of systemic autoimmune disease. Lancet. 2013;382(9894):819-31.

16. Morran MP, Vonberg A, Khadra A, Pietropaolo M. Immunogenetics of type 1 diabetes mellitus. Mol Aspects Med. 2015;42:42-60.

17. Chinoy H, Payne D, Poulton KV, Fertig N, Betteridge Z, Gunawardena H, et al. HLA-DPB1 associations differ between DRB1*03 positive anti-Jo-1 and anti-PM-Scl antibody positive idiopathic inflammatory myopathy. Rheumatology (Oxford). 2009;48(10):1213-7.

18. Taylor KE, Chung SA, Graham RR, Ortmann WA, Lee AT, Langefeld CD, et al. Risk alleles for systemic lupus erythematosus in a large case-control collection and associations with clinical subphenotypes. PLoS Genet. 2011;7(2):e1001311.

19. Diogo D, Okada Y, Plenge RM. Genome-wide association studies to advance our understanding of critical cell types and pathways in rheumatoid arthritis: recent findings and challenges. Curr Opin Rheumatol. 2014;26(1):85-92.

20. Srikanta S, Eisenbarth GS. Islet cell antigens. Initial studies of their biology and function. Mol Biol Med. 1986;3(2):113-27.

21. Roitt IM, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). Lancet. 1956;271(6947):820-1.

22. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford). 2007;46(7):1052-6.

23. Koffler D, Schur PH, Kunkel HG. Immunological studies concerning the nephritis of systemic lupus erythematosus. J Exp Med. 1967;126(4):607-24.

24. Maddison PJ, Reichlin M. Quantitation of precipitating antibodies to certain soluble nuclear antigens in SLE. Arthritis Rheum. 1977;20(3):819-24.

25. Zieve GW, Khusial PR. The anti-Sm immune response in autoimmunity and cell biology. Autoimmun Rev. 2003;2(5):235-40.

26. Greidinger EL, Flaherty KT, White B, Rosen A, Wigley FM, Wise RA. African-American race and antibodies to topoisomerase I are associated with increased severity of scleroderma lung disease. Chest. 1998;114(3):801-7.

27. Rosen A, Casciola-Rosen L. Autoantigens as Partners in Initiation and Propagation of Autoimmune Rheumatic Diseases. Annu Rev Immunol. 2016;34:395-420.

28. van Venrooij WJ, van Beers JJ, Pruijn GJ. Anti-CCP antibodies: the past, the present and the future. Nat Rev Rheumatol. 2011;7(7):391-8.

29. Targoff IN, Arnett FC. Clinical manifestations in patients with antibody to PL-12 antigen (alanyl-tRNA synthetase). Am J Med. 1990;88(3):241-51.

30. Nell VP, Machold KP, Stamm TA, Eberl G, Heinzl H, Uffmann M, et al. Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. Ann Rheum Dis. 2005;64(12):1731-6.

31. Barber KW, Rinehart J. The ABCs of PTMs. Nat Chem Biol. 2018;14(3):188-92.

32. Duan-Porter WD, Casciola-Rosen L, Rosen A. Autoantigens: the critical partner in initiating and propagating systemic autoimmunity. Ann N Y Acad Sci. 2005;1062:127-36.

33. Anderton SM. Post-translational modifications of self antigens: implications for autoimmunity. Curr Opin Immunol. 2004;16(6):753-8.

34. Carubbi F, Alunno A, Gerli R, Giacomelli R. Post-Translational Modifications of Proteins: Novel Insights in the Autoimmune Response in Rheumatoid Arthritis. Cells. 2019;8(7).

35. Casciola-Rosen L, Andrade F, Ulanet D, Wong WB, Rosen A. Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. J Exp Med. 1999;190(6):815-26.

36. Darrah E, Rosen A. Granzyme B cleavage of autoantigens in autoimmunity. Cell Death Differ. 2010;17(4):624-32.

37. Levine SM, Raben N, Xie D, Askin FB, Tuder R, Mullins M, et al. Novel conformation of histidyl-transfer RNA synthetase in the lung: the target tissue in Jo-1 autoantibody-associated myositis. Arthritis Rheum. 2007;56(8):2729-39.

38. Ulanet DB, Wigley FM, Gelber AC, Rosen A. Autoantibodies against B23, a nucleolar phosphoprotein, occur in scleroderma and are associated with pulmonary hypertension. Arthritis Rheum. 2003;49(1):85-92.

39. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med. 1994;179(4):1317-30.

40. Yang F, He Y, Zhai Z, Sun E. Programmed Cell Death Pathways in the Pathogenesis of Systemic Lupus Erythematosus. J Immunol Res. 2019;2019:3638562.

41. Kanduc D. The self/nonself issue: A confrontation between proteomes. Self Nonself. 2010;1(3):255-8.

42. Oldstone MB. Molecular mimicry and immune-mediated diseases. FASEB J. 1998;12(13):1255-65.

43. Ma WT, Chang C, Gershwin ME, Lian ZX. Development of autoantibodies precedes clinical manifestations of autoimmune diseases: A comprehensive review. J Autoimmun. 2017;83:95-112.

44. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S238-47.

45. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019;71(9):1400-12.

46. Choi MY, FitzPatrick RD, Buhler K, Mahler M, Fritzler MJ. A review and meta-analysis of anti-ribosomal P autoantibodies in systemic lupus erythematosus. Autoimmun Rev. 2020;19(3):102463.

47. Buyon JP, Clancy RM, Friedman DM. Autoimmune associated congenital heart block: integration of clinical and research clues in the management of the maternal / foetal dyad at risk. J Intern Med. 2009;265(6):653-62.

48. Clark CA, Laskin CA, Spitzer KA. Anticardiolipin antibodies and recurrent early pregnancy loss: a century of equivocal evidence. Hum Reprod Update. 2012;18(5):474-84.

49. Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. J Clin Invest. 2001;108(10):1417-22.

50. Sanders JS, Huitma MG, Kallenberg CG, Stegeman CA. Prediction of relapses in PR3-ANCA-associated vasculitis by assessing responses of ANCA titres to treatment. Rheumatology (Oxford). 2006;45(6):724-9.

51. Aletaha D, Bluml S. Therapeutic implications of autoantibodies in rheumatoid arthritis. RMD Open. 2016;2(1):e000009.

52. Shiozawa K, Kawasaki Y, Yamane T, Yoshihara R, Tanaka Y, Uto K, et al. Anticitrullinated protein antibody, but not its titer, is a predictor of radiographic progression and disease activity in rheumatoid arthritis. J Rheumatol. 2012;39(4):694-700.

53. Takahashi R, Isojima S, Umemura M, Miura Y, Oguro N, Ishii S, et al. Serum anticyclic citrullinated protein antibody titers are correlated with the response to biological agents in patients with rheumatoid arthritis. Open Access Rheumatol. 2014;6:57-64.

54. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.

55. Rahman A, Giles IP. Structure and function of autoantibodies and their role in autoimmune rheumatic diseases. Expert Rev Clin Immunol. 2006;2(2):225-36.

56. Weinstein E, Peeva E, Putterman C, Diamond B. B-cell biology. Rheum Dis Clin North Am. 2004;30(1):159-74.

57. Lipman NS, Jackson LR, Trudel LJ, Weis-Garcia F. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. ILAR J. 2005;46(3):258-68.

58. Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. Blood Transfus. 2007;5(4):227-40.

59. Fereidan-Esfahani M, Nayfeh T, Warrington A, Howe CL, Rodriguez M. IgM Natural Autoantibodies in Physiology and the Treatment of Disease. Methods Mol Biol. 2019;1904:53-81.

60. Jennewein MF, Alter G. The Immunoregulatory Roles of Antibody Glycosylation. Trends Immunol. 2017;38(5):358-72.

61. Dekkers G, Rispens T, Vidarsson G. Novel Concepts of Altered Immunoglobulin G Galactosylation in Autoimmune Diseases. Front Immunol. 2018;9:553.

62. McLachlan SM, Rapoport B. Breaking tolerance to thyroid antigens: changing concepts in thyroid autoimmunity. Endocr Rev. 2014;35(1):59-105.

63. Fujii T. Direct and indirect pathogenic roles of autoantibodies in systemic autoimmune diseases. Allergol Int. 2014;63(4):515-22.

64. Suurmond J, Diamond B. Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity. J Clin Invest. 2015;125(6):2194-202.

65. Naparstek Y, Plotz PH. The role of autoantibodies in autoimmune disease. Annu Rev Immunol. 1993;11:79-104.

66. Elkon K, Casali P. Nature and functions of autoantibodies. Nat Clin Pract Rheumatol. 2008;4(9):491-8.

67. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349(16):1526-33.

68. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003;48(10):2741-9.

69. Karlson EW, Chibnik LB, Tworoger SS, Lee IM, Buring JE, Shadick NA, et al. Biomarkers of inflammation and development of rheumatoid arthritis in women from two prospective cohort studies. Arthritis Rheum. 2009;60(3):641-52.

70. Gensous N, Charrier M, Duluc D, Contin-Bordes C, Truchetet ME, Lazaro E, et al. T Follicular Helper Cells in Autoimmune Disorders. Front Immunol. 2018;9:1637.

71. Deng Q, Luo Y, Chang C, Wu H, Ding Y, Xiao R. The Emerging Epigenetic Role of CD8+T Cells in Autoimmune Diseases: A Systematic Review. Front Immunol. 2019;10:856.

72. Liblau RS, Wong FS, Mars LT, Santamaria P. Autoreactive CD8 T cells in organ-specific autoimmunity: emerging targets for therapeutic intervention. Immunity. 2002;17(1):1-6.

73. Hill NJ, Hultcrantz M, Sarvetnick N, Flodstrom-Tullberg M. The target tissue in autoimmunity--an influential niche. Eur J Immunol. 2007;37(3):589-97.

74. Dalakas MC. Inflammatory muscle diseases. N Engl J Med. 2015;372(18):1734-47.

75. Schmidt J. Current Classification and Management of Inflammatory Myopathies. J Neuromuscul Dis. 2018;5(2):109-29.

76. Meyer A, Meyer N, Schaeffer M, Gottenberg JE, Geny B, Sibilia J. Incidence and prevalence of inflammatory myopathies: a systematic review. Rheumatology (Oxford). 2015;54(1):50-63.

77. Svensson J, Arkema EV, Lundberg IE, Holmqvist M. Incidence and prevalence of idiopathic inflammatory myopathies in Sweden: a nationwide population-based study. Rheumatology (Oxford). 2017;56(5):802-10.

78. Senecal JL, Raynauld JP, Troyanov Y. Editorial: A New Classification of Adult Autoimmune Myositis. Arthritis Rheumatol. 2017;69(5):878-84.

79. Lilleker JB, Vencovsky J, Wang G, Wedderburn LR, Diederichsen LP, Schmidt J, et al. The EuroMyositis registry: an international collaborative tool to facilitate myositis research. Ann Rheum Dis. 2018;77(1):30-9.

80. Hengstman GJ, van Venrooij WJ, Vencovsky J, Moutsopoulos HM, van Engelen BG. The relative prevalence of dermatomyositis and polymyositis in Europe exhibits a latitudinal gradient. Ann Rheum Dis. 2000;59(2):141-2.

81. Okada S, Weatherhead E, Targoff IN, Wesley R, Miller FW, International Myositis Collaborative Study G. Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. Arthritis Rheum. 2003;48(8):2285-93.

82. Needham M, Corbett A, Day T, Christiansen F, Fabian V, Mastaglia FL. Prevalence of sporadic inclusion body myositis and factors contributing to delayed diagnosis. J Clin Neurosci. 2008;15(12):1350-3.

83. Dobloug GC, Antal EA, Sveberg L, Garen T, Bitter H, Stjarne J, et al. High prevalence of inclusion body myositis in Norway; a population-based clinical epidemiology study. Eur J Neurol. 2015;22(4):672-e41.

84. Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med. 1975;292(8):403-7.

85. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med. 1975;292(7):344-7.

86. Leclair V, Lundberg IE. New Myositis Classification Criteria-What We Have Learned Since Bohan and Peter. Curr Rheumatol Rep. 2018;20(4):18.

87. Griggs RC, Askanas V, DiMauro S, Engel A, Karpati G, Mendell JR, et al. Inclusion body myositis and myopathies. Ann Neurol. 1995;38(5):705-13.

88. Lloyd TE, Mammen AL, Amato AA, Weiss MD, Needham M, Greenberg SA. Evaluation and construction of diagnostic criteria for inclusion body myositis. Neurology. 2014;83(5):426-33.

89. Bottai M, Tjarnlund A, Santoni G, Werth VP, Pilkington C, de Visser M, et al. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: a methodology report. RMD Open. 2017;3(2):e000507.

90. Mammen AL, Casciola-Rosen L, Christopher-Stine L, Lloyd TE, Wagner KR. Myositis-specific autoantibodies are specific for myositis compared to genetic muscle disease. Neurol Neuroimmunol Neuroinflamm. 2015;2(6):e172.

91. Lundberg IE. Myositis in 2016: New tools for diagnosis and therapy. Nat Rev Rheumatol. 2017;13(2):74-6.

92. Espinosa-Ortega F, Holmqvist M, Alexanderson H, Storfors H, Mimori T, Lundberg IE, et al. Comparison of autoantibody specificities tested by a line blot assay and immunoprecipitation-based algorithm in patients with idiopathic inflammatory myopathies. Ann Rheum Dis. 2019;78(6):858-60.

93. Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med. 2016;280(1):8-23.

94. Tansley SL, Simou S, Shaddick G, Betteridge ZE, Almeida B, Gunawardena H, et al. Autoantibodies in juvenile-onset myositis: Their diagnostic value and associated clinical phenotype in a large UK cohort. J Autoimmun. 2017;84:55-64.

95. Brouwer R, Hengstman GJ, Vree Egberts W, Ehrfeld H, Bozic B, Ghirardello A, et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis. 2001;60(2):116-23.

96. Ghirardello A, Doria A. New insights in myositis-specific autoantibodies. Curr Opin Rheumatol. 2018;30(6):614-22.

97. McHugh NJ, Tansley SL. Autoantibodies in myositis. Nat Rev Rheumatol. 2018;14(5):290-302.

98. Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, Albayda J, Paik JJ, Johnson C, et al. A longitudinal cohort study of the anti-synthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology (Oxford). 2017;56(6):999-1007.

99. Pinal-Fernandez I, Parks C, Werner JL, Albayda J, Paik J, Danoff SK, et al. Longitudinal Course of Disease in a Large Cohort of Myositis Patients With Autoantibodies Recognizing the Signal Recognition Particle. Arthritis Care Res (Hoboken). 2017;69(2):263-70.

100. Tiniakou E, Pinal-Fernandez I, Lloyd TE, Albayda J, Paik J, Werner JL, et al. More severe disease and slower recovery in younger patients with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Rheumatology (Oxford). 2017;56(5):787-94.

101. Selva-O'Callaghan A, Labrador-Horrillo M, Solans-Laque R, Simeon-Aznar CP, Martinez-Gomez X, Vilardell-Tarres M. Myositis-specific and myositis-associated antibodies in a series of eighty-eight Mediterranean patients with idiopathic inflammatory myopathy. Arthritis Rheum. 2006;55(5):791-8.

102. Selva-O'Callaghan A, Pinal-Fernandez I, Trallero-Araguas E, Milisenda JC, Grau-Junyent JM, Mammen AL. Classification and management of adult inflammatory myopathies. Lancet Neurol. 2018;17(9):816-28.

103. Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EK. A Comprehensive Overview on Myositis-Specific Antibodies: New and Old Biomarkers in Idiopathic Inflammatory Myopathy. Clin Rev Allergy Immunol. 2017;52(1):1-19.

104. Kurtzman DJB, Vleugels RA. Anti-melanoma differentiation-associated gene 5 (MDA5) dermatomyositis: A concise review with an emphasis on distinctive clinical features. J Am Acad Dermatol. 2018;78(4):776-85.

105. Komura K, Fujimoto M, Matsushita T, Kaji K, Kondo M, Hirano T, et al. Prevalence and clinical characteristics of anti-Mi-2 antibodies in Japanese patients with dermatomyositis. J Dermatol Sci. 2005;40(3):215-7.

106. Lundberg IE, de Visser M, Werth VP. Classification of myositis. Nat Rev Rheumatol. 2018;14(5):269-78.

107. Dimachkie MM, Barohn RJ. Inclusion body myositis. Curr Neurol Neurosci Rep. 2013;13(1):321.

108. Naddaf E, Barohn RJ, Dimachkie MM. Inclusion Body Myositis: Update on Pathogenesis and Treatment. Neurotherapeutics. 2018.

109. Aggarwal R, Moghadam-Kia S, Lacomis D, Malik A, Qi Z, Koontz D, et al. Anti-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) antibody in necrotizing myopathy: treatment outcomes, cancer risk, and role of autoantibody level. Scand J Rheumatol. 2019:1-7.

110. Mammen AL, Chung T, Christopher-Stine L, Rosen P, Rosen A, Doering KR, et al. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. Arthritis Rheum. 2011;63(3):713-21.

111. Takeguchi-Kikuchi S, Hayasaka T, Katayama T, Kano K, Takahashi K, Saito T, et al. Anti-signal Recognition Particle Antibody-positive Necrotizing Myopathy with Secondary Cardiomyopathy: The First Myocardial Biopsy- and Multimodal Imaging-proven Case. Intern Med. 2019;58(21):3189-94.

112. Jablonska S, Blaszyk M. Scleromyositis (scleroderma/polimyositis overlap) is an entity. J Eur Acad Dermatol Venereol. 2004;18(3):265-6.

113. Fredi M, Cavazzana I, Franceschini F. The clinico-serological spectrum of overlap myositis. Curr Opin Rheumatol. 2018;30(6):637-43.

114. Nuno-Nuno L, Joven BE, Carreira PE, Maldonado-Romero V, Larena-Grijalba C, Llorente Cubas I, et al. Overlap myositis, a distinct entity beyond primary inflammatory myositis: A retrospective analysis of a large cohort from the REMICAM registry. Int J Rheum Dis. 2019;22(8):1393-401.

115. Colafrancesco S, Priori R, Valesini G. Inflammatory myopathies and overlap syndromes: Update on histological and serological profile. Best Pract Res Clin Rheumatol. 2015;29(6):810-25.

116. Tiniakou E, Mammen AL. Idiopathic Inflammatory Myopathies and Malignancy: a Comprehensive Review. Clin Rev Allergy Immunol. 2017;52(1):20-33.

117. Yang Z, Lin F, Qin B, Liang Y, Zhong R. Polymyositis/dermatomyositis and malignancy risk: a metaanalysis study. J Rheumatol. 2015;42(2):282-91.

118. Hill CL, Zhang Y, Sigurgeirsson B, Pukkala E, Mellemkjaer L, Airio A, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet. 2001;357(9250):96-100.

119. Cherin P, Piette JC, Herson S, Bletry O, Wechsler B, Frances C, et al. Dermatomyositis and ovarian cancer: a report of 7 cases and literature review. J Rheumatol. 1993;20(11):1897-9.

120. Masuda H, Urushibara M, Kihara K. Successful treatment of dermatomyositis associated with adenocarcinoma of the prostate after radical prostatectomy. J Urol. 2003;169(3):1084.

121. Tallai B, Flasko T, Gyorgy T, Ponyi A, Andras C, Toth C, et al. Prostate cancer underlying acute, definitive dermatomyositis: successful treatment with radical perineal prostatectomy. Clin Rheumatol. 2006;25(1):119-20.

122. Labrador-Horrillo M, Martinez MA, Selva-O'Callaghan A, Trallero-Araguas E, Balada E, Vilardell-Tarres M, et al. Anti-TIF1gamma antibodies (anti-p155) in adult patients with dermatomyositis: comparison of different diagnostic assays. Ann Rheum Dis. 2012;71(6):993-6.

123. Trallero-Araguas E, Rodrigo-Pendas JA, Selva-O'Callaghan A, Martinez-Gomez X, Bosch X, Labrador-Horrillo M, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum. 2012;64(2):523-32.

124. Dani L, Holmqvist M, Martinez MA, Trallero-Araguas E, Dastmalchi M, Svensson J, et al. Anti-transcriptional intermediary factor 1 gamma antibodies in cancer-associated myositis: a longitudinal study. Clin Exp Rheumatol. 2020;38(1):67-73.

125. Bodoki L, Nagy-Vincze M, Griger Z, Betteridge Z, Szollosi L, Danko K. Four dermatomyositis-specific autoantibodies-anti-TIF1gamma, anti-NXP2, anti-SAE and anti-MDA5-in adult and juvenile patients with idiopathic inflammatory myopathies in a Hungarian cohort. Autoimmun Rev. 2014;13(12):1211-9.

126. Ceribelli A, Fredi M, Taraborelli M, Cavazzana I, Franceschini F, Quinzanini M, et al. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. Arthritis Res Ther. 2012;14(2):R97.

127. Fiorentino DF, Chung LS, Christopher-Stine L, Zaba L, Li S, Mammen AL, et al. Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1gamma. Arthritis Rheum. 2013;65(11):2954-62.

128. Ichimura Y, Matsushita T, Hamaguchi Y, Kaji K, Hasegawa M, Tanino Y, et al. Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. Ann Rheum Dis. 2012;71(5):710-3.

129. Gunawardena H, Wedderburn LR, Chinoy H, Betteridge ZE, North J, Ollier WE, et al. Autoantibodies to a 140-kd protein in juvenile dermatomyositis are associated with calcinosis. Arthritis Rheum. 2009;60(6):1807-14.

130. Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore). 1991;70(6):360-74.

131. Marguerie C, Bunn CC, Beynon HL, Bernstein RM, Hughes JM, So AK, et al. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. Q J Med. 1990;77(282):1019-38.

132. Giannini M, Notarnicola A, Dastmalchi M, Lundberg IE, Lopalco G, Iannone F. Heterogeneous clinical spectrum of interstitial lung disease in patients with anti-EJ anti-synthetase syndrome: a case series. Clin Rheumatol. 2016;35(9):2363-7.

133. Hervier B, Benveniste O. [Clinical phenotypes and prognosis of antisynthetase syndrome]. Rev Med Interne. 2014;35(7):453-60.

134. Mahler M, Miller FW, Fritzler MJ. Idiopathic inflammatory myopathies and the anti-synthetase syndrome: a comprehensive review. Autoimmun Rev. 2014;13(4-5):367-71.

135. Monti S, Montecucco C, Cavagna L. Clinical spectrum of anti-Jo-1-associated disease. Curr Opin Rheumatol. 2017;29(6):612-7.

136. Rider LG, Werth VP, Huber AM, Alexanderson H, Rao AP, Ruperto N, et al. Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: Physician and Patient/Parent Global Activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire (HAQ)/Childhood Health Assessment Questionnaire (C-HAQ), Childhood Myositis Assessment Scale (CMAS), Myositis Disease Activity Assessment Tool (MDAAT), Disease Activity Score (DAS), Short Form 36 (SF-36), Child Health Questionnaire (CHQ), physician global damage, Myositis Damage Index (MDI), Quantitative Muscle Testing (QMT), Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis Care Res (Hoboken). 2011;63 Suppl 11:S118-57.

137. Rider LG, Giannini EH, Brunner HI, Ruperto N, James-Newton L, Reed AM, et al. International consensus on preliminary definitions of improvement in adult and juvenile myositis. Arthritis Rheum. 2004;50(7):2281-90.

138. Rider LG, Aggarwal R, Pistorio A, Bayat N, Erman B, Feldman BM, et al. 2016 American College of Rheumatology/European League Against Rheumatism Criteria for Minimal, Moderate, and Major Clinical Response in Juvenile Dermatomyositis: An International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Ann Rheum Dis. 2017;76(5):782-91.

139. Rider LG, Lachenbruch PA, Monroe JB, Ravelli A, Cabalar I, Feldman BM, et al. Damage extent and predictors in adult and juvenile dermatomyositis and polymyositis as determined with the myositis damage index. Arthritis Rheum. 2009;60(11):3425-35.

140. Barsotti S, Lundberg IE. Current Treatment for Myositis. Curr Treatm Opt Rheumatol. 2018;4(4):299-315.

141. Oddis CV, Aggarwal R. Treatment in myositis. Nat Rev Rheumatol. 2018;14(5):279-89.

142. Moghadam-Kia S, Charlton D, Aggarwal R, Oddis CV. Management of refractory cutaneous dermatomyositis: potential role of Janus kinase inhibition with tofacitinib. Rheumatology (Oxford). 2019;58(6):1011-5.

143. Alexanderson H. Physical exercise as a treatment for adult and juvenile myositis. J Intern Med. 2016;280(1):75-96.

144. Munters LA, Loell I, Ossipova E, Raouf J, Dastmalchi M, Lindroos E, et al. Endurance Exercise Improves Molecular Pathways of Aerobic Metabolism in Patients With Myositis. Arthritis Rheumatol. 2016;68(7):1738-50.

145. Marie I. Morbidity and mortality in adult polymyositis and dermatomyositis. Curr Rheumatol Rep. 2012;14(3):275-85.

146. Bronner IM, van der Meulen MF, de Visser M, Kalmijn S, van Venrooij WJ, Voskuyl AE, et al. Long-term outcome in polymyositis and dermatomyositis. Ann Rheum Dis. 2006;65(11):1456-61.

147. Marie I, Hachulla E, Hatron PY, Hellot MF, Levesque H, Devulder B, et al. Polymyositis and dermatomyositis: short term and longterm outcome, and predictive factors of prognosis. J Rheumatol. 2001;28(10):2230-7.

148. Sultan SM, Ioannou Y, Moss K, Isenberg DA. Outcome in patients with idiopathic inflammatory myositis: morbidity and mortality. Rheumatology (Oxford). 2002;41(1):22-6.

149. Clarke AE, Bloch DA, Medsger TA, Jr., Oddis CV. A longitudinal study of functional disability in a national cohort of patients with polymyositis/dermatomyositis. Arthritis Rheum. 1995;38(9):1218-24.

150. Ponyi A, Borgulya G, Constantin T, Vancsa A, Gergely L, Danko K. Functional outcome and quality of life in adult patients with idiopathic inflammatory myositis. Rheumatology (Oxford). 2005;44(1):83-8.

151. Lundberg IE, Forbess CJ. Mortality in idiopathic inflammatory myopathies. Clin Exp Rheumatol. 2008;26(5 Suppl 51):S109-14.

152. Ceribelli A, De Santis M, Isailovic N, Gershwin ME, Selmi C. The Immune Response and the Pathogenesis of Idiopathic Inflammatory Myositis: a Critical Review. Clin Rev Allergy Immunol. 2017;52(1):58-70.

153. Miller FW, Lamb JA, Schmidt J, Nagaraju K. Risk factors and disease mechanisms in myositis. Nat Rev Rheumatol. 2018;14(5):255-68.

154. Miller FW, Chen W, O'Hanlon TP, Cooper RG, Vencovsky J, Rider LG, et al. Genome-wide association study identifies HLA 8.1 ancestral haplotype alleles as major genetic risk factors for myositis phenotypes. Genes Immun. 2015;16(7):470-80.

155. Miller FW, Cooper RG, Vencovsky J, Rider LG, Danko K, Wedderburn LR, et al. Genome-wide association study of dermatomyositis reveals genetic overlap with other autoimmune disorders. Arthritis Rheum. 2013;65(12):3239-47.

156. Jia X, Han B, Onengut-Gumuscu S, Chen WM, Concannon PJ, Rich SS, et al. Imputing amino acid polymorphisms in human leukocyte antigens. PLoS One. 2013;8(6):e64683.

157. Rothwell S, Cooper RG, Lundberg IE, Gregersen PK, Hanna MG, Machado PM, et al. Immune-Array Analysis in Sporadic Inclusion Body Myositis Reveals HLA-DRB1 Amino Acid Heterogeneity Across the Myositis Spectrum. Arthritis Rheumatol. 2017;69(5):1090-9.

158. Rothwell S, Cooper RG, Lundberg IE, Miller FW, Gregersen PK, Bowes J, et al. Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups. Ann Rheum Dis. 2016;75(8):1558-66.

159. Rothwell S, Lamb JA, Chinoy H. New developments in genetics of myositis. Curr Opin Rheumatol. 2016;28(6):651-6.

160. Svensson J, Holmqvist M, Lundberg IE, Arkema EV. Infections and respiratory tract disease as risk factors for idiopathic inflammatory myopathies: a population-based case-control study. Ann Rheum Dis. 2017;76(11):1803-8.

161. Chinoy H, Adimulam S, Marriage F, New P, Vincze M, Zilahi E, et al. Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. Ann Rheum Dis. 2012;71(6):961-5.

162. Love LA, Weinberg CR, McConnaughey DR, Oddis CV, Medsger TA, Jr., Reveille JD, et al. Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women. Arthritis Rheum. 2009;60(8):2499-504.

163. Shah M, Targoff IN, Rice MM, Miller FW, Rider LG, Childhood Myositis Heterogeneity Collaborative Study G. Brief report: ultraviolet radiation exposure is associated with clinical and autoantibody phenotypes in juvenile myositis. Arthritis Rheum. 2013;65(7):1934-41.

164. Goebels N, Michaelis D, Engelhardt M, Huber S, Bender A, Pongratz D, et al. Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. J Clin Invest. 1996;97(12):2905-10.

165. Orimo S, Koga R, Goto K, Nakamura K, Arai M, Tamaki M, et al. Immunohistochemical analysis of perforin and granzyme A in inflammatory myopathies. Neuromuscul Disord. 1994;4(3):219-26.

166. Reed AM, Ernste F. The inflammatory milieu in idiopathic inflammatory myositis. Curr Rheumatol Rep. 2009;11(4):295-301.

167. Fasth AE, Dastmalchi M, Rahbar A, Salomonsson S, Pandya JM, Lindroos E, et al. T cell infiltrates in the muscles of patients with dermatomyositis and polymyositis are dominated by CD28null T cells. J Immunol. 2009;183(7):4792-9.

168. Pandya JM, Fasth AE, Zong M, Arnardottir S, Dani L, Lindroos E, et al. Expanded T cell receptor Vbeta-restricted T cells from patients with sporadic inclusion body myositis are proinflammatory and cytotoxic CD28null T cells. Arthritis Rheum. 2010;62(11):3457-66.

169. Pandya JM, Venalis P, Al-Khalili L, Shahadat Hossain M, Stache V, Lundberg IE, et al. CD4+ and CD8+ CD28(null) T Cells Are Cytotoxic to Autologous Muscle Cells in Patients With Polymyositis. Arthritis Rheumatol. 2016;68(8):2016-26.

170. Pandya JM, Loell I, Hossain MS, Zong M, Alexanderson H, Raghavan S, et al. Effects of conventional immunosuppressive treatment on CD244+ (CD28null) and FOXP3+ T cells in the inflamed muscle of patients with polymyositis and dermatomyositis. Arthritis Res Ther. 2016;18:80.

171. Vercoulen Y, Bellutti Enders F, Meerding J, Plantinga M, Elst EF, Varsani H, et al. Increased presence of FOXP3+ regulatory T cells in inflamed muscle of patients with active juvenile dermatomyositis compared to peripheral blood. PLoS One. 2014;9(8):e105353.

172. Waschbisch A, Schwab N, Ruck T, Stenner MP, Wiendl H. FOXP3+ T regulatory cells in idiopathic inflammatory myopathies. J Neuroimmunol. 2010;225(1-2):137-42.

173. Gunawardena H, Betteridge ZE, McHugh NJ. Myositis-specific autoantibodies: their clinical and pathogenic significance in disease expression. Rheumatology (Oxford). 2009;48(6):607-12.

174. Ladislau L, Arouche-Delaperche L, Allenbach Y, Benveniste O. Potential Pathogenic Role of Anti-Signal Recognition Protein and Anti-3-hydroxy-3-methylglutaryl-CoA Reductase Antibodies in Immune-Mediated Necrotizing Myopathies. Curr Rheumatol Rep. 2018;20(9):56.

175. Tawara N, Yamashita S, Zhang X, Korogi M, Zhang Z, Doki T, et al. Pathomechanisms of anti-cytosolic 5'-nucleotidase 1A autoantibodies in sporadic inclusion body myositis. Ann Neurol. 2017;81(4):512-25.

176. Ekholm L, Vosslamber S, Tjarnlund A, de Jong TD, Betteridge Z, McHugh N, et al. Autoantibody Specificities and Type I Interferon Pathway Activation in Idiopathic Inflammatory Myopathies. Scand J Immunol. 2016;84(2):100-9.

177. Greenberg SA. Type 1 interferons and myositis. Arthritis Res Ther. 2010;12 Suppl 1:S4.

178. Tournadre A, Lenief V, Eljaafari A, Miossec P. Immature muscle precursors are a source of interferon-beta in myositis: role of Toll-like receptor 3 activation and contribution to HLA class I up-regulation. Arthritis Rheum. 2012;64(2):533-41.

179. Tournadre A, Lenief V, Miossec P. Expression of Toll-like receptor 3 and Toll-like receptor 7 in muscle is characteristic of inflammatory myopathy and is differentially regulated by Th1 and Th17 cytokines. Arthritis Rheum. 2010;62(7):2144-51.

180. Akeno N, Smith EP, Stefan M, Huber AK, Zhang W, Keddache M, et al. IFN-alpha mediates the development of autoimmunity both by direct tissue toxicity and through immune cell recruitment mechanisms. J Immunol. 2011;186(8):4693-706.

181. Meyer A, Laverny G, Allenbach Y, Grelet E, Ueberschlag V, Echaniz-Laguna A, et al. IFN-beta-induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in dermatomyositis. Acta Neuropathol. 2017;134(4):655-66.

182. Nie A, Sun B, Fu Z, Yu D. Roles of aminoacyl-tRNA synthetases in immune regulation and immune diseases. Cell Death Dis. 2019;10(12):901.

183. Rajendran V, Kalita P, Shukla H, Kumar A, Tripathi T. Aminoacyl-tRNA synthetases: Structure, function, and drug discovery. Int J Biol Macromol. 2018;111:400-14.

184. Kwon NH, Fox PL, Kim S. Aminoacyl-tRNA synthetases as therapeutic targets. Nat Rev Drug Discov. 2019;18(8):629-50.

185. Lee SW, Cho BH, Park SG, Kim S. Aminoacyl-tRNA synthetase complexes: beyond translation. J Cell Sci. 2004;117(Pt 17):3725-34.

186. Ascherman DP. Role of Jo-1 in the Immunopathogenesis of the Anti-synthetase Syndrome. Curr Rheumatol Rep. 2015;17(9):56.

187. Arif A, Terenzi F, Potdar AA, Jia J, Sacks J, China A, et al. EPRS is a critical mTORC1-S6K1 effector that influences adiposity in mice. Nature. 2017;542(7641):357-61.

188. Park SG, Schimmel P, Kim S. Aminoacyl tRNA synthetases and their connections to disease. Proc Natl Acad Sci U S A. 2008;105(32):11043-9.

189. Xu X, Shi Y, Zhang HM, Swindell EC, Marshall AG, Guo M, et al. Unique domain appended to vertebrate tRNA synthetase is essential for vascular development. Nat Commun. 2012;3:681.

190. Ahn YH, Park S, Choi JJ, Park BK, Rhee KH, Kang E, et al. Secreted tryptophanyl-tRNA synthetase as a primary defence system against infection. Nat Microbiol. 2016;2:16191.

191. Kim SB, Kim HR, Park MC, Cho S, Goughnour PC, Han D, et al. Caspase-8 controls the secretion of inflammatory lysyl-tRNA synthetase in exosomes from cancer cells. J Cell Biol. 2017;216(7):2201-16.

192. Park MC, Kang T, Jin D, Han JM, Kim SB, Park YJ, et al. Secreted human glycyl-tRNA synthetase implicated in defense against ERK-activated tumorigenesis. Proc Natl Acad Sci U S A. 2012;109(11):E640-7.

193. Kwon NH, Fox PL, Kim S. Aminoacyl-tRNA synthetases as therapeutic targets. Nat Rev Drug Discov. 2019.

194. Xu Z, Wei Z, Zhou JJ, Ye F, Lo WS, Wang F, et al. Internally deleted human tRNA synthetase suggests evolutionary pressure for repurposing. Structure. 2012;20(9):1470-7.

195. Zhou JJ, Wang F, Xu Z, Lo WS, Lau CF, Chiang KP, et al. Secreted histidyl-tRNA synthetase splice variants elaborate major epitopes for autoantibodies in inflammatory myositis. J Biol Chem. 2014;289(28):19269-75.

196. Lo WS, Gardiner E, Xu Z, Lau CF, Wang F, Zhou JJ, et al. Human tRNA synthetase catalytic nulls with diverse functions. Science. 2014;345(6194):328-32.

197. Datt M, Sharma A. Evolutionary and structural annotation of disease-associated mutations in human aminoacyl-tRNA synthetases. BMC Genomics. 2014;15:1063.

198. Puffenberger EG, Jinks RN, Sougnez C, Cibulskis K, Willert RA, Achilly NP, et al. Genetic mapping and exome sequencing identify variants associated with five novel diseases. PLoS One. 2012;7(1):e28936.

199. Miller FW, Twitty SA, Biswas T, Plotz PH. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotype stability, and isotype restriction of anti-Jo-1 autoantibodies. J Clin Invest. 1990;85(2):468-75.

200. Stone KB, Oddis CV, Fertig N, Katsumata Y, Lucas M, Vogt M, et al. Anti-Jo-1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy. Arthritis Rheum. 2007;56(9):3125-31.

201. Ghirardello A, Borella E, Beggio M, Franceschini F, Fredi M, Doria A. Myositis autoantibodies and clinical phenotypes. Auto Immun Highlights. 2014;5(3):69-75.

202. Casciola-Rosen L, Nagaraju K, Plotz P, Wang K, Levine S, Gabrielson E, et al. Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. J Exp Med. 2005;201(4):591-601.

203. Ascherman DP, Oriss TB, Oddis CV, Wright TM. Critical requirement for professional APCs in eliciting T cell responses to novel fragments of histidyl-tRNA synthetase (Jo-1) in Jo-1 antibody-positive polymyositis. J Immunol. 2002;169(12):7127-34.

204. Fahoum SK, Yang DC. Purification of mammalian histidyl-tRNA synthetase and its interaction with myositis-specific anti-Jo-1 antibodies. Biochemistry. 1987;26(18):5871-7.

205. Raben N, Nichols R, Dohlman J, McPhie P, Sridhar V, Hyde C, et al. A motif in human histidyl-tRNA synthetase which is shared among several aminoacyl-tRNA synthetases is a coiled-coil that is essential for enzymatic activity and contains the major autoantigenic epitope. J Biol Chem. 1994;269(39):24277-83.

206. Martin A, Shulman MJ, Tsui FW. Epitope studies indicate that histidyl-tRNA synthetase is a stimulating antigen in idiopathic myositis. FASEB J. 1995;9(12):1226-33.

207. Ramsden DA, Chen J, Miller FW, Misener V, Bernstein RM, Siminovitch KA, et al. Epitope mapping of the cloned human autoantigen, histidyl-tRNA synthetase. Analysis of the myositis-associated anti-Jo-1 autoimmune response. J Immunol. 1989;143(7):2267-72.

208. Galindo-Feria AS, Albrecht I, Fernandes-Cerqueira C, Notarnicola A, James EA, Herrath J, et al. Proinflammatory Histidyl-Transfer RNA Synthetase-Specific CD4+ T Cells in the Blood and Lungs of Patients With Idiopathic Inflammatory Myopathies. Arthritis Rheumatol. 2020;72(1):179-91.

209. Englund P, Wahlstrom J, Fathi M, Rasmussen E, Grunewald J, Tornling G, et al. Restricted T cell receptor BV gene usage in the lungs and muscles of patients with idiopathic inflammatory myopathies. Arthritis Rheum. 2007;56(1):372-83.

210. Halloran PF, Urmson J, Van der Meide PH, Autenried P. Regulation of MHC expression in vivo. II. IFN-alpha/beta inducers and recombinant IFN-alpha modulate MHC antigen expression in mouse tissues. J Immunol. 1989;142(12):4241-7.

211. Eloranta ML, Barbasso Helmers S, Ulfgren AK, Ronnblom L, Alm GV, Lundberg IE. A possible mechanism for endogenous activation of the type I interferon system in myositis patients with anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies. Arthritis Rheum. 2007;56(9):3112-24.

212. Isenberg DA, Rowe D, Shearer M, Novick D, Beverley PC. Localization of interferons and interleukin 2 in polymyositis and muscular dystrophy. Clin Exp Immunol. 1986;63(2):450-8.

213. Karpati G, Pouliot Y, Carpenter S. Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. Ann Neurol. 1988;23(1):64-72.

214. Howard OM, Dong HF, Yang D, Raben N, Nagaraju K, Rosen A, et al. Histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. J Exp Med. 2002;196(6):781-91.

215. Zhang S, Shu X, Tian X, Chen F, Lu X, Wang G. Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. Clin Exp Immunol. 2014;177(1):134-41.

216. Lundstrom SL, Fernandes-Cerqueira C, Ytterberg AJ, Ossipova E, Hensvold AH, Jakobsson PJ, et al. IgG antibodies to cyclic citrullinated peptides exhibit profiles specific in terms of IgG subclasses, Fc-glycans and a fab-Peptide sequence. PLoS One. 2014;9(11):e113924.

217. Scherer HU, van der Woude D, Ioan-Facsinay A, el Bannoudi H, Trouw LA, Wang J, et al. Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. Arthritis Rheum. 2010;62(6):1620-9.

218. Scherer HU, Wang J, Toes RE, van der Woude D, Koeleman CA, de Boer AR, et al. Immunoglobulin 1 (IgG1) Fc-glycosylation profiling of anti-citrullinated peptide antibodies from human serum. Proteomics Clin Appl. 2009;3(1):106-15.

219. Lundstrom SL, Hensvold AH, Rutishauser D, Klareskog L, Ytterberg AJ, Zubarev RA, et al. IgG Fc galactosylation predicts response to methotrexate in early rheumatoid arthritis. Arthritis Res Ther. 2017;19(1):182.

220. Heyder T, Wiklundh E, Eklund A, James A, Grunewald J, Zubarev RA, et al. Altered Fc galactosylation in IgG4 is a potential serum marker for chronic lung disease. ERJ Open Res. 2018;4(3).

221. Perdivara I, Peddada SD, Miller FW, Tomer KB, Deterding LJ. Mass spectrometric determination of IgG subclass-specific glycosylation profiles in siblings discordant for myositis syndromes. J Proteome Res. 2011;10(7):2969-78.

222. Nagaraju K, Plotz PH. Animal models of myositis. Rheum Dis Clin North Am. 2002;28(4):917-33.

223. Katsumata Y, Ascherman DP. Animal models in myositis. Curr Opin Rheumatol. 2008;20(6):681-5.

224. Blechynden LM, Lawson MA, Tabarias H, Garlepp MJ, Sherman J, Raben N, et al. Myositis induced by naked DNA immunization with the gene for histidyl-tRNA synthetase. Hum Gene Ther. 1997;8(12):1469-80.

225. Katsumata Y, Ridgway WM, Oriss T, Gu X, Chin D, Wu Y, et al. Species-specific immune responses generated by histidyl-tRNA synthetase immunization are associated with muscle and lung inflammation. J Autoimmun. 2007;29(2-3):174-86.

226. Sciorati C, Esposito A, Campana L, Canu T, Monno A, Palmisano A, et al. 7-Tesla magnetic resonance imaging precisely and noninvasively reflects inflammation and remodeling of the skeletal muscle in a mouse model of antisynthetase syndrome. Biomed Res Int. 2014;2014:879703.

227. Dobloug GC, Svensson J, Lundberg IE, Holmqvist M. Mortality in idiopathic inflammatory myopathy: results from a Swedish nationwide population-based cohort study. Ann Rheum Dis. 2018;77(1):40-7.

228. Holmqvist ME, Neovius M, Eriksson J, Mantel A, Wallberg-Jonsson S, Jacobsson LT, et al. Risk of venous thromboembolism in patients with rheumatoid arthritis and association with disease duration and hospitalization. JAMA. 2012;308(13):1350-6.

229. Montecucco F, Liberale L, Bonaventura A, Vecchie A, Dallegri F, Carbone F. The Role of Inflammation in Cardiovascular Outcome. Curr Atheroscler Rep. 2017;19(3):11.

230. Schoenfeld SR, Kasturi S, Costenbader KH. The epidemiology of atherosclerotic cardiovascular disease among patients with SLE: a systematic review. Semin Arthritis Rheum. 2013;43(1):77-95.

231. Symmons DP, Gabriel SE. Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. Nat Rev Rheumatol. 2011;7(7):399-408.

232. Rai SK, Choi HK, Sayre EC, Avina-Zubieta JA. Risk of myocardial infarction and ischaemic stroke in adults with polymyositis and dermatomyositis: a general population-based study. Rheumatology (Oxford). 2016;55(3):461-9.

233. Leclair V, Svensson J, Lundberg IE, Holmqvist M. Acute Coronary Syndrome in Idiopathic Inflammatory Myopathies: A Population-based Study. J Rheumatol. 2019;46(11):1509-14.

234. Carruthers EC, Choi HK, Sayre EC, Avina-Zubieta JA. Risk of deep venous thrombosis and pulmonary embolism in individuals with polymyositis and dermatomyositis: a general population-based study. Ann Rheum Dis. 2016;75(1):110-6.

235. Zoller B, Li X, Sundquist J, Sundquist K. Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. Lancet. 2012;379(9812):244-9.

236. Diederichsen LP, Diederichsen AC, Simonsen JA, Junker P, Sondergaard K, Lundberg IE, et al. Traditional cardiovascular risk factors and coronary artery calcification in adults with polymyositis and dermatomyositis: a Danish multicenter study. Arthritis Care Res (Hoboken). 2015;67(6):848-54.

237. Limaye VS, Lester S, Blumbergs P, Roberts-Thomson PJ. Idiopathic inflammatory myositis is associated with a high incidence of hypertension and diabetes mellitus. Int J Rheum Dis. 2010;13(2):132-7.

238. Wang H, Tang J, Chen X, Li F, Luo J. Lipid profiles in untreated patients with dermatomyositis. J Eur Acad Dermatol Venereol. 2013;27(2):175-9.

239. Barsotti S, Saponaro C, Gaggini M, Talarico R, Bianchini E, Di Lascio N, et al. Cardiometabolic risk and subclinical vascular damage assessment in idiopathic inflammatory myopathies: a challenge for the clinician. Clin Exp Rheumatol. 2019;37(6):1036-43.

240. Vincze M, Der H, Kerekes G, Szodoray P, Zeher M, Danko K, et al. Decreased flow-mediated dilatation with increased arterial stiffness and thickness as early signs of atherosclerosis in polymyositis and dermatomyositis patients. Clin Rheumatol. 2014;33(11):1635-41.

241. Arida A, Protogerou AD, Kitas GD, Sfikakis PP. Systemic Inflammatory Response and Atherosclerosis: The Paradigm of Chronic Inflammatory Rheumatic Diseases. Int J Mol Sci. 2018;19(7).

242. Carbone F, Bonaventura A, Liberale L, Paolino S, Torre F, Dallegri F, et al. Atherosclerosis in Rheumatoid Arthritis: Promoters and Opponents. Clin Rev Allergy Immunol. 2018.

243. Foley JH, Conway EM. Cross Talk Pathways Between Coagulation and Inflammation. Circ Res. 2016;118(9):1392-408.

244. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. Immunity. 2014;41(5):694-707.

245. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 2001;104(12):1336-42.

246. Shai I, Pischon T, Hu FB, Ascherio A, Rifai N, Rimm EB. Soluble intercellular adhesion molecules, soluble vascular cell adhesion molecules, and risk of coronary heart disease. Obesity (Silver Spring). 2006;14(11):2099-106.

247. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet. 1998;351(9096):88-92.

248. Pradhan AD, Rifai N, Ridker PM. Soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. Circulation. 2002;106(7):820-5.

249. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM, Jr., et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation. 1997;96(12):4219-25.

250. Khare A, Shetty S, Ghosh K, Mohanty D, Chatterjee S. Evaluation of markers of endothelial damage in cases of young myocardial infarction. Atherosclerosis. 2005;180(2):375-80.

251. Dzikowska-Diduch O, Domienik-Karlowicz J, Gorska E, Demkow U, Pruszczyk P, Kostrubiec M. E-selectin and sICAM-1, biomarkers of endothelial function, predict recurrence of venous thromboembolism. Thromb Res. 2017;157:173-80.

252. Torres C, Matos R, Morais S, Campos M, Lima M. Soluble endothelial cell molecules and circulating endothelial cells in patients with venous thromboembolism. Blood Coagul Fibrinolysis. 2017;28(8):589-95.

253. Cid MC, Grau JM, Casademont J, Tobias E, Picazo A, Coll-Vinent B, et al. Leucocyte/endothelial cell adhesion receptors in muscle biopsies from patients with idiopathic inflammatory myopathies (IIM). Clin Exp Immunol. 1996;104(3):467-73.

254. Figarella-Branger D, Schleinitz N, Boutiere-Albanese B, Camoin L, Bardin N, Guis S, et al. Platelet-endothelial cell adhesion molecule-1 and CD146: soluble levels and in situ expression of cellular adhesion molecules implicated in the cohesion of endothelial cells in idiopathic inflammatory myopathies. J Rheumatol. 2006;33(8):1623-30.

255. Grundtman C, Lundberg IE. Vascular involvement in the pathogenesis of idiopathic inflammatory myopathies. Autoimmunity. 2009;42(7):615-26.

256. Lundberg IE. The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. Curr Rheumatol Rep. 2000;2(3):216-24.

257. Ito T, Kumamoto T, Horinouchi H, Yukishige K, Sugihara R, Fujimoto S, et al. Adhesion molecule expression in experimental myositis. Muscle Nerve. 2002;25(3):409-18.

258. Lundberg I, Kratz AK, Alexanderson H, Patarroyo M. Decreased expression of interleukin-1alpha, interleukin-1beta, and cell adhesion molecules in muscle tissue following corticosteroid treatment in patients with polymyositis and dermatomyositis. Arthritis Rheum. 2000;43(2):336-48.

259. Limaye VS, Bonder CS, Sun WY, Lester S, Roberts-Thomson PJ, Blumbergs P. Levels of soluble adhesion molecules and their associations in inflammatory myositis. Int J Rheum Dis. 2013;16(1):99-101.

260. Murata K, Takayanagi T, Mano Y, Miyagawa S. [Serum levels of soluble adhesion molecules in patients with inflammatory myopathies]. Rinsho Shinkeigaku. 1996;36(1):12-6.

261. Jain A, Sharma MC, Sarkar C, Bhatia R, Singh S, Handa R. Increased expression of cell adhesion molecules in inflammatory myopathies: diagnostic utility and pathogenetic insights. Folia Neuropathol. 2009;47(1):33-42.

262. Sherer Y, Livneh A, Levy Y, Shoenfeld Y, Langevitz P. Dermatomyositis and polymyositis associated with the antiphospholipid syndrome-a novel overlap syndrome. Lupus. 2000;9(1):42-6.

263. Souza FH, Levy-Neto M, Shinjo SK. Antiphospholipid syndrome and dermatomyositis/polymyositis: a rare association. Rev Bras Reumatol. 2012;52(4):642-4.

264. Wang CH, Wang NC, Lin TY, Chen CH. Anti-Jo-1 myositis and the antiphospholipid syndrome showing right ventricular thrombus: a novel overlap syndrome with atypical presentation. Mod Rheumatol. 2014;24(5):865-8.

265. Connors GR, Christopher-Stine L, Oddis CV, Danoff SK. Interstitial lung disease associated with the idiopathic inflammatory myopathies: what progress has been made in the past 35 years? Chest. 2010;138(6):1464-74.

266. Travis WD, Costabel U, Hansell DM, Talmadge E. King J, Lynch DA, Nicholson AG, et al. An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias. American Journal of Respiratory and Critical Care Medicine. 2013;188(6):733-48.

267. Fischer A, Antoniou KM, Brown KK, Cadranel J, Corte TJ, du Bois RM, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976-87.

268. Eriksson JK, Askling J, Arkema EV. The Swedish Rheumatology Quality Register: optimisation of rheumatic disease assessments using register-enriched data. Clinical and experimental rheumatology. 2014;32(5 Suppl 85):S.

269. Keates T, Cooper CD, Savitsky P, Allerston CK, Phillips C, Hammarstrom M, et al. Expressing the human proteome for affinity proteomics: optimising expression of soluble protein domains and in vivo biotinylation. N Biotechnol. 2012;29(5):515-25.

270. Ossipova E, Cerqueira CF, Reed E, Kharlamova N, Israelsson L, Holmdahl R, et al. Affinity purified anti-citrullinated protein/peptide antibodies target antigens expressed in the rheumatoid joint. Arthritis Res Ther. 2014;16(4):R167.

271. Lundstrom SL, Yang H, Lyutvinskiy Y, Rutishauser D, Herukka SK, Soininen H, et al. Blood plasma IgG Fc glycans are significantly altered in Alzheimer's disease and progressive mild cognitive impairment. J Alzheimers Dis. 2014;38(3):567-79.

272. Lyutvinskiy Y, Yang H, Rutishauser D, Zubarev RA. In silico instrumental response correction improves precision of label-free proteomics and accuracy of proteomics-based predictive models. Mol Cell Proteomics. 2013;12(8):2324-31.

273. Ashcroft T, Simpson JM, Timbrell V. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. J Clin Pathol. 1988;41(4):467-70.

274. Chen G, Wang Y, Qin X, Li H, Guo Y, Wang Y, et al. Change in IgG1 Fc N-linked glycosylation in human lung cancer: age- and sex-related diagnostic potential. Electrophoresis. 2013;34(16):2407-16.

275. Davies J, Jiang L, Pan LZ, LaBarre MJ, Anderson D, Reff M. Expression of GnTIII in a recombinant anti-CD20 CHO production cell line: Expression of antibodies with altered glycoforms leads to an increase in ADCC through higher affinity for FC gamma RIII. Biotechnol Bioeng. 2001;74(4):288-94.

276. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem. 2002;277(30):26733-40.

277. Ramsden DA, Chen J, Miller FW, Misener V, Bernstein RM, Siminovitch KA, et al. Epitope mapping of the cloned human autoantigen, histidyl-tRNA synthetase. Analysis of the myositis-associated anti-Jo-1 autoimmune response. The Journal of Immunology. 1989;143(7):2267-72.

278. Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proceedings of the National Academy of Sciences. 1990;87(24):9933-7.

279. Miller FW, Twitty SA, Biswas T, Plotz PH. Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotype stability, and isotype restriction of anti-Jo-1 autoantibodies. The Journal of Clinical Investigation. 1990;85(2):468-75.

280. Raben N, Nichols R, Dohlman J, McPhie P, Sridhar V, Hyde C, et al. A motif in human histidyl-tRNA synthetase which is shared among several aminoacyl-tRNA synthetases is a coiled-coil that is essential for enzymatic activity and contains the major autoantigenic epitope. Journal of Biological Chemistry. 1994;269(39):24277-83.

281. Martin A, Shulman MJ, Tsui FW. Epitope studies indicate that histidyl-tRNA synthetase is a stimulating antigen in idiopathic myositis. The FASEB Journal. 1995;9(12):1226-33.

282. Engbers MJ, van Hylckama Vlieg A, Rosendaal FR. Venous thrombosis in the elderly: incidence, risk factors and risk groups. J Thromb Haemost. 2010;8(10):2105-12.

283. Silverstein RL, Bauer KA, Cushman M, Esmon CT, Ershler WB, Tracy RP. Venous thrombosis in the elderly: more questions than answers. Blood. 2007;110(9):3097-101.

284. Culmer DL, Dunbar ML, Hawley AE, Sood S, Sigler RE, Henke PK, et al. E-selectin inhibition with GMI-1271 decreases venous thrombosis without profoundly affecting tail vein bleeding in a mouse model. Thromb Haemost. 2017;117(6):1171-81.