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Walter, A.W.

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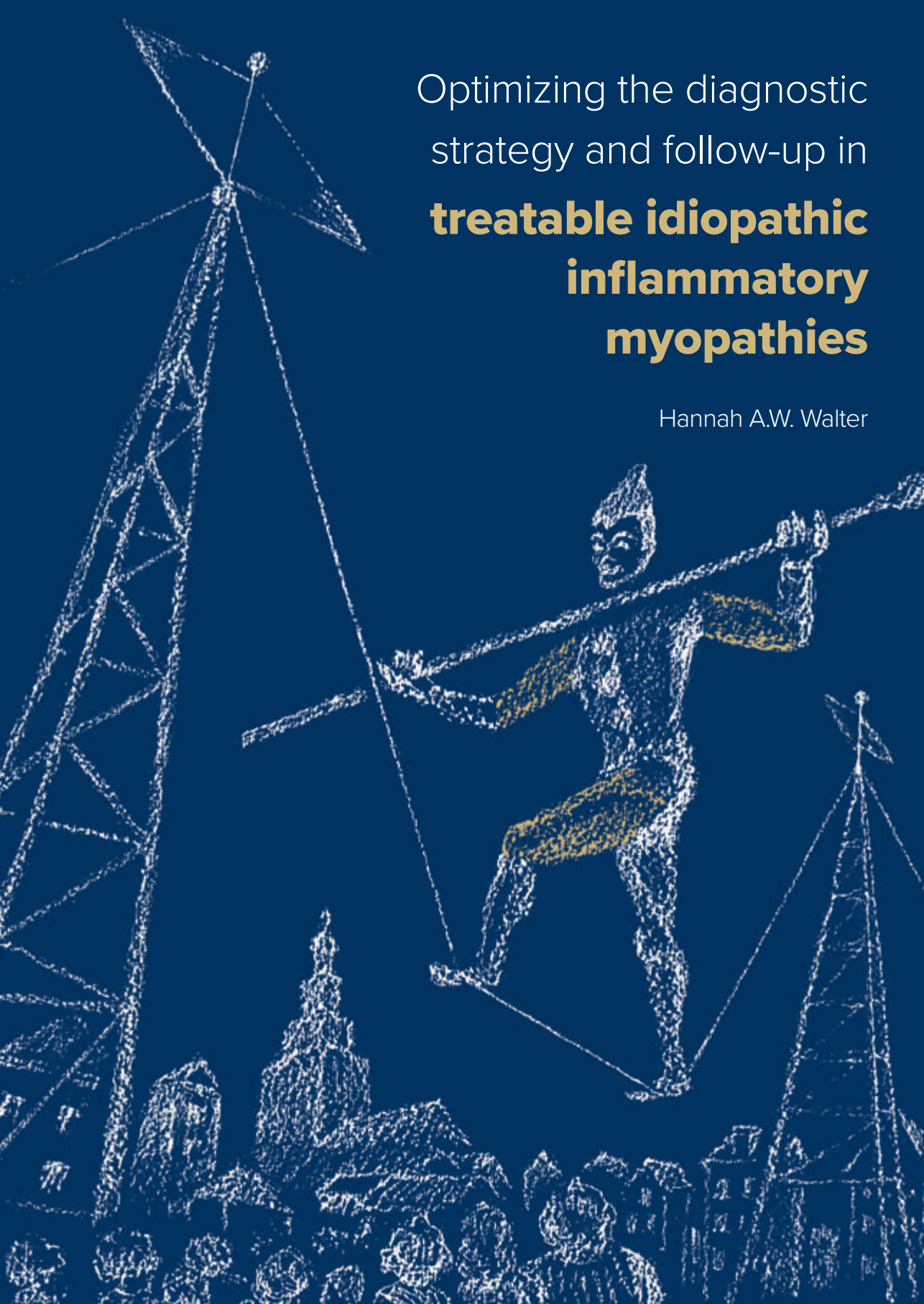

Optimizing the diagnostic strategy and follow-up in **treatable idiopathic inflammatory myopathies**

Hannah A.W. Walter

Optimizing the diagnostic strategy and follow-up in TREATABLE IDIOPATHIC INFLAMMATORY MYOPATHIES

Hannah A.W. Walter

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Optimizing the diagnostic strategy and follow-up in treatable idiopathic inflammatory myopathies

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Chapter 1

General introduction and thesis outline

Adapted from: Hannah A.W. Walter[1], Stefanie Glaubitz[2], Jens Schmidt[2], Anneke J. van der Kooi[1]. (2022). Emergencies in idiopathic inflammatory myopathies. In: Damian M and de Visser M. (Ed.), Emergencies in Neuromuscular Disorders (pp. 283-317), Cham, Switzerland: Springer Nature Switzerland.

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[1] DEPARTMENT OF NEUROLOGY, AMSTERDAM UNIVERSITY MEDICAL CENTRE, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF NEUROLOGY, UNIVERSITY MEDICAL CENTRE GÖTTINGEN, GERMANY

Idiopathic inflammatory myopathies (IIMs), often called myositis, are a group of rare, acquired myopathies with an auto-immune origin. IIMs are typically characterized by a subacute onset of muscle weakness in a symmetrical pattern affecting predominantly the proximal arm and leg muscles. Depending on the subtype, multiple organs such as skin, joints, lungs, and the heart can be involved in the inflammatory disease process. This results in a variety of signs and symptoms that different patients may experience. Moreover, myositis may occur as a paraneoplastic syndrome.

A classifying diagnosis is important for two main reasons: firstly to assign the right treatment, and secondly to provide a patient with accurate prognostic information.

Classification of idiopathic inflammatory myopathies

The classification of IIMs is based on a combination of clinical signs and symptoms, laboratory findings, myositis specific- and myositis-associated antibodies (MSA and MAA) and histological findings.[1-5]

Dermatomyositis (DM) is characterized by muscle weakness in proximal more than in distal muscles and in neck flexors more than in neck extensors, in combination with a typical skin rash, in particular Gottron papules/sign or heliotrope erythema. Often laboratory findings show an elevated creatine kinase (CK), but this may be normal. The occurrence of various antibodies is typical for DM (e.g. Mi2, TIF1γ, MDA5 and NXP2-antibodies). Muscle biopsy findings include perivascular inflammation and perifascicular atrophy.[1] In addition, sarcoplasmic MxA (myxovirus resistant A) expression in the cytoplasm of myofibres is a new immunohistochemical marker for the diagnosis of DM, indicating interferon type 1 pathology, with a specificity of 98%.[6] Dermatomyositis is best known for its occurrence as a paraneoplastic syndrome, called cancer associated myositis (CAM), which is defined as the diagnosis of cancer within 3 years of the diagnosis of IIM. In the presence of TIF1γ antibodies, the chance of cancer is 60-80%, and in case of anti-NXP2 24-38%.[7]

Antisynthetase syndrome (ASS) is defined by clinical features that include a combination of myositis, interstitial lung disease (ILD), and non-erosive arthritis (considered the 'triad'), and Raynaud's phenomenon, fever and/or mechanic's hands, in the presence of one or more antisynthetase-related antibodies (e.g. anti-Jo1, PL7, PL12, EJ, OJ). Arthritis or ILD are often the presenting symptoms.[8] Within the IIM spectrum, patients with antisynthetase syndrome antibodies are at significantly higher risk to develop interstitial lung disease (ILD).[9] A diagnosis

of ILD in patients with IMM is associated with higher morbidity and mortality compared to patients without ILD.[10]

Overlap-myositis (OM) is nowadays considered a distinct subtype, defined as patients fulfilling criteria for IIM plus criteria for another connective tissue disease (CTD), such as systemic sclerosis, systemic lupus erythematosus, mixed connective tissue disease, rheumatoid arthritis and primary Sjögren's syndrome.[5 11] The presence of autoantibodies such as anti-SSa, anti-SSb, anti-Ro52, anti-U1-nRNP, anti-PM-Scl and anti-Ku suggests an overlap syndrome and these patients may develop a CTD during the disease course.[12]

Immune-mediated necrotizing myopathy (IMNM) is characterized by often severe muscle weakness, very high serum CK (6000 IU/L or above)[13] activity, and muscle biopsy showing necrotic muscle fibres with a diffuse pattern in different stages of necrosis with regenerating fibres and without a specific, primary immune cell infiltration. Anti-SRP and HMGCR autoantibodies are associated with this subtype, but a substantial part of the patients is seronegative.[14] When anti-HMGCR antibodies are present, previous statin use is found in about half of the patients, but the antibody also frequently occurs in statin-naïve patients.[13] Patients with IMNM have an increased risk of CAM with an occurrence of malignancy in 29%, predominantly in anti-HMGCR positive and seronegative patients, but rarely in patients with anti-SRP antibodies.[7 15]

Inclusion body myositis (IBM) is a slowly progressive disease with involvement of the knee extensors more than hip flexors and finger flexors more than shoulder abduction. Typically, the muscle biopsy shows endomysial inflammatory infiltrates and, at the same time, rimmed vacuoles and protein accumulations.[2] It was shown that the combination of finger flexor or quadriceps weakness, and endomysial inflammation, and either invasion of non-necrotic muscle fibres or rimmed vacuoles, has a 90% sensitivity and 96% specificity for IBM.[16] The diagnosis may be supported by the presence of anti-cN1A (anti-cytosolic 5'-nucleotidase 1A) antibody, but a large study demonstrated a sensitivity of 30-50% and a low predictive value.[17] Subsequent studies showed that anti-cNA1 antibody is present in systemic autoimmune diseases as well, even in absence of any muscle impairment.[18]

Juvenile myositis affects children and adolescents. The diagnosis can (mostly) be based on clinico-pathological features and antibodies, and the same diagnostic criteria as in adults can be used.[3] MSA's and MAA's in children represent the same subgroups compared to adults, but the prevalence is different. The most common form is juvenile dermatomyositis (JDM),

representing approximately 80% of the juvenile myositis cases.[19 20] Juvenile overlap myositis represents the second largest group, occurring in 6-11% of the juvenile myositis patients, followed by juvenile polymyositis (IMNM and ASS), which is seen in 4-8% of the juvenile myositis cases. As juvenile myositis is often misdiagnosed in patients who actually have non-inflammatory myopathies, particularly muscular dystrophies, a muscle biopsy is often required for diagnosis.[21] In 70% of the patients with JDM, antibodies are present. There is no relation between JDM and cancer.

Polymyositis (PM) is a contested entity within the group of IIMs. It is the rarest form of myositis and currently considered a diagnosis of exclusion of all other forms of myositis.[5]

Epidemiology

IIMs account for the most common cause of acquired muscle diseases in adults, but is a rare disease in the general population.[22] The overall estimated prevalence is 14 cases per 100.000 inhabitants (range 2.4 – 33.8).[23] The incidence is estimated at 7.98 cases per million per year (range 1.16 – 19), which adds up to approximately 180 new myositis patients per year in The Netherlands. For adults, the peak age of onset is between 30 and 50 years of age. IIMs occur more commonly in women than in men (ratio F/M 2:1).

The prevalence of IBM is estimated at 1.85 cases per million per year, and the incidence at 2.01 cases per 100.000 per year.[23] IBM occurs more commonly in men than in women (ratio M/F 3:2) and predominantly Caucasian males are affected.[22 24] Disease onset in IBM is mainly above 50 years of age.

The estimated annual incidence for children is 2.5 to 4.1 cases per million per year.[7] The peak incidence for juvenile dermatomyositis is at the age of 7.[22]

Pathophysiology

IIMs are known to be auto-immune mediated diseases. The exact pathogenic cause and factors triggering the immune response in myositis remain unknown. Many studies have unravelled individual aspects of the pathophysiology such as antigen presentation and the myogenic concert of dendritic cells (DCs), T-cells, B cells and muscle fibres.

DM. From histological studies, it appears that CD4+ T-cells and B-cells play a main role in DM.[25] One of the main components of the pathophysiology are as yet unidentified antibodies against

endothelial cells with subsequent activation of the complement membrane attack complex C5b-9.[26] This leads to cell damage and reduced density of endomysial capillaries, ischemia, and muscle fibre destruction resembling micro-infarcts.[27] The resulting perifascicular atrophy is most prominent at the periphery of muscle fascicles. Activation of membrane attack complex is associated with release of pro-inflammatory cytokines which contribute towards migration of B-cells, CD4+ T-cells and plasmacytoid dendritic cells to the perimysial and endomysial space. The perifasicular muscle fibres are in a state of remodelling, regeneration and immune activation – by expressing major compatibility complex (MHC) class I and releasing pro-inflammatory chemokines.[28] However, there is evidence, that the perifascicular atrophy is not caused by the impaired blood supply.[29] An alternative explanatory approach for the pathogenesis is based on the role of type 1 interferons. In comparison to all other types of IIM, dermatomyositis shows an overexpression of type 1 interferon–inducible transcripts and proteins in muscle, like MxA and ISG15. The inappropriate intracellular production of type 1 interferon–inducible molecules leads to cell injury and atrophy in dermatomyositis.[30] Furthermore, dermatomyositis can be caused by cancer-induced auto-immunity as noted previously. T-cell response and antibodies against new tumour antigens cross-react with antigens in muscle and lead to the autoimmune phenotype. It is shown, that regenerating myoblasts in affected muscles in myositis express higher levels of these antigens than healthy controls.[31] In a large proportion of the patients with DM – up to 80-90% – autoantibodies can be detected[32], suggesting a pathophysiological relationship with the humoral immune system.[33] A role of B-cells in the pathogenesis of DM is evidenced by endomysial production of B-cell activating factor, B-cells and plasma cells in muscle tissue, and endomysial B-cell-maturation in part within the muscle itself.[34 35]

Other subtypes. In contrast, CD8+ T-cells play a predominant role in PM and IBM. MHC-I is upregulated at the cell surface of healthy appearing muscle fibres, probably in reaction to cytokine release from activated T-cells. Antigen-presenting dendritic cells contribute to endomysial activation of CD8+ T-cells, which form immunological synapses with muscle fibres via CD8 and MHC-I. As a result, CD8+ T-cells release cytotoxic molecules – perforin and granzyme B - into the muscle fibres. The subsequent muscle fibre necrosis adds to the inflammation by recruiting more lymphocytes and dendritic cells, thus, functioning as a vicious cycle that can maintain myoinflammation.[25]

IMNM. In immune-mediated necrotizing myopathies, SRP and HMGCR antibodies are directly linked to the myositis phenotype.[36] HMGCR is an enzyme, and the pharmacological target of statins.[37] Antibodies to HMGCR are associated with statin use in approximately half of the patients with this subtype.[38] SRP is a ribonucleoprotein mediating protein translocation to the endoplasmic reticulum. It was shown that inhibition of SRP by anti SRP antibodies reduced myoblast viability[39], induced myotubular atrophy and increased inflammatory cytokines.[33 40] Muscle biopsies of anti-SRP and anti-HMGCR positive patients contain necrotic fibres, with numerous macrophages and sparse lymphocytes. Additionally, the classical complement pathway is activated and sarcolemmal C5b-9 deposits correlated with necrosis of the muscle cells.[41] The presence of complement deposits on the sarcolemma of myofibers in IMNM patients may suggest an auto-antibody mediated pathophysiology instead of T-cell dependent cytotoxicity.[33] B cell involvement may not be as prominent compared to DM: low numbers of B cells are detected in muscle tissue of this subgroup, but B cell activating factor (BAFF) has been suggested as an important contributor to the muscle weakness.[42]

ASS. Recent findings suggest that ASS has a unique immunopathogenic pathway.[43] Antibodies such as histidyl t-RNA synthetases (Jo1, PL7, PL12, EJ) which are associated with anti-synthetase syndrome, can be expressed in different tissues, including muscles and lungs.[44] These antibodies induce the activation of innate immune cells and act as chemo-attractants, inducing the migration of immune cells into the lung.

OM. More evidence has emerged for overlap myositis, among which new autoantibodies that have been identified as potential markers of this subtype; i.e. anti-RuvBL1/2, poly-U-binding factor 60 kDa protein (PUF-60) and cytosolic 50-nucleotidase 1A (NT5C1A).[45] The exact immunopathological mechanism behind this subtype remains unclear.

IBM. Among the inflammatory findings, a degenerative mechanism plays an important role in IBM. In the muscle biopsy, β-amyloid deposits and rimmed vacuoles can be found. The aetiology of inflammation and degeneration is so far unresolved. It is currently assumed that an initial inflammatory trigger can cause production of β-amyloid. In this context of inflammatory/degenerative crosstalk, muscle fibres develop intracellular cell stress and may become irreversibly damaged.[46-48]

Clinical presentation and ancillary investigations

Muscle involvement

Clinically, IIMs are characterized by proximal, symmetrical muscle weakness in a limb-girdle pattern, which develops sub-acutely (weeks to months) in DM and ASS and frequently faster (within weeks) in IMNM. Mostly, proximal leg muscles show more weakness compared to the proximal arm muscles. There are some exceptions since DM associated with anti-MDA5 antibodies and ASS may be amyopathic. Due to muscle weakness, patients will increasingly experience problems in climbing stairs, rising from a (deep) chair, walking, and lifting objects and performing overhead activities such as combing their hair.[49 50] Myalgia and muscle tenderness are common. Neck flexor muscles can be involved in all subtypes, resulting in problems holding up the head (head drop). The involvement of oral, pharyngeal and proximal and distal oesophageal muscles may result in dysphagia, with sometimes excessive weight loss and the risk of choking and concomitant risk on aspiration pneumonia.[51] The vocal cords may be affected, resulting in a soft voice or hoarseness.

IBM develops in a different manner: this subtype has a gradual onset and is slowly progressive over years and characterized by asymmetric weakness of predominantly quadriceps and deep finger flexor muscles. However, IBM patients can also present with solitary dysphagia or asymmetrical weakness of distal leg muscles. In daily life, patients with IBM often have difficulties in buttoning or holding objects due to deep finger flexor involvement.[49] Muscular atrophy is commonly observed in finger flexors and quadriceps muscles.

Skin abnormalities

In dermatomyositis, skin changes can either precede or accompany muscle weakness. In some patients, abnormalities remain restricted to the skin, the so-called amyopathic dermatomyositis. Three abnormalities form the main characteristics for dermatomyositis, and include heliotrope erythema, periorbital edema, 'Gottron's papules', and 'Gottron sign', which are firm papules with a silvery superficial layer, which can be mistaken for psoriasis, or red rashes at the extensor side of the metacarpophalangeal and interphalangeal joints (**Figures 1, 2, 3).** Other typical abnormalities include erythema on the chest ('V-sign'), shoulders, arms, and upper back ('shawl-sign') or over the outside of the hip ('holster sign'). The skin of the fingertips may be cracked, and cuticles swollen, irregular, overgrowing the nails and in black

people they are often darkened. Atypical skin abnormalities may be present in anti-MDA5 dermatomyositis, including palmar papules, ulceration of the fingers and around the elbow and mechanic's hands (claimed to be characteristic for ASS, but also possible in TIF1γ positive DM patients) may occur. Skin changes in dermatomyositis may overlap with skin changes caused by connective tissue diseases such as systemic sclerosis or rheumatoid arthritis. In ASS, typical skin abnormalities include mechanic's hands, cracked skin or fissures at the lateral side of the index finger at thumb side. Of note, Gottron's sign and papules are found in 27% of the ASS-patients, heliotrope rash in 14% and both in 6%.[52] Furthermore, Raynaud's phenomenon may be present in patients with ASS or overlap myositis.

Calcinosis may be a skin manifestation of DM. Calcinosis is defined as a dystrophic calcification in injured tissue with normal serum calcium and serum phosphate levels.[53] It can occur in multiple forms, including circumscribed calcinosis (a superficial plaque or nodule), and tumoural calcinosis which are bigger and occur in deeper tissues. Calcinosis can also occur in tendons and lead to contractures, pain and immobility.[54] The subcutaneous calcifications may extrude and cause infections or ulcera.[50]

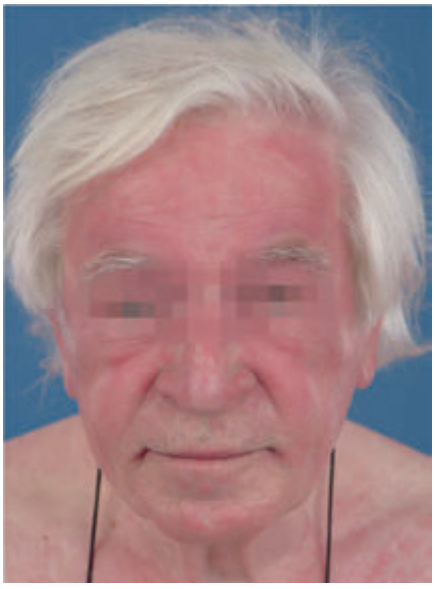

Figure 1. Heliotrope erythema in a dermatomyositis patient
**The patients' legal heir and publisher of the original book chapter gave approval for publication of these photos.*

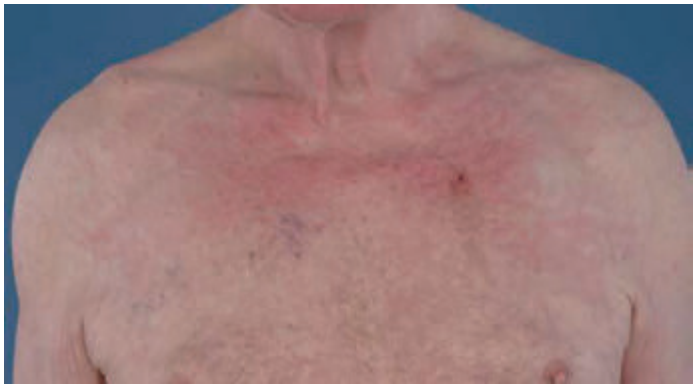

Figure 2. Erythema on the chest of a dermatomyositis patient, so called 'V-sign'

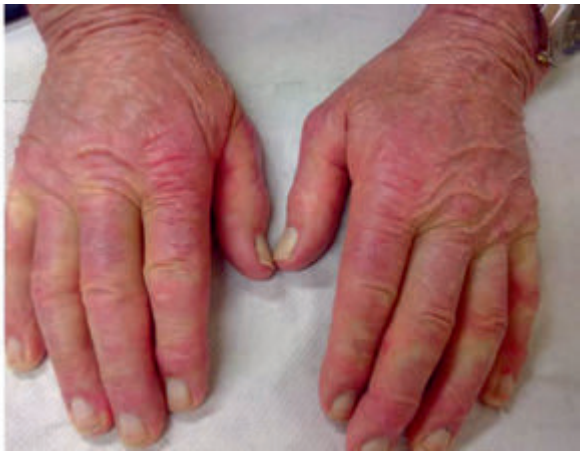

Figure 3. Gottron's papules on the metacarpophalangeal and interphalangeal joints of a dermatomyositis patient

Respiratory failure

In ASS, MDA5-associated dermatomyositis and overlap myositis, patients often experience dyspnoea. Respiratory failure has also been described in IMNM. This may be caused by ILD, myocarditis or respiratory muscle involvement. Acute respiratory distress (ARD) that needs ventilatory support on intensive care unit can be the initial presentation of ASS or anti-MDA5 dermatomyositis.[55] The diagnosis can be very challenging, especially when muscle weakness and cutaneous manifestations are lacking. In this specific patient group with ARD, the majority (96%) shows bilateral, predominantly lower condensation on a chest X-ray. On pulmonary

computerized tomography (CT), around 75% shows ground glass attenuation and alveolar condensation, and almost 40% shows lung fibrosis and mediastinal lymphadenopathies.[55]

Dysphagia

Dysphagia is a clinical sign that is often present in IIM[56] and affects on average 40% of the patients with DM, ASS, OM and IMNM.[57] Dysphagia in IBM is more frequent (60%) and it may be the initial symptom.[58] The pharynx and upper oesophageal sphincter are affected as these consist of skeletal muscle.[59] Motility disturbances of the distal oesophagus (which contains predominantly

smooth muscle rather than skeletal muscle) causes dysphagia in two-third of the myositis patients.[51] Recent studies in IBM indicate a reduced upper oesophageal sphincter opening, possibly caused by fibrosis of crico-pharyngeal muscle and weakness of suprahyoid muscles. Early symptoms include problems with clearing one's throat with solids and later liquids, coughing during eating and a prolonged duration of food intake, whereas choking or unwanted weight loss are later symptoms.[3] Other symptoms include hoarseness. Dysphagia increases the risk of aspiration pneumonia and death and, therefore, swallowing function should be tested and one should specifically ask for symptoms of dysphagia (there are excellent scales, e.g. EAT-10). Two questions reliably predict impaired swallowing: 'Does food get stuck in your throat' and 'Do you have to swallow repeatedly in order to get rid of food'.[60] Diagnostic tests such as video fluoroscopic swallow studies (VFSS) and fibre-optic endoscopic evaluation of swallowing (FEES) can confirm the diagnosis.[61] Real time MRI or manometry may have future potential[17], but – so far – are mostly considered beyond clinical practice. The presence of Mi2, TIF1γ, NXP2, U1RNP, and PM-Scl antibodies is strongly associated with the presence of dysphagia, but it may also develop in absence of antibodies.[9]

Systemic symptoms

Fever, fatigue, arthralgia or arthritis may occur in all subtypes, but are more common in ASS and OM.[57] Arthralgia or arthritis is the presenting symptom in 21-35% of ASS patients.[62] Within the ASS spectrum, arthritis is most common in anti-Jo1 positive patients; 61% show arthritis or arthralgia at onset, and this is mostly poly-articular and symmetrical.[63] Subluxation arthropathy has been described in about one-fifth of anti-Jo1 ASS patients.

Cardiac involvement

Cardiac manifestations of IIM develop in the coronary arteries, epicardium, myocardium as well as the endocardium. The myocardium is mostly affected. Myocarditis may subsequently evolve into cardiomyopathy, cardiac conduction system and automatism disorders and supra- and ventricular arrhythmias.[64]

According to the Euromyositis registry, 9% of the IIM patients suffer from cardiac involvement, including pericarditis, myocarditis, arrhythmia or sinus tachycardia.[57] However, this may be an underestimation since inflammation of cardiac muscle was detected in 25–30% of patients with IIM in post-mortem studies.[64]

Elevated cardiac enzymes (troponin T and troponin I) indicate myocardial damage, but have divergent specificity and sensitivity. Troponins may originate from regenerating skeletal muscle; therefore serum elevation can erroneously point to myocardial involvement. Cardiac troponin I appears to be more specific for myocardial damage than troponin T.[65] Troponin assessment may offer guidance for myocarditis screening.

Elevated NT-pro-BNP is correlated with reduced cardiac function in IIM.[66] The presence of anti-Ro and anti-SRP antibodies is suggested to be associated with cardiac complications.[64]

Diagnostic testing

No gold standard exists to establish the diagnosis of IIMs. To establish a valid diagnosis, a patient suspected of an IIM should undergo extensive muscle strength testing, laboratory testing, including at least creatine kinase (CK), ASAT, ALAT, LDH, aldolase troponin T and autoantibodies, and preferably muscle imaging or EMG before a muscle biopsy.

Muscle imaging can be performed either by muscle ultrasound or by muscle MRI. Muscle ultrasound can detect structural muscle changes, such as oedema, atrophy and fibrosis and has proven to be useful in juvenile inflammatory myopathies but its value in the diagnosis of adult IIMs remains unclear.[67 68] A whole body muscle MRI, or MRI of the upper arms and/or thighs can detect muscle oedema (inflammation) and fatty replacement of muscle.[69] Muscle imaging should be preferably performed to guide the biopsy by detecting localized muscle inflammation in order to decrease the chance of a false negative biopsy.[70] Since the specificity of some of the MSA's is not fully known, a muscle biopsy still remains needed in order to achieve a correct diagnosis. Muscle biopsy is of great importance, not only because the classification criteria[1] rely on histological features, but also because some congenital,

dystrophic, toxic or metabolic myopathies can be mistaken for IIM. There are some exceptions: when dermatomyositis is suspected and classical skin abnormalities are present, and when anti-Jo1 or other tRNA-synthetases antibodies are present.[3]

In addition, EMG usually shows myopathic changes and is useful to differentiate between myopathies and neuropathies.[71] However, EMG changes are non-specific, may be missed when a low number of muscles is sampled and do not help to differentiate between the different myopathies.

Autoantibody testing can be performed with a myositis line blot including anti-HMGCR and/or anti-cN-1A antibodies. In 70% of the IIM patients, an autoantibody, either myositis specific (MSA) or myositis associated (MAA), can be detected in the serum; in 50% of the cases this is a MSA.[72] MSA's are associated with rather well defined subtypes (DM, ASS, IMNM) and include MDA5, NXP-2, Mi-2, TIF1γ, SAE1, Jo-1, PL-7, PL-12, OJ, KS, ZO, EJ, SRP, HMGCR and cN1A. MAA's include PM-Scl, Ku, U1RNP, U1/U2RNP, U3RNP, Ro52 and Ro60, are not specific for myositis and may occur within all IIM subtypes, and in particular in overlap myositis. Existence of an antibody simplifies the differentiation of myositis subtypes, in particular when typical clinical and histological findings are present. Albeit they may be present in healthy people.

If ILD is suspected, either because clinical symptoms are present or antibodies are associated with a risk of ILD, pulmonary function tests including diffusion capacity should be performed and, in most cases, also a pulmonary high-resolution CT should be conducted.

When cardiomyopathy is suspected, cardiac imaging can confirm the diagnosis. Cardiac magnetic resonance imaging (CMR) is recommended as the primary non-invasive method.[73] An echocardiography can provide useful information, but the findings lack specificity. More information can be found in the part 'Management of cardiac involvement'.

Cancer screening is advocated in all IIM patients except ASS, OM, IBM, and SRP-IMNM. In particular, an annual cancer screening for three years after diagnosis is recommended in myositis patients with presence of TIF1-γ or NXP-2 antibodies, IMNM with HMGCR antibodies or without myositis antibodies.[74] When IMNM is clearly statin induced cancer screening is probably not necessary.

Conventional tumour screening is performed by clinical history and physical examination, CT of chest, abdomen and pelvis, urine cytology, faecal occult blood testing and mammography and gynaecological examination for females.[75] Additional test such as gastroscopy or colonoscopy should be considered, depending on the age, sex and medical history of the

patient. In Asian patients, nasopharyngeal cancer is prevalent. Particularly in patients with TIF1-γ and NXP-2-antibodies or b-symptoms (fever, weight loss, night sweats), a PET-CT could be considered.

General principles of treatment for inflammatory myopathies

Corticosteroids are the first line treatment in all IIMs except for IBM. In IBM, there is no evidence of sustained treatment effects, although a few treatment studies and case reports have shown positive effects on dysphagia in selected patients. Usually, the therapy starts with oral prednisolone (1mg/kg/day) for 4 weeks with tapering over weeks until disease remission is achieved. The dosage of prednisolone in maintenance therapy should be as low as possible to avoid any side effects. Oral dexamethasone pulse therapy (six cycles of 40mg/day for four consecutive days with 28 day intervals) could replace prednisone, and showed less side effects in a randomized controlled trial compared to prednisone.[76] Long term corticosteroid therapy should be avoided because of the significant side effects. Timely start of corticosteroid-sparing therapy is advocated e.g. azathioprine, mycophenolate mofetil or methotrexate. Typical side effects of long-term corticosteroid therapy are diabetes, hypertension, steroid-induced muscle weakness, osteoporosis, adrenal insufficiency, cataract and glaucoma.[77]

In a rapidly progressive myositis, when bulbar symptoms are present, or life-threatening comorbidities such as ILD are present methylprednisolone pulse therapy (0.5-1g/day for 3-5 days) instead of oral corticosteroids is advocated. If required, treatment can be intensified by other immunosuppressive or immunomodulating agents e.g. rituximab or IVIg (**figure 4** and paragraph 'Emergencies in IIM and their management'). Especially IMNM often responds insufficiently to corticosteroid monotherapy and requires additional immunosuppressants or IVIg. Most existing therapies are based on expert opinions and case series. The benefit of early treatment of rituximab in refractory dermatomyositis in comparison to a later initiation of rituximab therapy was tested in a randomized controlled trial and showed no significant differences, but true effects may have been masked by the trial design; and further controlled studies are needed.[78]

If the clinical condition of the patient allows, one should always try to postpone the start of immunosuppressive therapy until the diagnosis has been confirmed by a muscle biopsy (with the exception of DM with typical skin lesions or ASS in presence of antibodies).

Treatment of cancer associated myositis (CAM)

The association between IIMs and cancer is well known and extensively reported. The muscular and skin involvement can be severe and often rapidly progressive. Therefore, early diagnosis of the tumour is important. The standard treatment of myositis is not sufficient in CAM, while treatment of the tumour usually improves the myositis symptoms. Relapse of cancer typically leads to a relapse of CAM.[79]

The malignancy should be treated according to specific therapeutic standards (surgery, chemotherapy, radiotherapy, hormone therapy). It was shown that, after tumour surgery, the serum CK activity was reduced significantly and symptoms improved.[80] In addition, immunosuppressive treatment is part of the standard care. Glucocorticoids and/or IVIg do not pose a risk of tumour development as side-effect. In addition, triple therapy with prednisone, IVIg and cyclosporine is given in some hospitals.[81]

Data on treatment with immune checkpoint inhibitors, which can induce myositis, in patients with paraneoplastic IIM is scarce, and should therefore be discussed within a multidisciplinary team.

The overall prognosis of CAM is poor and depends on the nature of the tumour and its progression.

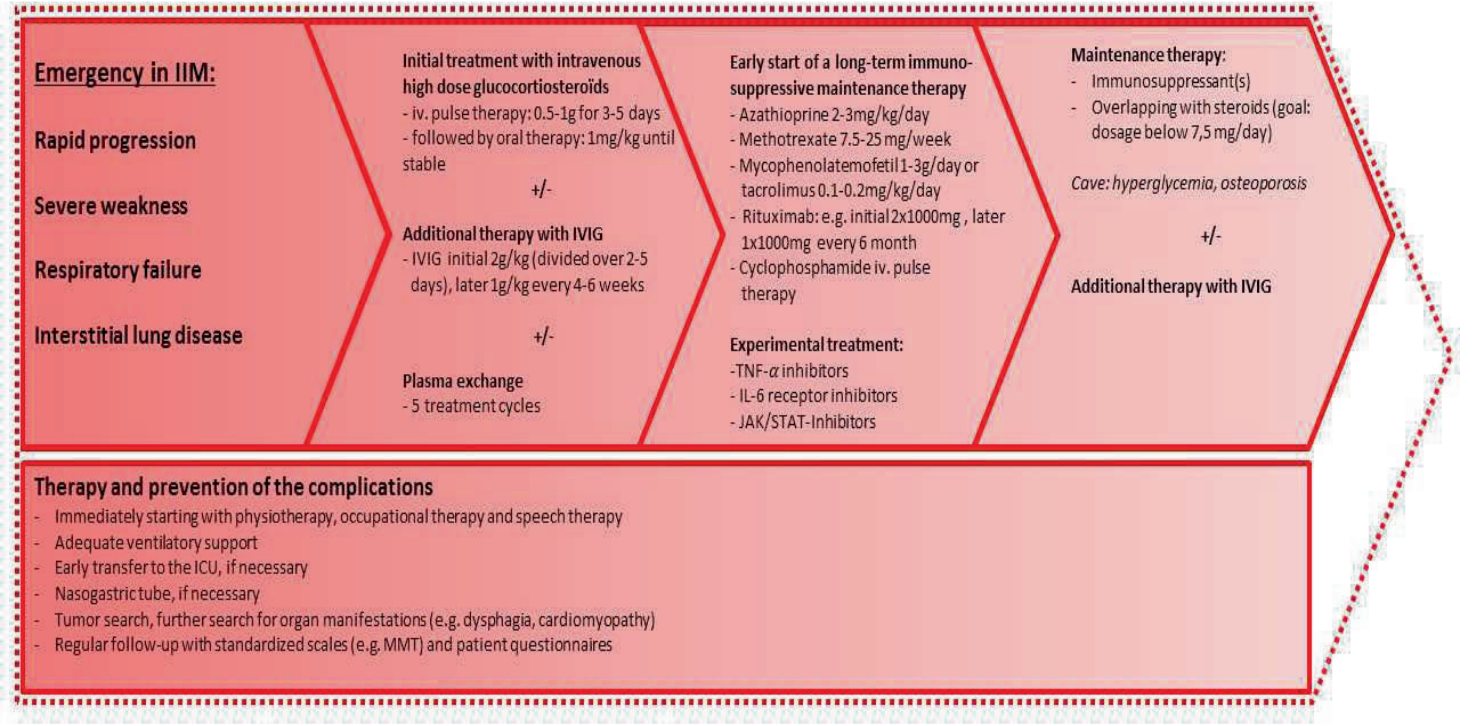

Figure 4. Treatment of emergencies in idiopathic inflammatory myopathy, some elements modified from Glaubitz et al.[82]

Emergencies in IIM and their management

As idiopathic inflammatory myopathies are a spectrum of diseases, emergencies can present in many different ways. During the course of IIMs, interstitial lung disease, malignancy and cardiac involvement are the three most common causes of death and, thus, these need to be considered during emergency treatment in IIM.[64]

Case vignette 1: Muscle weakness and ILD

A 42-year-old Afro-American woman started experiencing malaise, arthralgia and dyspnea one month before her visit to the outpatient clinic. Laboratory tests showed anti-SSA, anti-SM and anti-RNP antibodies. A pulmonary CT showed infiltrative abnormalities consistent with an organizing pneumonia and the patient was diagnosed with Sjögren/Mixed Connective Tissue Disease. Prednisone 15mg/day was started, without improvement of dyspnea and arthralgia. Although a new CT showed a decrease of the pulmonary abnormalities, her dyspnea became incapacitating, with a vital capacity (VC) of 1.2L (42%). She was then referred to the pulmonology outpatient clinic of an academic hospital.
Additional pulmonary diffusion tests showed a diffusing capacity for carbon monoxide of 25% with a total lung capacity of 1.99 l/44%. Ultrasonography of diaphragm showed decreased excursions. Pulmonary CT showed interstitial abnormalities and NSIP (Nonspecific Interstitial Pneumonitis). Laboratory investigations showed a CK of 1800 U/l. A myositis immunoblot was performed and showed strongly positive anti-EJ and anti-Ro52 antibodies. Examination at the Neurology outpatient clinic revealed symmetrical proximal muscle weakness of deltoid, biceps brachii, iliopsoas, gluteus medius and maximus muscles (MRC grade 4), and dyspnea. She could only get up from a chair with the support of her arms. Her cuticles were darkened and showed small lesions. A whole-body MRI was performed and showed edema in the musculus obturatorius, gluteal musculature, hamstrings and gastrocnemius muscles, without atrophy or fatty replacement (**figure 5**). Because of the combination of proximal muscle weakness, elevated CK, muscle edema on MRI, positive anti-EJ antibodies and reduced pulmonary function, she was diagnosed with antisynthetasesyndrome.
Troponine-T was elevated (0.384µg/L), but cardiac MRI appeared normal. Because of incapacitating ILD and concomitant myositis, she was treated with intravenous methylprednisolone pulse therapy, and continued at home with 20mg prednisone per day. Tacrolimus was added after three cycles of methylprednisolone, five months after disease onset. Clinically she markedly improved and was able to resume normal daily activities. Repeat pulmonary function tests four months later also showed improvement.

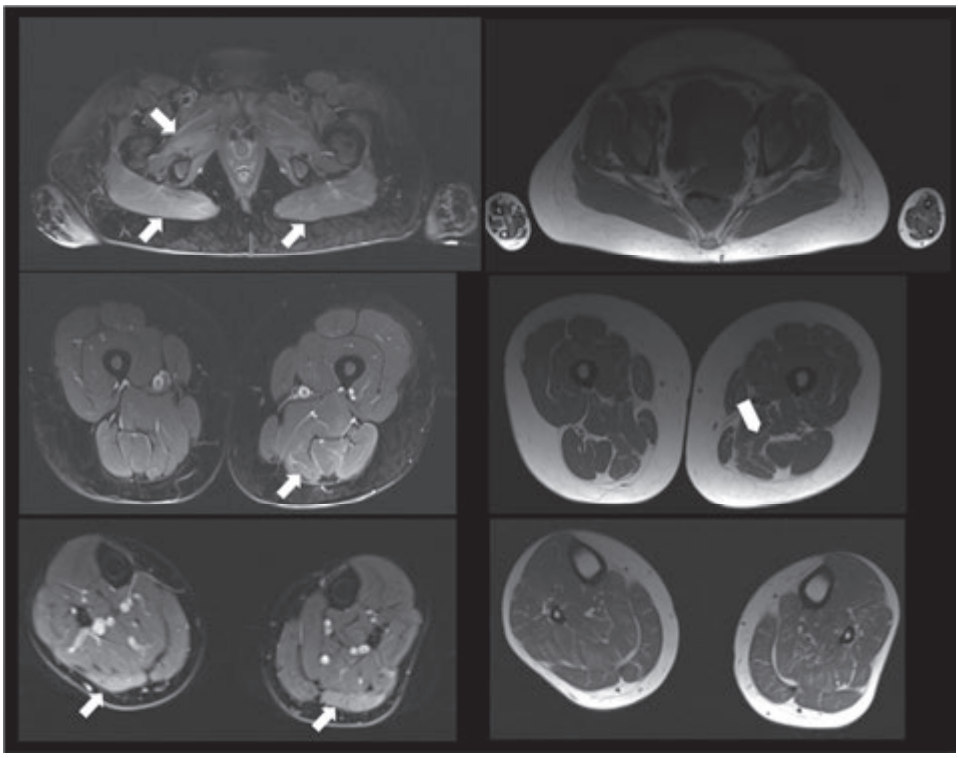

Figure 5. Axial images of lower limbs by T2 Dixon muscle MRI of the described patient in case vignette 1.

Upper level: white arrows show oedema in gluteus musculature bilaterally (left image). No fatty infiltration was shown (right image). Middle level: Oedema in hamstring musculature (left image). Starting of fatty infiltration (right image). Lower level: Oedema in gastrocnemius muscles bilaterally (left image). No fatty infiltration was found (right image).

Management of severe muscle weakness

The disease course of IIM is often subacute, but particularly IMNM may lead to rapid, severe weakness. Delayed treatment may cause irreversible muscle damage and atrophy, and contractures and disability may ensue. To prevent this, severe muscle weakness needs to be treated quickly and according to an adequate, rigorous treatment escalation scheme.[76] Initial oral or intravenous glucocorticosteroids are usually complemented early with immunosuppressants such as azathioprine or methotrexate. Add-on treatment with IVIg and escalation treatment with rituximab and other drugs is often required **(Figure 4)**. For anti-SRP as well as anti-HMGCR associated necrotizing myopathies, the addition of a second agent (such as methotrexate) is advised within one month if there is no significant improvement.[13] For anti-SRP myopathy rituximab should always be considered within six months if initial corticosteroids and immunosuppressants are insufficient. For anti-HMGCR IVIg should be added if other therapeutics are not effective. The decision for the type of add-on immunosuppressant treatment is mostly based on retrospective studies and expert opinion.
The intensive-care unit (ICU) team must be reassured that chances for good recovery are high, even with severe prolonged weakness.[83] Timely physiotherapy and suitable splints are very important to avoid disabling contractures.

Management of severe dysphagia

Dysphagia is associated with a high risk for chocking, and concomitant aspiration, pneumonia, and severe weight loss. Immunosuppressive treatment of dysphagia is similar to that of the other manifestations of IIM, albeit in severe dysphagia intravenous methylprednisolone instead of oral corticosteroids should be administered. In addition, nutritional advices, and counselling for speech therapy is necessary. When the risk of choking is high, one needs to consider a nasogastric feeding-tube until the swallowing function has improved. A PEG-tube should be considered if swallowing does not improve.

Management of interstitial lung disease

Early recognition of interstitial lung disease (ILD) in IIM patients is the key to prevent irreversible lung damage. Once a patient with IIM is admitted to the (outpatient) clinic and ILD is suspected, the patient should undergo pulmonary function tests including (sitting and

supine) vital capacity and diffusion/restriction and/or a HRCT of the lungs to detect interstitial pneumonia.

In case of unknown cause of acute respiratory distress (ARD), broncho-alveolar lavage is not very useful to diagnose ASS or anti-MDA5 IIM but can be performed to rule out other diagnoses. The presence of increased C-reactive protein, in the absence of increased serum procalcitonine could indicate a non-infectious inflammatory process. Demonstration of myositis-specific antibodies using myositis immunoblot may support the existence of a myositis-related respiratory distress.

The poor prognosis of untreated ILD calls for quick and rigorous treatment. Knowledge about immunosuppressant treatment is mostly based on retrospective studies due to the absence of randomized clinical trials. High doses (intravenous) steroids are the cornerstone of the treatment. There is consensus to start a steroid-saving immunosuppressant drug early on. As such, mycophenolate mofetil, tacrolimus, rituximab or cyclophosphamide can be given.[44 55] Since methotrexate can occasionally be associated with lung injury, i.e. pneumonitis, this drug should be used with caution in ILD.[84] In addition, plasmapheresis and IVIg can be used. Escalation to rituximab is often required. One should be aware that ILD in anti-MDA5 positive patients can progress very fast and is often refractory to standard immunosuppressive treatment.[85] ILD in ASS often responds to immunosuppressive therapy, but tends to show a recurrent clinical course. Beside drug treatment, referral to a lung transplant specialist is advocated to inform and counsel the patient in a non-urgent setting. If the pulmonary status is severe, a lung transplant specialist should be consulted directly.
Lung transplantation may be an option when, despite immunosuppressive therapies, patients suffer from progressive decline in pulmonary function.[86]
Importantly, inflammation of respiratory muscles can cause weakness, and subsequently dyspnoea and respiratory failure may develop. This needs to be considered during diagnosis of patients with IIM and respiratory failure.

Management of cardiac involvement

Electrocardiogram, ultrasonography and cardiac MRI (CMR) can show abnormalities in IIM patients. CMR is suggested to be a useful and non-invasive modality to diagnose and monitor

myocardial inflammation in IIM. CMR detects subclinical involvement of the myocardium in patients with myositis more precisely than cardiac muscle scintigraphy or ultrasonography,[87] [88] and contrast-enhanced MRI with gadolinium can differentiate between myocardial infarction and inflammatory tissue from myocarditis.[89]

Treatment of myocarditis relates to the underlying pathophysiology[89], i.e., the systemic standard treatment consisting of corticosteroids and other immunosuppressive therapy is applied to treat cardiac involvement. Cyclophosphamide, cyclosporine, methotrexate and azathioprine have been used as treatment of cardiac involvement, but their efficacy remains unsure since reliable study data are not available. IVIg may be considered, but interdisciplinary care including the cardiologist is necessary to prevent volume overload.[88]

Research about the effects of corticosteroid treatment or other treatment options on cardiac complications is limited. No guidelines or randomized trials exist. Studies show conflicting results: one study showed normalization of heart failure and CMR abnormalities, but another study reported poor response of ECG abnormalities on corticosteroid treatment.[90] [91] When congestive heart failure is present, traditional cardiac medication including β-blockers, diuretics, angiotensin-converting enzyme (ACE) inhibitors or angiotensin-II receptor blockers (ARBs) can be used.[89]

Management of severe cutaneous lesions

To characterize the diseases severity and the activity of the cutaneous lesions, the 'Cutaneous Dermatomyositis Disease Area and Severity Index' (CDASI) can be used. It rates the existence of erythema, scale, erosion and ulceration in 15 anatomical locations, as well as problems of calcinosis, poikiloderma and rates the severity of alopecia and Gottron papules.[92]

The initial therapy of IIM consisting of corticosteroids and immunosuppressants should improve the skin changes and the muscle symptoms.[93] Furthermore, topical calcineurin inhibitors like tacrolimus ointment (0,1%) may lead to improvement of the skin lesions.[101] Antihistamines can help patients with pruritus.[94] In addition to the systemic therapy, topical substances like mild-potency steroids, menthol or pramoxine can ameliorate generalized pruritus.[95]

When this therapy fails, a combination of antimalarial medication like hydroxychloroquine and quinacrine can further improve clinical manifestations.[94] [96] However, cutaneous drug eruption

is a common adverse event in patients with DM taking antimalarial medication.[97] In case of non-responders, the use of low-dose MTX[98] or mycophenolate mofetil was shown to be effective.[99] Patients have to use sun protection (sun protection factor (SPF) 50 minimum) and should always avoid direct sunlight.[100]

In patients with a rapid progression and refractory cutaneous lesions, IVIg can be effective.[101] IVIg has shown beneficial effects on the cutaneous lesions in up to 83% of the patients with DM.[102] Rituximab also displayed beneficial effects regarding refractory skin rashes.[103]

Some patients with severe cutaneous lesions do not respond to the standard treatment with corticosteroids, antimalarial medication, various immunosuppressant and IVIg. For these cases, JAK-inhibitors can be an experimental treatment alternative. JAK inhibitors like tofacitinib affect the 'JAK-STAT pathway' ('Janus kinase' [JAK], 'signal transducer and activator of transcription' [STAT]), which controls the pathways of different cytokines.[104] Several case series suggest a benefit from JAK inhibitors in non-responders with DM and severe cutaneous lesion.[105 106]

Therapy recommendation for calcinosis consists of immunosuppressive therapy in addition with calcium channel blockers and bisphosphonates.[107] Mechanical therapies like surgery in addition to diltiazem[108 109] or electric shock wave lithotripsy can alleviate the pain and immobility.[108]

AIM AND OUTLINE OF THE THESIS

Aim

The focus of this thesis is on diagnostic testing and the evaluation of possible modalities for the follow-up of patients with IIM. The overarching aim of this thesis is to improve the strategy leading to a complete and accurate diagnosis in IIM and identify useful markers for follow up.

Outline

Within the possible diagnostic tests for IIM, muscle imaging plays an important role. In **chapter 2**, we evaluate the diagnostic accuracy of quantitative, semi-quantitative and qualitative muscle ultrasound and muscle magnetic resonance imaging (MRI) in the diagnosis of IIM and compare their potential in the follow-up of myositis patients.

Cardiac involvement is a common complication in IIM, and results in a mortality rate of approximately 4%.[110] Cardiac screening in patients with IIM is advised, yet standardized screening strategies are lacking. In **chapter 3**, we investigate different diagnostic modalities to trace cardiac involvement – (peri)myocarditis – in patients with IIM. By using Constructing Classification and Regression Tree (CART) analysis, a diagnostic strategy of sequentially added diagnostic tests is proposed.

Other currently used diagnostic tests for IIM – i.e. muscle imaging, EMG, antibody testing and muscle biopsy – have undergone technical improvement during the past decades, but a muscle biopsy is still considered necessary to diagnose an IIM in most patients. In **chapter 4** we describe the rationale and study protocol for the ongoing ADAPT study, which aims for a diagnostic strategy with a high diagnostic accuracy and the least patient burden. We describe a prospective overcomplete diagnostic study design, using muscle imaging, EMG, antibody testing and muscle biopsy.

In **chapter 5**, we explore a new diagnostic field for myositis: B-cell receptor (BcR) sequencing. We investigate if BcR repertoires show overlap between muscle tissue and blood of myositis patients, and we show preliminary evidence for a correlation between characteristics of the clonal repertoire and response to treatment.

The International Myositis Assessment and Clinical Studies Group (IMACS) questionnaire[111] is an internationally accepted and validated outcome measure for treatment evaluation in patients with IIM. One of the limitations of this scale is that it measures improvement, but not deterioration. In **chapter 6**, we introduce the Academic Medical Centre (AMC) Linear Disability Scale (ALDS) to evaluate treatment response in IIM and describe it's clinimetric qualities.

In **chapter 7**, we summarize the results of the different studies and provide a general discussion and directions for future research.

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AW, SG, JS and AK drafted the manuscript. AW, SG, JS and AK accept responsibility for conduct of research and gave final approval.

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2

Chapter 2

Ultrasound and MR muscle imaging in new onset idiopathic inflammatory myopathies at diagnosis and after treatment: a comparative pilot study

Hannah A.W. Walter[1]*, Johan Lim[1]*, Joost Raaphorst[1], Frank F. Smithuis[2], J. Michiel den Harder[2], Filip Eftimov[1], Wouter Potters[1], Christiaan G.J. Saris[4], Marianne de Visser[1], Ivo N. van Schaik[1,5], Rob J. de Haan[3], Anneke J. van der Kooi[1], Camiel Verhamme[1]

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[1] DEPARTMENT OF NEUROLOGY, AMSTERDAM UNIVERSITY MEDICAL CENTRE, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF RADIOLOGY AND NUCLEAR MEDICINE, AMSTERDAM UNIVERSITY MEDICAL CENTRE, AMSTERDAM MOVEMENT SCIENCES, AMSTERDAM, THE NETHERLANDS
[3] DEPARTMENT OF CLINICAL EPIDEMIOLOGY, BIOSTATISTICS AND BIOINFORMATICS, AMSTERDAM UNIVERSITY MEDICAL CENTRE, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[4] DEPARTMENT OF NEUROLOGY, RADBOUD UMC, DONDERS INSTITUTE FOR BRAIN COGNITION AND BEHAVIOUR, RADBOUD UNIVERSITY, NIJMEGEN, THE NETHERLANDS
[5] SPAARNE GASTHUIS, HAARLEM, THE NETHERLANDS

* SHARED FIRST AUTHORSHIP

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ABSTRACT

Objectives: To prospectively compare ultrasound (US) and whole-body Magnetic Resonance Imaging (MRI) for detection of muscle abnormalities compatible with idiopathic inflammatory myopathies (IIM).

Methods: Newly diagnosed IIM patients underwent US (14 muscles) and MRI (36 muscles) at diagnosis and after nine weeks monotherapy with intravenous immunoglobulin. Muscles were compatible with IIM when quantitative US echo-intensity (EI) z-score was ≥1.5, semi-quantitative US Heckmatt score was ≥2, qualitative US was abnormal, or when MRI showed oedema on T2-weighted images. At patient level, findings were classified as abnormal when quantitative US EI z-score was >1.5 (n=3 muscles), >2.5 (n=2 muscles) or >3.5 (n=1 muscle), or if ≥3 muscles showed abnormalities as described above for the other diagnostic methods.

Results: At diagnosis, in 18 patients US of 252 muscles revealed abnormalities in 36 muscles (14%) with quantitative, in 153 (61%) with semi-quantitative and in 168 (67%) with qualitative analysis. MRI showed oedema in 476 out of 623 muscles (76%). Five patients (28%) reached abnormal classification with quantitative US, 16 (89%) with semi-quantitative and qualitative US, and all patients (100%) with MRI. Nine-week follow-up of 12 patients showed no change over time with quantitative US or MRI, and a decrease in abnormalities with semi-quantitative US ($p<0.01$), and qualitative US ($p<0.01$).

Conclusion: At diagnosis, MRI was more sensitive than US to detect muscle abnormalities compatible with IIM. Semi-quantitative US and qualitative US detected abnormalities in the majority of the patients while evaluating fewer muscles than MRI and showed change over time after nine weeks of treatment.

INTRODUCTION

Dermatomyositis, antisynthetasesyndrome, immune-mediated necrotizing myopathy and non-specific /overlap myositis are treatable idiopathic inflammatory myopathies (IIMs) that typically present with proximal muscle weakness.[1] Muscle imaging has become an increasingly important tool in the diagnostic work-up and follow-up of IIMs. Both ultrasound (US) and magnetic resonance imaging (MRI) can demonstrate abnormalities compatible with IIMs in the muscle and subcutaneous fat and fasciitis, which is usually present in the active (early) phase of IIMs as oedema.[2-4] Oedema can be seen as a hyperintense signal on T2 MRI and may result in echo-intensity changes on US. Other detectable muscle abnormalities, such as muscle atrophy and fatty replacement, typically occurring as signs of chronic damage, may be found in later disease phases, and are less specific for IIMs.[5-9]

Whereas US is easily accessible, and without claustrophobic issues, whole-body MRI provides a representation of a large number of muscles including deep muscles that are not assessable by US. US acquisition is operator dependent, MRI protocols may differ between centres, and US and MRI image analysis both require experienced evaluators.

Studies comparing the usefulness of muscle US as compared to MRI in IIMs are scarce: some studies compared US to MRI in only one muscle via grayscale analysis, muscle perfusion or strain sono-elastography at diagnosis[10 11] and another study compared the diagnostic value of US and MRI cross-sectionally during the disease process in mainly amyopathic DM patients [2]. To date, no studies systematically compared both techniques during follow-up. A recent expert review states that the position of US as compared to MRI in the diagnosis of IIMs is unknown and comparative longitudinal studies are needed.[12]

We aimed to compare the ability of US and whole-body MRI to detect muscle abnormalities in IIM patients at diagnosis and correlated these abnormalities with markers used in daily clinical practice such as muscle strength and serum creatine kinase (sCK). Additionally, we investigated changes in these parameters at follow-up after a nine-week period of treatment with intravenous immunoglobulins (IVIg).

METHODS

Patients

We used data of patients in a phase-2 open-label cohort study on IVIg as first-line treatment. [13] The patients had newly diagnosed, biopsy-proven IIM, were assessed clinically before and

during treatment and underwent US and MRI at diagnosis and after 9 weeks of treatment. All patients signed informed consent prior to inclusion. The study protocol has been approved by the medical ethics committee of the Academic Medical Centre, Amsterdam, The Netherlands, and was conducted in accordance with the declaration of Helsinki.

Clinical examination

Six core set measures of the International Myositis Assessment and Clinical Studies (IMACS) Group[14] were collected at baseline and during follow-up. These were the Physician Global Activity (PhGA), Patient Global Activity (PGA), Extra Muscular Activity (EMA), Muscle enzymes (among which sCK), Health Assessment Questionnaire Disability Index (HAQ-DI) and Manual Muscle Testing (MMT13). The MMT scores the individual muscle strength ranging from 0-10, and the total score per patient ranged from 0 to 260, with higher scores representing a better strength.

Ultrasound

The standardized US examination was done by trained neurophysiologists (C.V. and C.S.) using an ultrasound scanner with an 8–14 MHz broadband linear transducer with a 53-mm footprint and an axial resolution of around 0.2 mm (MyLabTwice, Esaote SpA, Genoa, Italy). Images were anonymized prior to scoring for patient identification and for the moment of examination (i.e. baseline or follow-up). [15 16] For the assessment of muscle abnormalities with quantitative, semi-quantitative and qualitative analysis, we used the same ultrasound images at standardized anatomical sites.[17]

Echo-intensity of 14 muscles (bilateral deltoid, biceps brachii, flexor carpi radialis, flexor digitorum profundus, rectus femoris, vastus lateralis and tibialis anterior) was scored with three methods:

Quantitative analysis: mean echo-intensity (EI) of standardized predefined regions of interest were compared to muscle-specific reference values from a healthy control population and expressed as z-scores. Abnormal EI was defined as a z-score ≥1.5 for an individual muscle. [17 18] Regions of interest were drawn manually in the muscles by A.W., J.L, and C.V.

Semi-quantitative analysis: EI was visually rated using the 4-point Heckmatt grading scale (1-4); a score ≥2 was considered abnormal.[6] Scoring was performed by C.S.

Qualitative analysis: EI was visually rated as normal or abnormal. An abnormal score was reached when there were visual changes in EI. These visual changes also included a 'shine-through' appearance or 'see-through echogenicity increase' as described for muscle oedema, which can be missed by the semi-quantitative Heckmatt grading, and/or focal changes i.e focal areas of increased echogenicity, loss of definition of perimysial septa and focal change in echotexture.[12] Scoring was performed by C.S.

Muscle thickness (MT) of the same 14 muscles was scored quantitatively and decreased muscle thickness was defined as a z-score < –2 for an individual muscle.[17 19] Fascial thickness was scored quantitatively for the deltoid, rectus femoris and vastus lateralis muscles and considered abnormal when showing a z-score >2 compared to previous published normal values.[16] The callipers to measure the MT and FT were positioned manually by A.W, J.L, and C.V with 0.25 cm interval. In addition, fascial thickness was scored qualitatively for the fasciae of all 14 muscles.

At the patient level, quantitative US analysis was abnormal in case of an EI z-score ≥1.5SD in at least three muscles, a z-score of ≥2.5 in two muscles or a z-score of ≥3.5 in one muscle.[18] Semi-quantitative and qualitative US analysis were scored abnormal, when at least three muscles showed abnormalities according to the EI criteria described above. The number of abnormal muscles per patient were calculated for quantitative, semi-quantitative and qualitative US. For semi-quantitative analysis a Heckmatt sumscore per patient was calculated ranging from 0 to 42. To calculate this sumscore, Heckmatt scores 1 – 4 of individual muscles were recoded to 0 – 3 and summed.

The number of muscles with maximal muscle strength according to manual muscle strength evaluation and with abnormal muscles according to semi-quantitative US (Heckmatt score ≥2) were calculated for ten muscles (or muscle groups) that were evaluated with MMT and US; i.e. the bilateral deltoid, biceps brachii, forearm flexors, quadriceps femoris and tibialis anterior muscles.

MRI

The standardized whole body MRI (WB-MRI) protocol, performed on a 3.0 Tesla Ingenia MRI scanner (Philips, Best, The Netherlands), included water and fat imaging using 2D coronal and

axial T2-weighted two-point Dixon scans; an equivalent MRI scanning protocol was used by Sigmund *et al.*[20] Sequences were anonymized for patient identification and for the moment of examination, and scored by a trained musculoskeletal radiologist (F.F.S) according to a previously described method.[3]

The extent of muscle oedema was semi-quantitatively scored in 36 muscle groups: cervical, deltoid, supraspinatus, infraspinatus, biceps, triceps, forearm flexors, forearm extensors, gluteal, iliopsoas, sartorius, hip adductors, quadriceps, hamstring, tensor fasciae latae, tibialis anterior, peroneus and gastrocnemius. Muscles were bilaterally scored as: 0) no oedema, 1) oedema in <50% of the muscle area, and 2) oedema in ≥50% of the muscle area.[3] The presence of muscular fatty infiltration was noted. Four fascial areas and subcutaneous areas were scored for 0) no oedema and 1) oedema.[3]

At the patient level, MRI was considered abnormal when at least three muscles showed oedema. This criterion was chosen to enable comparison with US. The number of abnormal muscles per patient was calculated. Additionally, an MRI oedema sumscore per patient was calculated ranging from 0 to 72. To calculate this MRI oedema sumscore the 0 – 2 MRI scores were added together.

The number of muscles with maximal muscle strength according to manual muscle strength evaluation and with muscle oedema on MRI (oedema score ≥1) were calculated for 19 muscles, i.e. neck flexors, deltoid, biceps brachii, forearm flexors, gluteus musculature, iliopsoas, quadriceps, hamstrings, tibialis anterior and gastrocnemius muscles.

Statistical analysis

Patient baseline characteristics, IMACS core set measures, scores of the different imaging modalities (quantitative US, semi-quantitative US, semi-quantitative sumscore, qualitative US, US fascia thickness, US muscle thickness, MRI oedema score, MRI oedema (average) sumscore, MRI subcutaneous oedema and MRI fascial oedema) at baseline and follow-up, and number of muscles showing maximal MMT and US or MRI abnormalities at baseline were summarized using descriptive statistics. An MRI oedema average sumscore, was calculated in case of missing data: the sumscore was divided by the amount of valid muscles per patient and subsequently multiplied by the total amount of muscles.

Differences between follow-up scores and baseline scores of the IMACS core set measures, and different imaging modalities described above were expressed in median change scores with interquartile ranges. For both the MRI data and US data, differences between non-normally distributed continuous baseline and follow-up parameters were analysed using the Wilcoxon signed rank test.

Friedman ANOVA, for three or more non-parametric variables and with an ordinal variable as the dependant variable, was used to compare the baseline scores of quantitative, semi-quantitative, qualitative US and MRI for five muscle groups that were measured with both imaging modalities; deltoid, biceps, forearm flexors (US: flexor carpi radialis and flexor digitorum profundus), quadriceps femoris (US: rectus femoris and vastus lateralis) and tibialis anterior. When Friedman ANOVA showed statistically significant differences ($p<0.05$) between the groups, we performed post hoc pairwise comparisons by Wilcoxon signed rank tests.

Correlations at baseline and follow-up

Spearman's rank correlation (r_s) was used to assess the association between the semi-quantitative US sumscore and MRI oedema sumscore on the one hand, and muscle strength (MMT13) and sCK on the other hand.

Spearman's rank correlation (r_s) was used to assess the association between the change scores (follow-up – baseline) of semi-quantitative US sumscores and MRI oedema sumscores with change scores of MMT13 and change of sCK over time. A bootstrap procedure was used to compare the correlations between the change scores.

Statistical significance was defined as a two-sided p-value < 0.05. In view of the explorative nature of this pilot study we did not correct for multiple comparisons.[21] All analyses were performed in SPSS version 26 (IBM, Inc., Chicago, Illinois), apart from the bootstrap procedure which was performed in RStudio 3.6.1. (www.rstudio.org).

RESULTS

Patients

Twenty newly diagnosed, biopsy-proven IIM patients participated in the IMMEDIATE study.[22] Eighteen out of 20 patients underwent both US and MRI at diagnosis. Due to logistical issues, one patient missed MRI and another patient missed US at baseline. Baseline characteristics are summarized in **Table 1**. All patients had muscle weakness. Nine had dermatomyositis (50%), four immune mediated necrotizing myopathy (22%), four non-specific/overlap myositis (22%) and one antisynthetasesyndrome (6%).

Baseline

At baseline, US and MRI were performed with a median of three days in between (range 0-21 days). In three patients, one or both examinations were performed after initiation of IVIg: US after 19 days in one patient, MRI after one day in one patient, and both US and MRI after five days in another patient. **Figure 1** shows images of US and MRI in one patient.

Ultrasound

At the muscle level, quantitative US analysis showed EI abnormalities in 36 out of 252 muscles (14%). Semi-quantitative US showed EI changes in 153 out of 252 muscles (61%), and in qualitative US EI changes were found in in 168 out of 252 muscles (67%), among which focal changes were present in 12 muscles (5%) (Supplementary Table S1 and S2). Quantitative US showed that MT was reduced in 47 out of 252 muscles (19%). Increased fascia thickness was found in 17 out of 170 fasciae (10%). Qualitative US showed increased thickness in 8 out of 252 fasciae (3%).

At the patient level, abnormality criteria based on EI were fulfilled in five patients (28%) based on quantitative US, and in 16 patients (89%) based on both semi-quantitative US and qualitative US.

Table 2 shows the median number of abnormal muscles per patient per US method, and the semi-quantitative US sumscore.

Semi-quantitative US showed EI abnormalities in 59 out of 72 muscles (82%) with a maximal MMT, predominantly in forearm flexors and tibialis anterior.

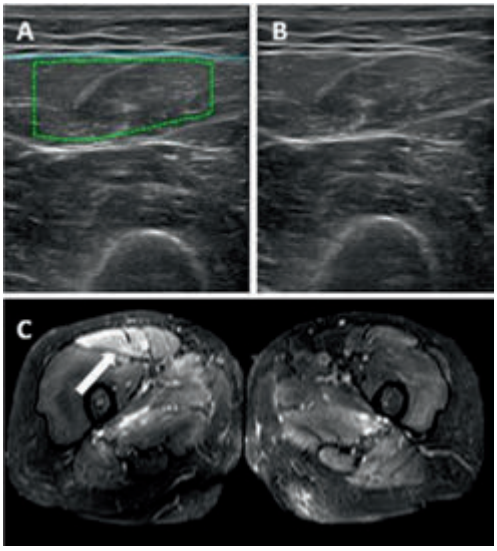

Figure 1. Images of the right rectus femoris of the same patient using different imaging modalities. Scoring for right rectus femoris muscle showed in figure: Panel A, quantitative US z-score 0.44; Panel B, qualitative US visually abnormal, Heckmatt score 2; Panel C, MRI score 2 (white arrow).

MRI

At the muscle level, oedema was present in 476 out of 623 muscle groups (76%); 206 muscles (33%) showed ≤50% oedema, 270 muscles (43%) showed >50% oedema (Supplementary Table S3).

At the patient level, all 18 patients (100%) fulfilled our predefined criteria for muscle MRI abnormality. Two patients (11%) showed some fatty infiltration one in the gluteus medius and another in quadriceps and hamstring muscles.

Fascial oedema was present in 47 out of 132 regions (36%) and subcutaneous oedema in 44 out of 136 regions (32%), both were present amongst all IIM subtypes (**Table 2)**.

MRI showed oedema in 45 out of 98 muscles (46%) with a maximum MMT, predominantly in tibialis anterior and gastrocnemius muscles.

Table 1. Baseline characteristics of 18 included patients in baseline analysis.

Characteristic	Outcome
Age in years at diagnosis, median (IQR)	55 (36 – 68)
Time between first symptom and diagnosis, months; median (IQR)	4.5 (3.8 – 6.3)
Gender, male n (%)	8 (44.4)
Dysphagia, n (%)	13 (72.2)
Cancer, n (%)	1 (5.6)
Connective tissue disorder, n (%)	3 (16.7)
sCK, U/L median (IQR)	960 (160 – 4971)
MMT13 score, median (IQR)	214 (185 - 227)
Neck flexors	7 (4 – 8)
Neck extensors	9 (8 – 10)
Trapezius	10 (10 – 10)
Deltoid	9 (4 – 9)
Biceps brachii	9 (7.8 – 9)
Wrist extensors	9.5 (8 – 10)
Wrist flexors	10 (8 – 10)
Iliopsoas	6 (6 – 7.3)
Quadriceps	9 (9 – 9)
Gluteus maximus	8 (3 – 8)
Gluteus medius	8 (4 – 8.3)
Hamstrings	8 (7 – 8)
Tibialis anterior	10 (10 – 10)
Gastrocnemius	10 (10 – 10)

sCK, serum creatine kinase; MMT, manual muscle testing according to Kendall. Score is based on MMT13, MMT scores of the right muscles are displayed for the limb muscles (no statistically significant difference between right and left muscle MMT score).

Table 2. Clinical measures and abnormalities found by US and MRI in twelve patients at baseline, at follow-up and changes over time.

	Score range	Baseline n=12, median; IQR	Follow-up n=12, median; IQR	Change score, follow-up - baseline, median; IQR	p-value [a]
IMACS core set measures					
PhGA	0 -10	3.6; 3.3 – 4.0	2.1; 1.0 – 3.7	-1.5; -2.2 – -0.2	<0.01
PaGA	0 - 10	6.0; 5.0 – 7.6	3.4; 1.6 – 6.5	-2.8; -3.9 – 0.6	0.07
MMT	0 – 260	213; 189.0 – 234.0	229; 209.0 – 240.0	11.0; -6.0 – 29	0.09
EMA	0 -10	2.1; 0.8 – 2.9	1.4; 0.3 – 2.7	-0.4; -0.8 – 0.0	0.20
HAQ	0 - 3	2.0; 1.4 – 2.5	1.1; 0.3 – 2.0	-0.7; -1.1 – -0.2	<0.01
sCK (U/L)		960; 102.0 – 8559.0	490; 84.0 – 4733.0	-61.0; -2722.0 – -13.0	0.08
Ultrasound					
Muscle echo-intensity					
Quantitative [b]	0 - 14	2; 0 – 3	1; 0 – 2	-1; -1 – 0	0.22
Semi-quantitative [c]					
Score ≥2 [d]	0 – 14	11; 6 – 14	5; 4 – 8	-4; -7 – -1	<0.01
Sumscore [e]	0 - 42	13; 6 – 14	5; 4 – 9	-4; -8 – -2	<0.01
Qualitative					
Visual [f]	0 – 14	11; 6 – 14	5; 4 – 10	-4; -4 – -1	<0.01
Focal abnormalities	0 – 14	0; 0 – 1	0; 0 – 1	0	1.0
Fascia thickness					
Quantitative [g]	0 – 10	1; 0 – 2	1; 0 – 1	0; -1 – 1	0.60
Qualitative	0 – 10	0; 0 – 0	0; 0 – 0	0	0.41
Muscle thickness [h]					
Quantitative	0 – 10	3; 1 – 4	4; 1 – 7	1; -1 – 2	0.17
MRI					
Muscle oedema					
Score ≥1 [i]	0 – 36	26; 16 – 34	23; 7.5 – 31	-3; -14 – 0	0.14
Sumscore [j]	0 – 72	33; 20 – 60	29; 11 – 46	-6; -19 –1	0.08
Average sumscore	0-72	35; 20 – 60	31; 12 – 46	-6; -19 – 1	0.09
Subcutaneous oedema					
Subcutaneous score	0 – 8	3; 0 – 4	1.5; 0 – 5	0; -2 – 0	0.92
Fascial oedema					
Fascial score	0 – 8	2; 0 – 4	2; 1 – 4	0; -1 – 2	0.23

IMACS: International Myositis Assessment and Clinical Studies Group; IQR: interquartile range; PhGA: physician global activity; PaGA: patient global activity; MMT: manual muscle testing; EMA Extramuscular Assessment; HAQ: health assessment questionnaire; sCK: serum creatine kinase.

[a] Wilcoxon signed rank test, p<0.05 considered significant;
[b] Echo-intensity z-score >1.5;
[c] Heckmatt grading;
[d] Numbers of abnormal muscles per patient;
[e] Sum of recoded Heckmatt scores (1–4 to 0–3);
[f] Based on visually increased echo-intensity;
[g] Based on fascia thickness >2SD;
[h] Based on muscle thickness <2SD;

[i] Numbers of abnormal muscles per patient;
[j] Sum of MRI scores of individual muscles.

Comparison of five muscle groups between US and MRI

Analysis of five muscle groups that were measured with both modalities showed abnormal muscles in 33 out of 180 (18%) by quantitative US, in 129 out of 180 (72%) by semi-quantitative US, in 129 out of 180 (72%) in qualitative US, and in 134 out of 180 (74%) by MRI (Friedman ANOVA = 35.8, p <0.001), respectively. Quantitative US showed significantly less abnormal muscles as compared to qualitative US, semi quantitative US, and MRI (Wilcoxon signed rank test; p <0.001). Post-hoc Wilcoxon signed rank tests showed no statistically significant difference in numbers of abnormal muscles between semi-quantitative US, qualitative US and MRI for these five muscle groups.

All muscle groups showing focal changes on qualitative US showed oedema on MRI. **Table 3** shows the number of abnormally scored muscles of quantitative, semi-quantitative and qualitative ultrasound compared to MRI grading.

Correlations between imaging modalities muscle strength and sCK

At baseline, semi-quantative US sumscore (r_s = -0.674 p = 0.002), and MRI oedema sumscore (r_s =-0.583 p=0.009) correlated with MMT13. No statistically significant correlation was found between the US sumscore or MRI oedema sumscore and sCK (r_s = 0.207 p = 0.41 and r_s = 0.067 p = 0.786 respectively).

Table 3. Comparison between US and MRI in 5 muscle groups.

	MRI no oedema	**MRI <50% oedema**	**MRI ≥50% oedema**
MRI	35	53	81
Quantitative US, n (%)	5 (14%)	8 (15%)	20 (25%)
Semi-quantitative US, n (%)	23 (66%)	40 (75%)	66 (81%)
Qualitative US, n (%)	23 (66%)	40 (75%)	66 (81%)

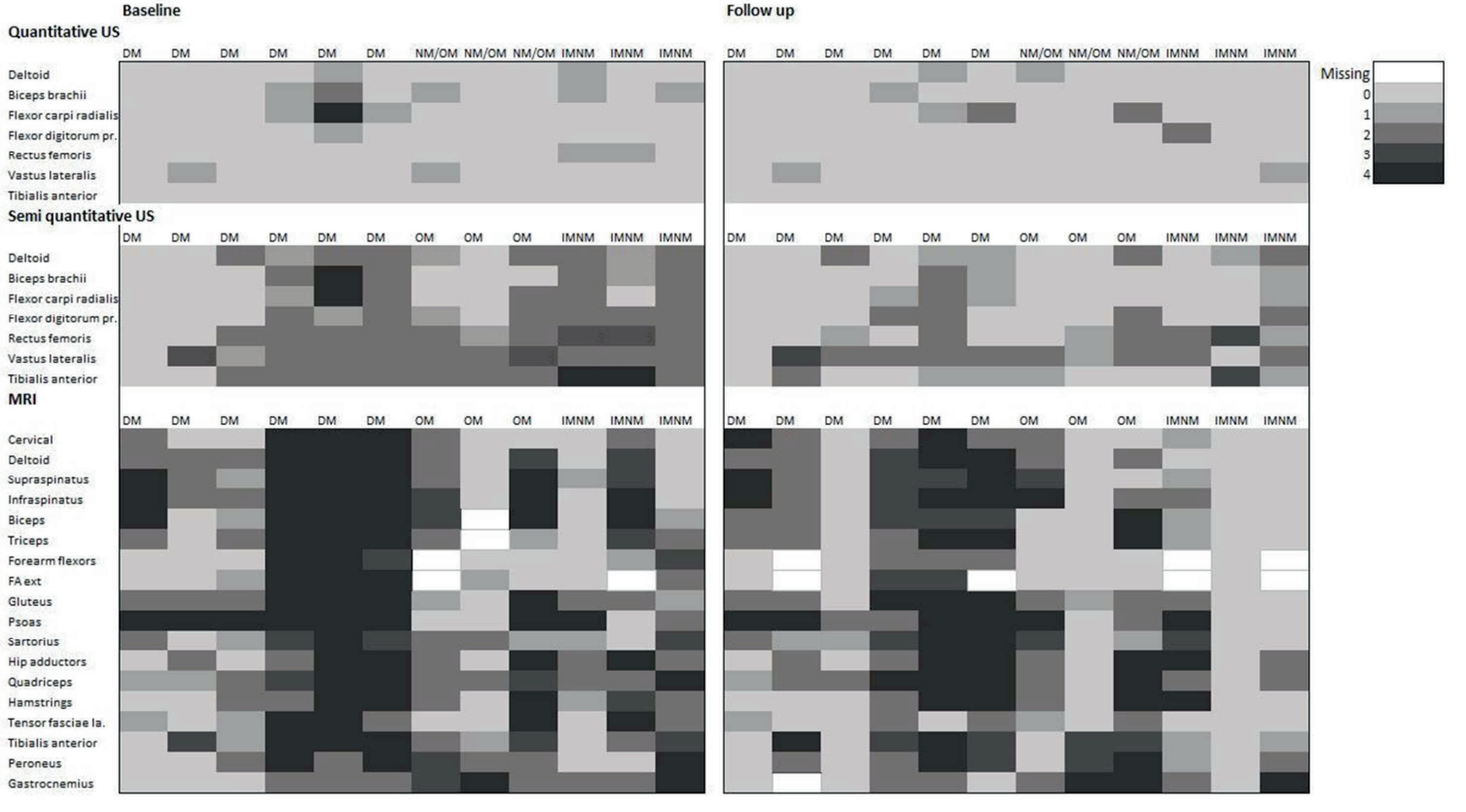

Figure 2. Heatmap of muscle abnormalities with quantitative US, semi-quantitative US, and MRI in twelve patients at baseline and at follow-up.

Patients: DM = dermatomyositis. NM/OM = non-specific/ overlap myositis. IMNM = immune mediated necrotizing myopathy. Colors: a darker shade of grey up to black represents a more abnormal muscle. Scores of the muscles of the left and right side are summed; Quantitative US: z-score <1.4 = 0; 1.5-2.4 = 1; 2.5-3.4 = 2; ≥3.5 = 3. Semi-quantitative: Heckmatt 1-4 scores were recoded to 0-3. For quantitative US and semi quantitative US the maximum score obtained was 4 (5 and 6 were not reached)
MRI: 0-2 score.

Follow-up

Twelve patients underwent both US and MRI at follow-up after a nine-week period of IVIg, with a median of 0 days in between (range 0-15 days). Median age, sCK and MMT score at baseline did not differ according to Mann-Whitney U test (all p-values >0.3) between these 12 patients and the group of six patients that was excluded at follow-up because either US or MRI were not performed, which occurred mostly due to logistical issues.

Table 2 shows the median number of abnormal muscles of these 12 patients, the change score and the p-value of change over time. **Figure 2** shows a comparison of quantitative, semi-quantitative US analysis and MRI analysis at baseline and follow-up in a heatmap.

Ultrasound

EI was assessed in 168 muscles at follow-up and compared to baseline. Quantitative US analysis showed a non-significant decrease in the number of abnormal muscles over time from 20 (12%) to 13 (8%), p = 0.22 (Supplementary Table S1). Semi-quantitative and qualitative US analysis showed a statistically significant decrease in the number of abnormal muscles over time; from 111 (66%) to 68 (40%), p= 0.01, and from 111 (66%) to 76 (55%), p = 0.01, respectively. The number of focal abnormalities (n=7; 4%) did not change over time, p = 1.0. Semi-quantitative sumscore decreased from 124 to 72 over time, p = 0.01.

The number of muscles with decreased thickness as assessed quantitatively for 168 muscles did not statistically significant change over time from 36 (19%) at baseline to 45 (23%) at follow-up (p = 0.17). The number of fasciae with increased thickness as assessed for 120 fasciae did not statistically significant change over time; for quantitative analysis from 12 (10%) at baseline to 10 (8%) at follow-up (p = 0.60) and for qualitative analysis from 2 (2%) at baseline to 5 (4%) at follow-up (p = 0.41).

MRI

The total number of oedematous muscles as assessed in 432 muscles decreased non-significantly over time from 295 out of 420 (70%) to 240 out of 412 (58%), Wilcocon signed rank test; p = 0.14. (Supplementary Table S3). The total MRI oedema average sumscore decreased from 455 to 362 over time; p = 0.09.

The number of regions with fascial oedema as assessed in 96 muscle regions did not statistically significant change over time from 33 (36%) at baseline to 25 (28%) at follow-up, p

= 0.23. The number of regions with subcutaneous oedema did not statistically significant change over time from 30 (31%) at baseline to 28 (29%) at follow-up, p = 0.92, **table 2**. Missing values are shown below Supplementary Table S3.

Correlations over time

The change score of the semi-quantitative US sumscore correlated with change MMT13 score (r_s =

-0.624 ; p = 0.03). The change MRI oedema sumscore showed no statistically significant correlation with change MMT13 score (r_s = -0.489; p = 0.11). The observed difference between these correlations was -0.15 (bootstrap 95% CI -0.70 - 0.36), showing no statistically significant difference.

The change scores of the semi-quantitative US sumscore and MRI oedema sumscore showed a correlation (r_s = 0.661, p = 0.02). The change scores of the imaging modalities showed no statistically significant correlation with the sCK change over time; change semi-quantitative US sumscore r_s = -0.114, p = 0.723 and change MRI oedema sumscore r_s = -0.070, p = 0.829.The observed difference between these correlations was -0.04 (bootstrap 95% CI -0.56 – 0.49), showing no statistically significant difference.

DISCUSSION

In this prospective, longitudinal study in patients with biopsy proven IMM we showed that semi-quantitative, qualitative US and MRI often revealed muscle abnormalities in the acute phase of IMM, while this was not the case for quantitative US. Semi-quantitative and qualitative US were able to detect changes over nine weeks of follow-up, while MRI detected no significant change over this relatively short follow-up period. MRI detected abnormalities in all patients at diagnosis, while semi-quantitative and qualitative US detected abnormalities in most patients and quantitative US detected abnormalities in a minority of the patients. Our results for MRI are in line with previous results that showed that WB-MRI is a sensitive modality in the early phases of IIM.[23-25] From our data we can derive that semi-quantitative or qualitative muscle US analysis are a reasonable alternative to identify muscle abnormalities at diagnosis, if muscle MRI is not routinely available in clinical practice.

In our study, quantitative US only detected abnormalities in a minority of adult IMM patients at diagnosis. Apparently, muscle oedema in acute myositis in adult patients may be accompanied by subtle changes in echo-intensity, which are not yet be detectable with quantitative US, while these are detectable with semi-quantitative and qualitative analysis. In a previous study quantitative US did not show an increase in EI in any of eight acute dermatomyositis patients[26], another study on juvenile dermatomyositis found increased muscle EI in >2 muscles per patient in only 28% of the patients using quantitative US at diagnosis.[19] Thus, current quantitative US methods alone may not be the best way forward to standardize outcome assessment in clinical trials in IIM, as was stated before [27]. The presence of mostly subtle changes in echo-intensity in our patient group was also reflected in semi-quantitative analysis: the higher end of the scale, reflecting a more abnormal muscle, was not reached. A study on eleven treatment naive IIM patients showed similar results compared to our cohort; semi-quantitative grading at baseline showed an increased Heckmatt score in 9 out of 11 patients, and none of the patients reached the most abnormal score.[28]

Our study showed that muscle US and MRI abnormalities were not limited to clinically weak muscles, as was shown before in juvenile dermatomyositis[3] and in adult patients.[29] In addition to previous studies in adults, our study describes which muscles with normal strength show abnormalities on imaging: predominantly forearm flexors, tibialis anterior and gastrocnemius muscles. This finding underlines the importance of imaging as an add-on to clinical examination in the diagnosis of IIMs, as it may reveal subclinical muscle abnormalities, and as such could facilitate the selection of a muscle for biopsy.

Semi-quantitative and qualitative US were able to detect changes over time during follow-up. Previous longitudinal studies on imaging in IIM are scarce. For semi-quantitative analysis, our findings are in line with the above-mentioned study[28], which showed normalisation of semi-quantitative US scores in six out of seven patients after six months.
Data on follow-up MRI in IIMs is limited, and the timing of follow-up MRI in previous literature was not standardized as in our cohort. A significant decrease in MRI intensity and MRI oedema sumscore over time during treatment was reported in a follow-up MRI after an average of 9.4 months in one study and after 2-6 months in another study[3 30], as compared to the relatively short follow-up (nine weeks) in our study which may have precluded the detection of changes

over time. Another report, in which follow-up MRI was only performed in patients who clinically did not respond to therapy, found no decrease in oedema score.[31] Currently, there is no literature that defines a clinically important difference over time; neither for US, nor for MRI, future studies are needed to investigate this clinical important difference so that it can be used in longitudinal studies.

Regarding fascia, it was shown before that thickened fascia (FT) on muscle ultrasound, and circumferential increased signal surrounding muscles on MRI, scored as fascial oedema (FE), can indicate the presence of a fasciitis.[32 33] Increased FT was found in around 10% of assessed fasciae with quantitative US, in 3% with qualitative US, and about one third of the fasciae showed FE on MRI. Changes over time were not detected for FT of FE. Previous reports described FE as an early abnormality in IIM patients, even in the absence of muscle inflammation.[4 34 35] Myofascial oedema has been reported as a risk factor for rapid onset interstitial lung disease in myositis.[36] Thus, fascial oedema may have clinical relevance and is better detected by MRI as compared to US in our cohort.

Both semi-quantitative US sumscore and MRI oedema sumscore correlated with muscle strength at baseline, which was described before for semi-quantitative US[26] and MRI.[3 31 36] A new finding of our study is a correlation between change scores of semi-quantitative US and changes scores of muscle strength. This strengthens the suggestion that muscle imaging could serve as a biomarker in IIM in treatment evaluation.[31]

Strengths of our study were the prospective, longitudinal design including a standardized intervention, the inclusion of newly diagnosed treatment-naive patients, the exploration of multiple standardised protocols for both imaging methods, the standardized time between baseline and follow-up and the blinded evaluation of baseline and follow-up. Limitations were the small sample size, the fact that only patients with biopsy-proven myositis were included which may have led to an over-estimation of muscle abnormalities, the relatively short follow-up duration, and no evaluation of intra-rater or inter-rater reliability. However, previous studies have shown a good inter-observer reliability (inter-rater intra-class correlation coefficient 0.76, CI 0.67-0.82) for semi-quantitative muscle ultrasound when performed by experienced staff physicians, which was the case in our study.[37] In addition, for MRI, a

substantial to excellent inter-observer agreement (Cohen's Kappa 0.7-0.9) was described in the used protocol for the muscles we analysed.[3]

A direct comparison of the imaging modalities was challenged by the higher number of muscles that were evaluated with MRI as compared to US and differences in scoring systems of muscles for both modalities, among which a difference in analysed muscle volume which was higher for MRI than for US.

In conclusion, this pilot study favours WB-MRI as diagnostic imaging modality in IIM patients. Semi-quantitative and qualitative US may be a reasonable alternative to WB-MRI at diagnosis and showed changes over time. Quantitative US was insensitive at diagnosis and follow-up in IIM patients.

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A.W., J.L, J.H. J.D., F.E., I.S., R.H., M.V. A.K. and C.V. had substantial contributions to the conception and design of the work.
C.S., F.S., W.P., had a substantial contribution in the data acquisition.
A.W., J.R. A.K. and C.V. drafted the manuscript and all authors critically revised it critically for important intellectual content and gave final approval of this version to be published, and gave agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interest
A.W., F.S., J.H., F.E., W.P., C.S., and R.H. report no competing interests.
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SUPPLEMENTAL MATERIAL

Supplementary Table S1. Quantitative ultrasound: numbers of muscles and fasciae showing abnormalities at baseline, at follow-up, and changes over time.

	Baseline, n=18				**Baseline, n=12**				**Follow-up, n=12**				**Change; follow-up – baseline, n=12**	
	Echo-intensity			**Muscle thickness**	**Echo-intensity**			**Muscle thickness**	**Echo-intensity**			**Muscle thickness**	**Echo-intensity**	**Muscle thickness**
Z-score	1.5 - 2.4	2.5 - 3.4	>3.5		1.5 - 2.4	2.5 - 3.4	>3.5		1.5 - 2.4	2.5 - 3.4	>3.5			
Abnormal muscle echo-intensity and thickness														
Biceps brachii	7	1		0	4	1		0	2			2	-3	2
Deltoid	5	1		15	2			10	2			13	0	3
Flexor carpi radialis	5	1	2	5	3	1	2	3	1	1		4	-2	1
Flexor digitorum profundus	2	1		0	1			0	2			0	1	0
Rectus femoris	3			9	3			8	2			9	-1	1
Tibialis anterior	3			4	0			4	0			7	0	3
Vastus lateralis	5			14	3			11	3			10	0	1
Total	**36 (14%)**			**47 (16%)**	**20 (12%)**			**36 (19%)**	**13 (8%)**			**45 (23%)**	**-7**	**9**
Abnormal fascial thickness														
Deltoid	10				8				5				-3	
Rectus femoris- superficial	0				0				0				0	
Rectus femoris - deep	7				4				5				1	
Vastus lateralis - superficial	0				0				0				0	
Vastus lateralis - deep	0				0				0				0	
Total	**17 (10%)**				**12 (10%)**				**10 (8.3%)**				**-2**	

The numbers in the table above are the sum of right and left side.

Supplementary Table S2. Quantitative ultrasound data of echo-intensity, muscle thickness and fascia thickness at baseline, at follow-up, and changes over time.

	Baseline	**Baseline**	**Follow-up**	**Δ-score◆**	**p-value**
	n=18	n=12	n=12		
Muscle thickness					
Biceps brachii	2.5 (0.59)	2.5 (0.57)	2.4 (0.4)	-0.1	0.229
Deltoid	1.3 (0.4)	1.3 (0.32)	1.3 (0.4)	0	0.810
Flexor carpi radialis	1.1 (0.3)	1.1 (0.30)	1.2 (0.3)	0.1	0.433
Flexor digitorum profundus	2.6 (0.2)	2.6 (0.28)	2.5 (0.3)	-0.1	0.439
Rectus femoris	3.1 (9.4)	3.1 (1.0)	3.3 (0.8)	0.2	0.283
Tibialis anterior	2.5 (0.3)	2.5 (0.3)	2.4 (0.3)	-0.1	0.107
Vastus lateralis	2.7 (0.7)	2.7 (0.6)	2.6 (0.6)	-0.1	0.214
Muscle thickness z-score					
Biceps brachii	0.61 (1.4)	0.51 (1.3)	0.2 (1.3)	-0.31	0.224
Deltoid	-1.65 (1.2)	-1.73 (0.9)	-1.8 (1.2)	-0.07	0.811
Flexor carpi radialis	-0.6 (1.4)	-0.62 (1.5)	-0.3 (1.4)	0.32	0.315
Flexor digitorum profundus	1.1 (0.9)	0.91 (0.96)	0.7 (0.6)	-0.21	0.446
Rectus femoris	-1.2 (1.7)	-1.67 (1.6)	-1.3 (1.3)	0.37	0.279
Tibialis anterior	-0.1 (1.1)	-0.15 (1.1)	-0.4 (1.5)	-0.25	0.111
Vastus lateralis	-1.3 (1.3)	-1.6 (0.93)	-1.9 (0.8)	-0.3	0.215
Echo-intensity					
Biceps brachii	68.0 (13.4)	64.2 (14.1)	63.0 (10.0)	-1.2	0.694
Deltoid	61.8 (14.0)	59.3 (13.5)	56.3 (16.5)	-3	0.329
Flexor carpi radialis	57.1 (13.3)	54.0 (13.6)	52.6 (10.7)	-1.4	0.563
Flexor digitorum profundus	59.9 (14.7)	55.1 (12.3)	60.0 (12.1)	4.9	0.052
Rectus femoris	64.0 (15.0)	66.7 (16.2)	67.2 (13.2)	0.5	0.719
Tibialis anterior	77.2 (14.4)	73.4 (12.8)	73.7 (11.9)	0.3	0.701
Vastus lateralis	70.9 (10.5)	71.5 (11.5)	69.8 (15.5)	-1.7	0.808
Echo-intensity z-score					
Biceps brachii	-0.37 (2.0)	-0.7 (2.2)	-0.92 (1.7)	-0.22	0.684
Deltoid	-0.35 (1.5)	-0.38 (1.4)	-0.79 (1.6)	-0.41	0.305
Flexor carpi radialis	0.42 (1.9)	-0.07 (1.7)	-0.13 (1.4)	-0.04	0.569
Flexor digitorum profundus	-0.64 (1.7)	-0.03 (1.4)	-0.44 (1.5)	-0.41	0.054
Rectus femoris	-0.71 (1.8)	-0.24 (1.8)	-0.19 (1.6)	0.2	0.832
Tibialis anterior	-0.25 (1.4)	-0.50 (1.2)	-0.46 (1.1)	0.1	0.817
Vastus lateralis	0.1 (1.2)	0.3 (1.3)	0.14 (1.77)	0.44	0.554
Fascia thickness					
Deltoid	0.63 (0.01)	0.64 (0.02)	0.57 (0.01)	-0.07	0.388
Rectus femoris - superficial	0.87 (0.02)	0.89 (0.02)	0.84 (0.02)	-0.05	0.433

Rectus femoris - deep	1.5 (0.06)	1.4 (0.03)	1.3 (0.03)	-0.1	0.272
Vastus lateralis - superficial	0.91 (0.02)	0.94 (0.02)	1.0 (0.03)	0.04	0.48
Vastus lateralis - deep	0.95 (0.02)	0.96 (0.02)	1.1 (0.03)	0.14	0.937
	*n=17 301 no fascia at baseline.				

Fascia thickness z-score					
Deltoid	1.1 (1.9)	1.23 (2.1)	0.4 (1.4)	-0.83	0.388
Rectus femoris - superficial	-0.9 (1.0)	-0.8 (1.1)	-1.1 (1.1)	-0.3	0.433
Rectus femoris - deep	1.6 (3.3)	1.3 (1.9)	0.4 (1.0)	-0.9	0.272
Vastus lateralis - superficial	-1.2 (0.7)	-1.4 (0.7)	-0.9 (1.1)	0.5	0.48
Vastus lateralis - deep	-1.1 (0.8)	-1.0 (0.8)	-0.7 (1.0)	0.3	0.937

No statistical difference found between measures of right and left muscle, hence the right muscle value is shown. Mean (SD) is shown, p-value is paired sample t-test. Exception for fascia thickness, median (SE), for fascia thickness p-value is Wilcoxon rank test. Delta score = follow-up - baseline, based on 12 patients.

Supplementary Table S3. MRI: Numbers of muscles showing oedema and numbers of regions showing fascial oedema and subcutaneous oedema at baseline, at follow-up, and changes over time.

	Baseline, n=18			**Baseline, n=12**			**Follow-up, n=12**			**Change sumscore of muscles, n=12**
	Score:			**Score:**			**Score:**			
	1	2	% abnormal	1	2	% abnormal	1	2	% abnormal	
Muscle oedema										
Cervical	10	10	59%	6	6	50%	9	4	54%	-1
Deltoideus	12	18	83%	10	8	75%	9	5	58%	-7
Supraspinatus	7	23	83%	7	11	75%	6	7	54%	-9
Infraspinatus	6	24	83%	5	13	75%	7	9	67%	-6
Biceps brachii	7	20	84%	5	11	67%	8	5	57%	-9
Triceps	12	17	85%	10	7	71%	7	6	57%	-5
Forearm flexors	5	10	52%	3	6	38%	6	0	35%	-9
Forearm extensors	8	8	59%	4	6	42%	2	2	27%	-10
Gluteus musculature	18	14	89%	12	8	83%	11	6	71%	-5
Psoas	6	24	83%	2	16	75%	6	12	75%	-4
Sartorius	17	9	72%	12	5	71%	8	7	63%	0
Hip adductor	14	14	80%	10	8	75%	8	8	67%	-2
Quadriceps femoris	18	15	92%	14	8	92%	11	8	79%	-3
Hamstrings	14	14	78%	10	7	71%	4	8	50%	-4
TFL	10	12	61%	6	8	58%	8	0	33%	-16
Tibialis anterior	11	18	81%	7	10	71%	6	8	58%	-5
Peroneus	14	11	69%	9	7	67%	9	6	63%	-2
Gastrocnemius	17	9	72%	13	5	75%	8	6	64%	-3
Total	206	270	76%	145	150	68%	133	107	58%	-5.5

Fascial oedema and subcutaneous oedema								
	FE, n=18	**SE, n=18**	**FE, n=12**	**SE, n=12**	**FE, n=12**	**SE, n=12**	**Change FE n=12**	**Change SE n=12**
Upper arm	4	5	3	3	3	0	0	-3
Lower arm	2	4	2	2	5	0	+3	-2
Upper leg	22	15	14	11	11	10	-3	-1
Lower leg	19	20	14	14	6	18	-8	+4
Total	47 (36%)	44 (32%)	33(36%)	30 (33%)	25 (28%)	28 (31%)	-8	-2

TFL= tensor fasciae latae. FE = fascial oedema. SE= subcutaneous oedema. All muscles, fascia and subcutaneous regions were scored bilaterally. Change sumscore of muscles = sumscore follow-up – sumscore baseline. Change FE/ Change SE= muscles with fascial/subcutaneous oedema at follow-up – muscles with fascial/subcutaneous oedema at baseline.

Missing values (mostly due to artefacts on MRI): Muscles: Baseline (n=18) cervical 2, biceps 4, triceps 2, forearm flexors 7, forearm extensors 9; missing = 24/648. Baseline (n=12): biceps 3, triceps 2, forearm flexors 3, forearm extensors 4; missing = 12/432. Follow-up (n=12): biceps 1, triceps 1, forearm flexors 7, forearm extensors 9, gastrocnemius 2; missing = 20/432. Fascia: baseline (n=18); upper arm 2, lower arm 10; missing = 12/144. Fascia Baseline n=12; lower arm 4; missing = 4/96. Fascia follow-up n=12; upper arm 2, lower arm 4, lower leg 2; missing = 8/96. Subcutis: baseline (n=18); lower arm 8; missing = 8/144. Subcutis baseline n=12; lower arm 4; missing = 4/96. Subcutis follow-up n=12: upper arm 2, lower arm 4; missing = 6/96.

3

Chapter 3

Multimodality screening for (peri) myocarditis in newly diagnosed idiopathic inflammatory myopathies: a cross-sectional study

Johan Lim[1*], Hannah A.W. Walter[1*], Rianne A.C.M. de Bruin-Bon[2], Myrthe C. Jarings[1], R. Nils Planken[3], Wouter E.M. Kok[2], Joost Raaphorst[1], Yigal M. Pinto[2], Ahmad S. Amin[2], S. Matthijs Boekholdt[2], Anneke J. van der Kooi[1]

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[1] DEPARTMENT OF NEUROLOGY, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF CARDIOLOGY, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[3] DEPARTMENT OF RADIOLOGY AND NUCLEAR MEDICINE, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS

* SHARED FIRST AUTHORSHIP

SUBMITTED

ABSTRACT

Background: Cardiac involvement in idiopathic inflammatory myopathy (IIM or "myositis") is associated with an approximate 4% mortality, but standardised screening strategies are lacking.

Objective: We explored a multimodality screening focussing on potentially reversible cardiac involvement – i.e. active (peri)myocarditis – in newly diagnosed IIM.

Methods: We included adult IIM patients from 2017 to 2020. At time of diagnosis, patients underwent cardiac evaluation including laboratory biomarkers, electrocardiography, echocardiography, and cardiac magnetic resonance imaging (CMR). Based on 2019 consensus criteria for myocarditis, an adjudication committee made diagnoses of definite, probable, possible or no (peri)myocarditis. We explored diagnostic values of sequentially added diagnostic modalities by Constructing Classification and Regression Tree (CART) analysis in patients with definite/probable versus no (peri)myocarditis.

Results: We included 34 IIM patients, in whom diagnoses of definite (six, 18%), probable (two, 6%), possible (11, 32%), or no (peri)myocarditis (15, 44%) were adjudicated. CART-analysis in patients with probable/definite or no (peri)myocarditis diagnosis showed that high-sensitivity cardiac troponin T (cut-off value <113 ng/L) ruled out (peri)myocarditis with a sensitivity of 88%, while high-sensitivity troponin I (cut-off value >35 ng/L) ruled in (peri)myocarditis with a specificity of 100%. Applying high-sensitivity cardiac troponins with these cut-off values in a diagnostic algorithm without and with a CMR to the total population of 34 patients demonstrated a diagnostic accuracy for a clear diagnosis of probable/definite or no (peri)myocarditis of 59% and 68%, respectively.

Conclusion: A diagnostic algorithm for detection of (peri)myocarditis in adult IIM may consist of sequential testing with high-sensitivity cardiac troponins and CMR.

INTRODUCTION

Cardiac involvement in patients with idiopathic inflammatory myopathy (IIM; commonly referred to as "myositis"), may result in arrhythmias and/or cardiac failure with subsequent mortality in approximately 4% of patients, especially if left untreated.[1] A subset of patients with early stage cardiac involvement – i.e. active (peri)myocarditis – may be amenable to treatment.[2]

The diagnosis of myocarditis (from various causes) has been based in the past on the Lake Louise criteria of 2009, targeting on tissue inflammation mainly with the help of conventional cardiac magnetic resonance imaging (CMR) techniques by assessment of late gadolinium enhancement (LGE) and by T2-weighted imaging.[3] In 2018, the criteria have been refined using novel CMR techniques for demonstrating myocardial oedema on T2-weighted imaging and native T2 mapping and for diffuse fibrosis and infiltrations using T1-imaging.[4] With these newer criteria, sensitivity for diagnosing acute myocarditis has increased from 74% to 85% by adding T1-mapping [5]. The latter criteria have also been adopted in a multimodality diagnostic strategy to detect immune-mediated myocarditis in cancer therapeutics, that translated probabilities in diagnostic categories of definite or probable versus possible and no myocarditis [6]. The Lake Louise criteria of 2009 and the newer CMR criteria of 2018 for myocarditis have both been validated with clinical criteria and endomyocardial biopsy.[3-5] Although it would suffice to perform CMR to diagnose acute myocarditis in IIM patients, a multimodality cardiac screening is often performed using laboratory testing, electrocardiography, echocardiography, and coronary angiography in addition to CMR, because of the predisposition of patients to other (cardio)vascular disease which may contribute to the CMR findings.[7-9] Diagnosing active (peri)myocarditis within a multimodality approach however has a priority for treatment with immune-suppressant agents and is the focus of the present study. We tested a multimodality screening strategy in newly diagnosed IIM patients with the hypothesis that it would 1) identify a considerable proportion of patients with active (peri)myocarditis and 2) make it possible to devise screening strategies for (peri)myocarditis. For these reasons, we designed this cross-sectional study.

METHODS

Patients

We included newly diagnosed adult IIM patients between February 2017 and February 2020 at a single referral centre for IIM in Amsterdam, The Netherlands. All patients were evaluated by an IIM specialist. Inclusion criteria were as follows: newly diagnosed adult patients with IIM based on the 2004 European Neuromuscular Centre (ENMC) criteria except inclusion body myositis.[10] This study was conducted in the setting of routine clinical practice and in accordance with the local research code provided by the IRB, national legislation, and the declaration of Helsinki. We registered the following baseline data at time of diagnosis: IIM subtype, age, sex, disease duration (time between first symptoms and diagnosis), presence of interstitial lung disease (ILD) as confirmed by high-resolution computer tomography, presence of an associated connective tissue disorder (CTD), presence of associated cancer, presence of myositis related autoantibodies as assessed by a line blot assay, presence of anti-mitochondrial autoantibodies (AMAs) as assessed by immunofluorescence, and prior or active immunosuppressive treatment.

Cardiac screening

All patients were evaluated by a cardiologist with expertise in cardiomyopathies. We registered the following items at time of diagnosis: pre-existing cardiovascular disease (CVD), cardiovascular risk factors (CVRF; e.g. smoking, diabetes, hypertension), presence of symptoms of possible cardiac origin (i.e. complaints of dyspnoea, palpitations, peripheral oedema, chest pain, or syncope not attributable to another non-(peri)myocarditis diagnosis), and timing of cardiac evaluation.

At time of IIM diagnosis, patients underwent laboratory investigations, ECG, echocardiography, and CMR. Laboratory biomarkers included high-sensitivity cardiac troponin T (hs-TnT; reference value <50 ng/L; Elecsys® Troponin T high sensitive, Roche Diagnostics, Rotkreuz, Switzerland), high-sensitivity cardiac troponin I (hs-TnI; reference value females <12 ng/L and males <20 ng/L; high-sensitivity Troponin I, Beckman Coulter, Brea, California, USA), and N-terminal pro B-type natriuretic peptide (NT-proBNP; reference value <130 ng/L; Elecsys® proBNP II, Roche Diagnostics, Rotkreuz, Switzerland).

A standard 12-lead ECG was recorded. Echocardiography was performed by a dedicated

technician (R.B.). Echocardiographic views were acquired on a Vivid 9 or 9.5 (GE Healthcare, Horten, Norway) and were evaluated for wall motion abnormalities, systolic and diastolic ventricular function and pulmonary artery hypertension (PAH) according to guideline recommendations.[11 12] Additional strain analyses – with strain abnormalities based on global longitudinal strain and mechanical dispersion – were performed offline on EchoPAC PC software v.201 (GE Healthcare, Horten, Norway).[13]

CMR images were acquired using 1.5-Tesla CMR (Magnetom Avanto, Siemens Medical Systems, Erlangen, Germany). CMR investigations consisted of steady-state free precession cine imaging in standard long axis images (2-chamber, 3-chamber, 4-chamber orientations) and a stack of short axis images. T2-weighted turbo spin echo or spectral attenuated inversion recovery sequences were acquired for detection of myocardial oedema. Post-contrast images were acquired for the detection of late gadolinium enhancement (LGE). Short axis cine imaging with full right ventricle and left ventricle coverage and a 20% inter-slice gap were segmented using dedicated post-processing software (Circle CV, Calgary, Canada). Papillary muscles were included in the left ventricular volume, not separately segmented. Additional parametric mapping consisting of T1/T2 mapping and calculation of extracellular volume (ECV) was performed in part of the patients.[14 15] Parametric T1/T2 maps were obtained in 3 short axis views, and were assessed visually for regional differences. If these were observed, a ROI was used to quantify the regional T1/T2 values respectively. No average T1/T2 values across the entire myocardium in the short axis views were quantified, as this approach may mask regional abnormalities. Pending definitive local T1/T2 mapping reference values, <1050 ms for T1-mapping and <45 ms for T2-mapping were considered normal, while >1100 ms for T1-mapping and >50 ms for T2-mapping were considered abnormal. Additional coronary angiography to exclude coronary heart disease was performed at the discretion of the treating cardiologist.

An adjudication committee (J.L., H.B., A.A., Y.P., S.B, A.K.) assessed the presence of IIM-related (peri)myocarditis using consensus criteria for myocarditis, with designations of definite, probable, possible, or no (peri)myocarditis (Supplemental material S1).[6]

Statistical analysis

Results were described using descriptive statistics. Sample size: convenience sample. Diagnostic values of sequential diagnostic modalities were explored by Constructing Classification and Regression Tree (CART) analysis using SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA). CART analysis was first performed in a selection of patients with clear diagnoses of probable/definite or no (peri)myocarditis. Cut-off values of the troponins were determined as those with high specificity for the diagnosis (peri)myocarditis (rule in), and cut-off values with high sensitivity were used to rule out (peri)myocarditis. The most useful diagnostic modalities in CART were then ranked in order of appearance, to establish a diagnostic algorithm that was then tested for its accuracy in the total cohort of patients. Any data not published within the article – e.g. the CMR exam card/sequence protocol – will (after anonymization) be shared upon request from any qualified investigator.

RESULTS

Patient characteristics

We included 34 newly diagnosed IIM patients of whom the demographics and clinical features are summarised in Table 1. There were 10 patients (29%) who had received prior treatment with immunosuppressants, of whom five patients with partial but insufficient treatment response.

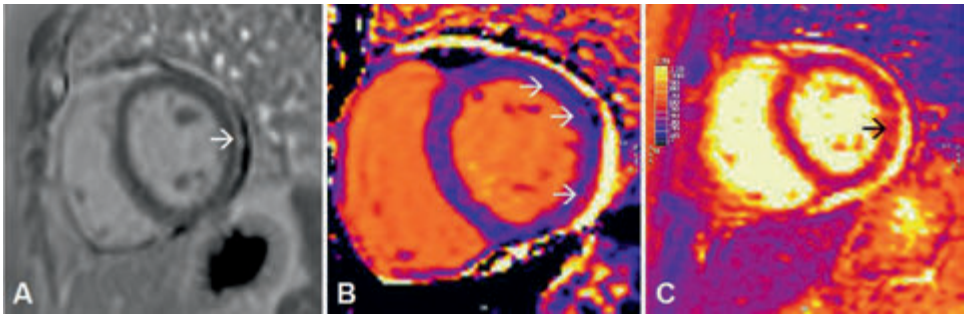


Figure 1. Abnormalities on cardiac magnetic resonance imaging (CMR) consistent with (peri)myocarditis in a patient with non-specific myositis/overlap myositis in the setting of a mixed connective tissue disease

Classic CMR abnormalities consistent with (peri)myocarditis, i.e. late gadolinium enhancement, is seen epicardial in the basal-mid inferolateraal segment on T1-weighted imaging (short-axis view), which indicates myocardial fibrosis (arrow Figure 1A). Recently, parametric T1/T2-mapping have been developed, which allows for quantitative (re-)evaluation of abnormalities consistent with (peri)myocarditis. Increased values on T1-mapping (arrows Figure 1B) and T2-mapping (arrow Figure 1C) are (focally) seen in the inferolateral segment (short axis view), which indicate myocardial oedema. Of note, this patient did not have chest pain or palpitations, and had an unremarkable electrocardiography and echocardiography at time of the CMR.

Table 1. Demographics and clinical features of the 34 included patients.

	All (n=34)	Probable/definite (peri)myocarditis (n=8)	Possible (peri)myocarditis (n=11)	No (peri)myocarditis (n=15)
IIM subtype	- DM (n=17) - ASS (n=4) - IMNM (n=7) - NM/OM (n=6)	- DM (n=2) - ASS (n=1) - IMNM (n=2) - NM/OM (n=3)	- DM (n=4) - ASS (n=2) - IMNM (n=4) - NM/OM (n=1)	- DM (n=11) - ASS (n=1) - IMNM (n=1) - NM/OM (n=2)
Age (median years, IQR)	53 (42 to 62)	50 (34-59)	60 (47-66)	50 (37-62)
Females (n, %)	24 (71%)	5 (63%)	6 (55%)	13 (87%)
Disease duration (median months, IQR)	5 (3-10)	5 (3-10)	6 (2-8)	5 (3-13)
CTD (n, %)	4 (12%)	4 (50%)	0 (0%)	0 (0%)
Cancer (n, %)	1 (3%)	0 (0%)	0 (0%)	1 (7%)
MSAs/MAAs	AMAs (n=1) anti-EJ (n=1) anti-HMGCR (n=3) anti-Jo1 (n=2) anti-Ku (n=2) anti-MDA5 (n=3) anti-Mi2 (n=4) anti-NXP2 (n=2) anti-PL7 (n=1) anti-PMScl (n=1) anti-Ro52 (n=4) anti-SRP (n=2) anti-TIF1γ (n=2) seronegative (n=9)	AMAs (n=0) anti-EJ (n=1) anti-Mi2 (n=1) anti-TIF1γ+anti-Ku (n=1) anti-Ro52 (n=2) anti-SRP (n=1) seronegative (n=3)	AMAs (n=0) anti-HMGCR (n=2) anti-Jo1 (n=1) anti-Ku (n=1) anti-Mi2 (n=1) anti-NXP2 (n=1) anti-PL7 (n=1) anti-SRP (n=1) seronegative (n=2)	AMAs (n=1)[d] anti-HMGCR (n=1) anti-Jo1 (n=1) anti-Ku (n=1) anti-MDA5 (n=3) anti-Mi2 (n=2) anti-NXP2 (n=1) anti-PMScl (n=1) anti-Ro52 (n=2) anti-TIF1γ (n=1) seronegative (n=4)
AMAs (n, %)	1 (3%)	0 (0%)	1 (9%)	0 (0%)
prior CVD (n, %)	7 (21%)	1 (13%)	5 (45%)	1 (7%)
CVRF (n, %)	16 (47%)	4 (50%)	9 (82%)	3 (20%)
Symptoms of possible cardiac origin[a] (n, %)	23 (68%)	6 (75%)	10 (91%)	7 (47%)
hs-TnT (median ng/L, IQR)	156 (47-333)	531 (153-861)	159 (99-333)	50 (31-205)[d]
hs-TnI (median ng/L, IQR)	13 (4-35)	39 (12-91)	16 (5-77)	7 (2-19)[d]
NT-proBNP	137 (80-283)	202 (89-410)	135 (72-549)	138 (79-186)[d]

(median ng/L, IQR)				
abnormal ECG rest (n, %)	8 (24%)	4 (50%)	4 (36%)	0 (0%)
abnormal ECG strain (n, %)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
abnormal echocardiography wall motion[b] (n, %)	5 (15%)	2 (25%)	1 (9%)	2 (13%)[d]
abnormal echocardiography LVDD[b] (n, %)	3 (9%)	0 (0%)	2 (18%)	1 (7%)[d]
abnormal echocardiography strain[b] (n, %)	17 (50%)	5 (63%)	6 (55%)	6 (40%)[d]
CMR[c] (n, %)	9 (26%)	8 (100%)	1 (100%)	0 (0%)

Abbreviations (alphabetical order): AMAs = anti-mitochondrial autoantibodies; ASS = anti-synthetase syndrome; CMR = cardiac magnetic resonance imaging; CTD = connective tissue disease; CVD = cardiovascular disease; CVRF = cardiovascular risk factors; DM = dermatomyositis; ECG = electrocardiography; hs-TnI = high-sensitivity cardiac troponin I (reference value females <12 ng/L and males < 20 ng/L); hs-TnT = high-sensitivity cardiac troponin T (reference value <50 ng/L); IMNM = immune-mediated necrotizing myopathy; MAAs = myositis-associated autoantibodies; MSAs = myositis-specific autoantibodies; NM/OM = non-specific/overlap myositis; NT-proBNP = N-terminal pro hormone B-type natriuretic peptid (reference value <130 ng/L).

[a] Symptoms of possible cardiac origin: any complaints of dyspnea, palpitations, peripheral oedema, chest pain, or syncope not entirely attributable to another non-myocarditis diagnosis. Of the patients with one or more complaints of possible cardiac origin, four patients also suffered from ILD.

[b] Conventional and strain echocardiography were qualitatively and quantitatively assessed by a dedicated technician (R.B.). Strain echocardiography was considered abnormal based on global longitudinal strain (reference value -18 to-22%) and mechanical dispersion (reference value <38 ms).

[c] Conventional and parametric T1/T2 mapping using CMR were qualitatively assessed by a dedicated cardiologist (S.B.) and radiologist (R.P.). Absolute values for parametric T1/T2 mapping are shown in parentheses when considered abnormal. Pending definitive local T1/T2 mapping reference values, <1050 ms for T1-mapping and <45 ms for T2-mapping were considered normal, while >1100 ms for T1-mapping and >50 ms for T2-mapping were considered abnormal.

[d] Abnormalities were not considered disease-related by the adjudication committee (J.L., H.B., A.A., Y.P., S.B, A.K).

Cardiac screening results

Seven patients (21%) had pre-existing CVD and 16 (47%) had one or more CVRFs at time of diagnosis. Twenty-three patients (68%) had one or more symptoms of possible cardiac origin, mostly consisting of dyspnoea (19 patients), to a lesser degree palpitations (nine patients) and chest pain (six patients), and no symptoms of syncope or peripheral oedema. Median time between start of treatment and ancillary investigations was as follows: zero days for laboratory biomarkers (IQR -3 to 0 days), one day for ECG (IQR -0.5 to 28 days), one day for echocardiography (IQR -2 to 12 days), and 23 days for CMR (IQR 2 to 48 days).

A diagnosis of definite (peri)myocarditis was adjudicated in six patients (18%), probable (peri)myocarditis in two patients (6%), possible (peri)myocarditis in 11 patients (32%), and no (peri)myocarditis in 15 patients (44%).The eight cases with diagnoses of probable/definite (peri)myocarditis were found in patients of all IIM subtypes (Table 2). Two out of these eight (25%) were without any cardiac symptoms, of whom one patient with progressive conduction abnormalities for which a pacemaker implantation was required (patient IMNM2 Table 2). One patient with probable (peri)myocarditis had PAH (patient IMNM9 Table 2), none of the patients with a diagnosis of probable/definite (peri)myocarditis suffered from heart failure or had left ventricular diastolic dysfunction (LVDD; Table 2).

Eleven patients (32%) had a diagnosis of possible (peri)myocarditis based on abnormal hs-TnT and/or hs-TnI levels with either symptoms or ECG abnormalities of possible (peri)myocarditis origin. CMR was normal in all but one patient with long-standing CMR abnormalities suggestive of (peri)myocarditis in whom other causes of cardiomyopathy – e.g. genetic – could not be completely ruled out. Of the 15 patients without a diagnosis of (peri)myocarditis, seven patients (47%) had abnormal levels of hs-TnT and five patients (33%) had abnormal levels of hs-TnI.

Table 2. Patient characteristics of 8 patients with a probable/definite diagnosis of (peri)myocarditis

Patient	age	sex	history[a]	cardiac symptoms[b]	lab biomarkers (ng/L)	ECG	echo-cardiography[b]	CMR[c]	vascular imaging[d]	(peri) myocarditis
DM10, anti-Mi2+	31	F	-	chest pain	- hs-TnT: 714 - hs-TnI: 64 - NTproBNP: 108	normal	- WMA: no - LVDD: no - strain: normal	- T2/LGE abnormalities: no - parametric T1/T2 mapping abnormalities: T1M (anteroseptal 1109 ms), ECV (31%), and T2M anteroseptal (61 ms)	NA	definite
DM15, anti-TIF1γ+, anti-Ku+	60	M	-	dyspnea, palpitations	- hs-TnT: 113 - hs-TnI: 9.1 - NTproBNP: 59	normal	NA	- T2/LGE abnormalities: suggestive LGE inferobasal-subendocardial - parametric T1/T2 mapping abnormalities: T1M inferobasal (1165 ms) and T2M inferobasal (58 ms)	CAG: normal	definite
ASS6, anti-EJ+, anti-Ro52+	46	F	-	Chest pain, dyspnea	- hs-TnT: 348 - hs-TnI: 19 - NTproBNP: 83	normal	- WMA: yes - LVDD: no -strain: abnormal GLS (-14%) and MD (50 ms)	- T2/LGE abnormalities: no - parametric T1M/T2 mapping abnormalities: T1M diffuse (1141 ms), ECV (33%), T2M septal (57 ms)	NA	definite

IMNM2, anti-Ro52+	56	M	smoking, SSc	-	- hs-TnT: 1470 - hs-TnI: 758 - NTproBNP: 758	abnormal conduction requiring pacemaker	- WMA: no - LVDD: no -strain: abnormal MD (40 ms)	- T2/LGE abnormalities: LGE anterolateral-subendocardial parametric T1/T2 mapping abnormalities: T1M subendocardial (1158 ms) and ECV (37%)	CAG: normal	definite
IMNM9, anti-SRP+	53	M	smoking, OSAS	-	- hs-TnT: 825 - hs-TnI: 100 - NTproBNP: 187	aspecific VPBs[e]	- WMA: no - LVDD: no -strain: abnormal GLS (-17.5%) and MD (59 ms)	- T2/LGE abnormalities: suggestive LGE basolateral-epi/midmyocardial - parametric T1/T2 mapping abnormalities: T1M inferobasoseptal (1070 ms) and ECV (37%)	chest CT: dilated pulmonary trunk of 3.8 cm	probable
NM/OM1, seronegative	72	F	MCTD	palpitations	- hs-TnT: 274 - hs-TnI: 42 - NTproBNP: 456	abnormal Q waves consistent with CHD[e]	- WMA: no - LVDD: no -strain: abnormal GLS (-14%) and MD (49 ms)	-T2/LGE abnormalities: T2 hyperintensity and suggestive LGE inferobasolateral-epicardial - parametric T1/T2 mapping abnormalities: T1M inferobasolateral (1160ms)	NA	definite
NM/OM4, seronegative	25	F	MCTD	chest pain, dyspnoea, palpitations	- hs-TnT: 873 - hs-TnI: 36 - NTproBNP: 217	abnormal T waves	- WMA: yes - LVDD: no -strain: abnormal GLS (-17%) and MD (43 ms)	- T2/LGE abnormalities: LGE basal pericardial - parametric T1/T2 mapping abnormalities: no	NA	definite

								- minimal PE			
NM/OM6, seronegative	41	F	-	dyspnea	- hs-TnT: 12 - hs-TnI: 1.7 - NTproBNP: 272	normal	- WMA: no - LVDD: no - strain: normal	- T2/LGE abnormalities: no - parametric T1/T2 mapping abnormalities: suggestive T2M septal (55 ms)	NA	probable	

Abbreviations (alphabetical order): ASS = anti-synthetase syndrome; CHD = coronary heart disease; CMR = cardiac magnetic resonance imaging; ECG = electrocardiography; ECV = extracellular volume; F= female, GLS = global longitudinal strain (reference value -18 to-22%); hs-TnI = high-sensitivity cardiac troponin I (reference value females <12 ng/L and males < 20 ng/L); hs-TnT = high-sensitivity cardiac troponin T (reference value <50 ng/L); IMNM = immune-mediated necrotizing myopathy; LGE = late gadolinium enhancement; LVDD = left ventricular diastolic dysfunction; M = male; MD = mechanical dispersion (reference value <38 ms); NA = not available; NM/OM = non-specific/overlap myositis; NT-proBNP = N-terminal pro hormone B-type natriuretic peptid (reference value <130 ng/L); PE = pericardial effusion; T1M = native T1-mapping; T2 = T2-weighted imaging; T2M = native T2 mapping.

[a] history of pre-existing cardiovascular disease, cardiovascular risk factors (i.e. smoking, diabetes, hypertension), associated cancer/connective tissue disease

[b] Conventional and strain echocardiography were qualitatively and quantitatively assessed by a dedicated technician (R.B.). When considered abnormal, absolute strain values are shown in parentheses.

[c] Conventional and parametric T1/T2 mapping using CMR were qualitatively assessed by a dedicated cardiologist (S.B.) and radiologist (R.P.). Absolute values for parametric T1/T2 mapping are shown in parentheses when considered abnormal. Pending definitive local T1/T2 mapping reference values, <1050 ms for T1-mapping and <45 ms for T2-mapping were considered normal, while >1100 ms for T1-mapping and >50 ms for T2-mapping were considered abnormal.

[d] Additional coronary angiography to exclude coronary artery disease was performed at the disretion of the treating cardiologist.

[e] Abnormalities were not considered disease-related by the adjudication committee (J.L., H.B., A.A., Y.P., S.B, A.K.).

Screening strategies for (peri)myocarditis

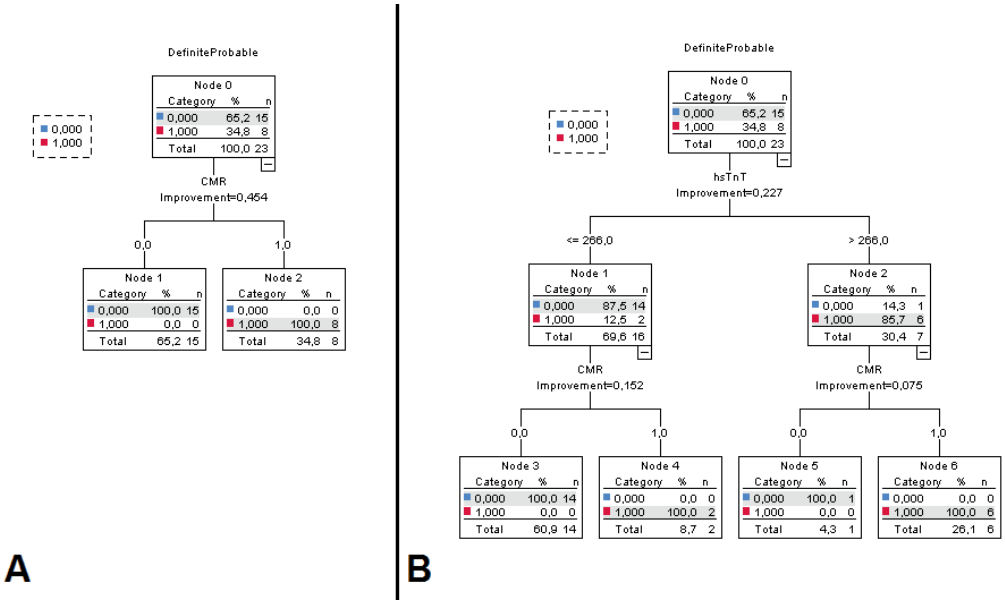
CART analysis confirmed that CMR was the diagnostic modality identifying all patients with a clear diagnosis of probable/definite (peri)myocarditis or no (peri)myocarditis (Figure 2A). In contrast, neither abnormally elevated NT-proBNP, the presence of AMAs, abnormalities on ECG, nor abnormalities on echocardiography appeared to have added value in discriminating between patients with a diagnosis of probable/definite (peri)myocarditis and patients with no evidence of (peri)myocarditis (Figure 2A-B). On the other hand, CART analysis showed that both high-sensitivity cardiac troponins were useful as a first step in a diagnostic algorithm, with hs-TnT most useful to rule-out (peri)myocarditis and hs-TnI most useful to rule-in (peri)myocarditis (Figure 2B-D).

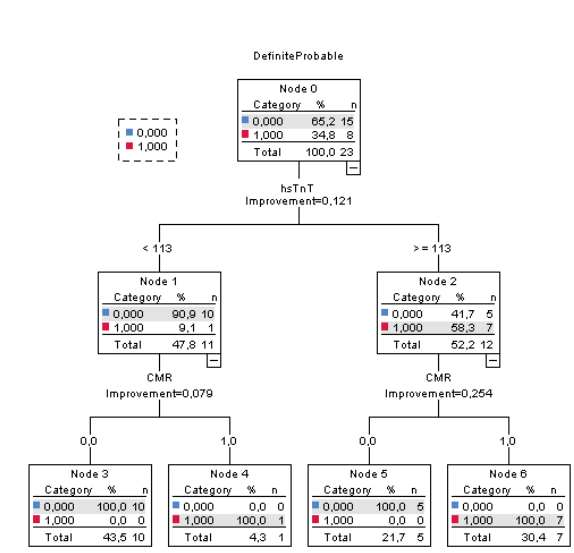
To rule-out (peri)myocarditis, CART analysis showed that hs-TnT with a cut-off value of <266 ng/L as a first step would have resulted in two false-negative diagnoses of probable/definite (peri)myocarditis (Figure 2B; patients DM15 and NM/OM6 Table 2). One of these patients had a diagnosis of probable (peri)myocarditis, a hs-TnT of 12 ng/L, and mild symptoms of possible cardiac origin in whom standard treatment with high-dose glucocorticoids resulted in improvement of these symptoms and resolution of CMR abnormalities (patient NM/OM6 Table 2). The other patient had a diagnosis of definite (peri)myocarditis, hs-TnT of 113 ng/L, and clinically relevant disease based on CMR abnormalities in the absence of an alternative ischemic cause (patient DM15 Table 2). We optimised the cut-off value by manually choosing <113 ng/L, resulting in one false-negative diagnosis/mild case of probable (peri)myocarditis (patient NM/OM6 Table 2). Hence, hs-TnT with a cut-off value of <113 ng/L was deemed optimal to rule-out (peri)myocarditis with sensitivity of 88%, specificity of 67%, and negative predictive value of 91% (Figure 2C).

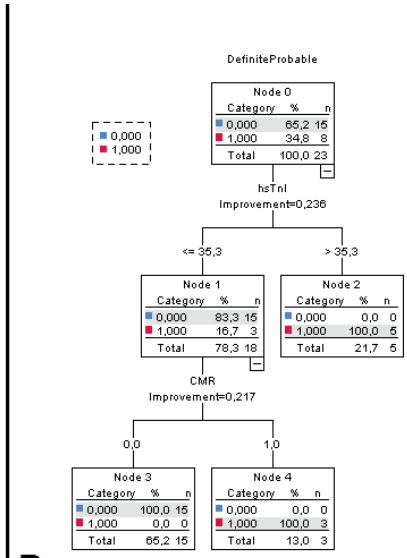
To rule-in (peri)myocarditis, CART analysis showed that hs-TnI with a cut-off value of >35 ng/L as a first step resulted in five patients diagnosed as (peri)myocarditis and zero false-positive diagnoses (Figure 2D). There were three false-negative diagnoses: apart from the two patients described above as having a hs-TnT of < 266 ng/L, one additional patient with low hs-TnI had a clinically relevant (peri)myocarditis, based on CMR results and symptoms of possible cardiac origin (patient ASS6 Table 2). Nevertheless, hs-TnI with a cut-off value of >35 ng/L was selected

as optimal to rule-in (peri)myocarditis with sensitivity of 63%, specificity of 100% and positive predictive value of 100%.

We then assessed the combined diagnostic accuracy of hs-TnT to rule-out (peri)myocarditis and hs-TnI to rule-in (peri)myocarditis in the 23 patients selected with a clear diagnosis of probable/definite or no (peri)myocarditis (Figure 2E). All patients with hs-TnT of <113 ng/L also had hs-TnI of ≤35 ng/L, while patients with hs-TnT of ≥113 ng/L could be divided in those with hs-TnI of ≤35 ng/L and >35 ng/L. This resulted in a diagnostic accuracy of 87% (20 of 23 patients) in those with a clear diagnosis (Figure 2E), and a diagnostic accuracy of 59% (20 of 34 patients) in the total population that included patients with a diagnosis of possible (peri)myocarditis. When combined in a diagnostic algorithm with CMR as second step, diagnostic accuracy increased to 68% (23 of 34 patients; Figure 3).







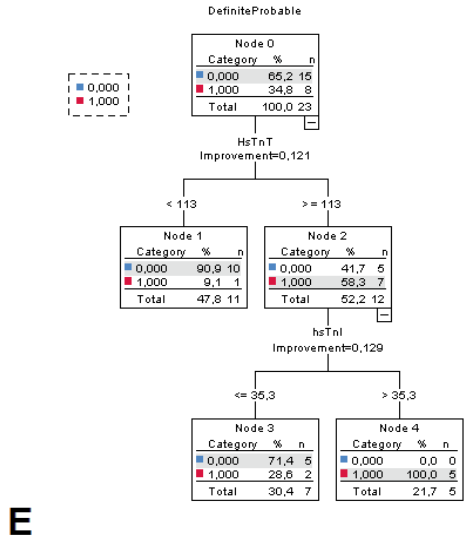


Figure 2. Diagnostic values of sequential diagnostic modalities were explored by Constructing Classification and Regression Tree (CART) analysis in patients with a diagnosis probable/definite (peri)myocarditis and patients with a diagnosis of no (peri)myocarditis

Cardiac magnetic resonance imaging (CMR) was the most useful diagnostic modality identifying all patients with a diagnosis of probable/definite (peri)myocarditis and those with a diagnosis of no (peri)myocarditis (Figure 2A). As gatekeepers for CMR, manually chosen hs-TnT <113 ng/L was more useful than hs-TnT <266 ng/L to rule-out (peri)myocarditis (Figure 2B-C), while hs-TnI >35 ng/L was useful to rule-in (peri)myocarditis (Figure 2D). Twenty of 23 patients had a clearly predicted outcome combining hs-TnT to rule-out (peri)myocarditis and hs-TnI to rule-

in (peri)myocarditis, resulting in a combined diagnostic accuracy of 87% (Figure 2E). Abbreviations (alphabetical order): CMR = cardiac magnetic resonance imaging; DefiniteProbable = probable/definite diagnosis of (peri)myocarditis in which 0 = no (blue) and 1 = yes (red); hs-TnI = high-sensitivity cardiac troponin I; hs-TnT = high-sensitivity cardiac troponin T.

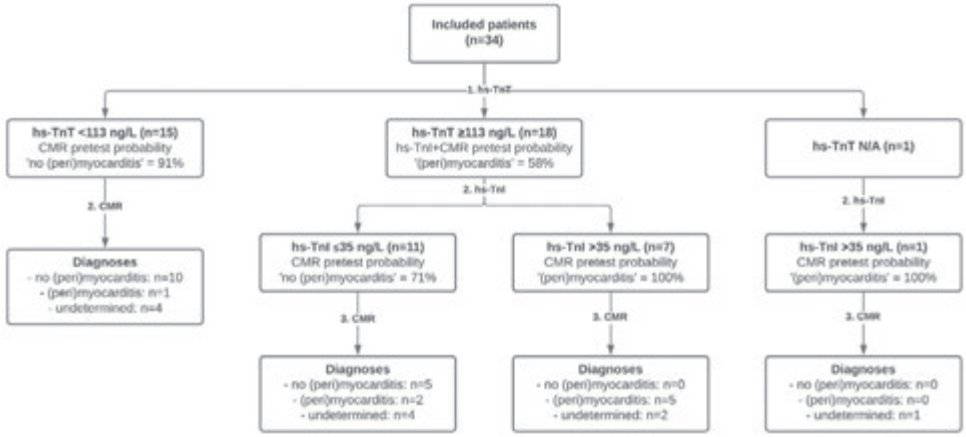


Figure 3. Diagnostic accuracy of combining high-sensitivity cardiac troponins T and I as first step, and cardiac magnetic resonance imaging as second step in the total population of 34 included patients
This resulted in a diagnostic accuracy of 68% (23 of 34 patients), leaving diagnosis undetermined in 11 patients (32%) despite performing a CMR. Abbreviations (alphabetical order): CMR = cardiac magnetic resonance imaging; hs-TnI = high-sensitivity cardiac troponin; hs-TnT = high-sensitivity cardiac troponin T; N/A = not available.

DISCUSSION

Our study confirms the findings of previous reports that (peri)myocarditis/early myocardial involvement is found in a considerable proportion (almost one fifth) of newly diagnosed IIM patients. [2 7-9 16-18] Our study adds to the literature that a multimodality screening strategy for the purpose of diagnosing active (peri)myocarditis in IIM may be modified towards a strategy using two initial major diagnostic tools: a laboratory test (including both hs-TnT and hs-TnI) in combination with a comprehensive CMR investigation (that includes parametric T1/T2 mapping). Compared to two histopathological autopsy studies demonstrating active myocarditis in 25 to 30% of IIM patients[19 20], we describe a somewhat lower frequency of active (peri)myocarditis in 18% of patients. Our lower frequency may be a result of selection of less severe patients than those of the autopsy reports, but may to some extent also be a result of an underestimation by restricting the diagnosis of (peri)myocarditis to those with a

clear diagnosis and diagnostic delay of CMR in part of the patients. Our results may also be compared to a single study using CMR that demonstrated myocarditis in 75% of 20 acute IIM patients, following the Lake Louise CMR criteria for myocarditis of 2009.[7] Although some overestimation of true prevalence of active myocarditis is expected using the older criteria, the difference in false positive rates between older and the newer criteria is not larger than 3% and cannot fully explain the difference in prevalence between the studies.[5] Therefore we believe our results present a conservative estimate of the true prevalence of active myocarditis in IIM. Still other studies found cardiac abnormalities in up to 65% of IIM patients but their results were not restricted to (peri)myocarditis.[9 16 18]

The importance of CMR in a standardised screening strategy that includes parametric T1/T2-mapping is illustrated by the detection of (peri)myocarditis in approximately a quarter to a third of IIM patients in whom (peri)myocarditis was not suspected based on symptoms of possible cardiac origin or conventional ancillary investigations – i.e. ECG, cardiac echography, and LGE on CMR - alone. As expected from the criteria in the definition that are used for the diagnosis of myocarditis that includes CMR findings.[6] CMR was the most useful diagnostic modality (Figure 2).[8] Similarly, our finding that approximately a third of patients with a diagnosis of probable/definite (peri)myocarditis had abnormalities on parametric T1/T2-mapping in CMR, but not with conventional LGE on CMR, is in accordance with earlier reports.[2 21] Still, in 11 of 34 (32%) of our patients who had a CMR, diagnosis was classified as possible (peri)myocarditis, underlining the need for additional clues to confirm/exclude the diagnosis.[6 8 22]

The limitations of CMR – that include availability, quality and costs – call for a gatekeeper to guide additional diagnostic workup or for a diagnostic equivalent for diagnosing (peri)myocarditis. The CART-analyses in our study suggest that both hs-TnT and hs-TnI have potential as gatekeepers to rule-out (peri)myocarditis and to rule-in (peri)myocarditis, respectively (Figure 2). Our results – suggesting high sensitivity of hs-TnT and high specificity of hs-TnI – are in accordance with an earlier report that used normal reference ranges of second generation cardiac troponins as cut-off levels.[23] As of yet, there are no 100% accurate cut-off values for cardiac troponins in IIM [24]. Our CART analysis suggested a cut-off value of hs-TnT <113 ng/L for rule-out with a sensitivity of 88% and negative predictive value of 94%.

When further validated, this cut-off level of hs-TnT may considerably reduce the number of CMRs needed (in our study reducing the number of CMRs from 42 to 23, an absolute reduction of 45%). Although CART analysis further suggested that hs-TnI with cut-off value of >35 ng/L may identify 63% of patients with probable/definite (peri)myocarditis with reasonable certainty (positive predictive value = 63%), there remains a need to confirm the diagnosis with CMR. There also remains a need to better delineate patients with intermediate levels of hs-TnT and hs-TnI and one also has to consider the added functional and structural information that CMR may provide.

Of note, it is unclear whether the "false positive" rates of hs-TnT found in our study and that of earlier reports reflect only "true" false positives, i.e. cross-reactivity of skeletal muscle troponin. It may also be that "false positive" hs-TnT and hs-TnI levels reflect subclinical (peri)myocarditis at least for a proportion of patients, in whom (peri)myocarditis is not (yet) visible even on state of the art CMR.[25] Other abnormalities, such as abnormally elevated levels of NT-proBNP, or the presence of AMAs, abnormalities on ECG, and abnormalities on echocardiography were not valuable in our diagnostic flowchart for a positive diagnosis of (peri)myocarditis in patients with newly diagnosed IIM (Figure 2). Our findings appear to be in conflict with reports that suggested NT-proBNP and AMAs for discriminating between patients with and without a diagnosis of (peri)myocarditis.[18 26] Differences in the applied criteria for defining (peri)myocarditis/myocardial involvement, the techniques of the ancillary investigations performed, and the studied populations may explain some of the differences in diagnostic accuracy.

Furthermore, while we did not find patients with cardiac failure at time of diagnosis, we did find LVDD in approximately one tenth of patients – none of whom had a diagnosis of probable/definite (peri)myocarditis – and PAH in one patient with a probable diagnosis of (peri)myocarditis. It is hypothesised that both LVDD and PAH are precursors of cardiac failure in IIM patients, leading to subsequent morbidity and mortality.[18 27 28] While the prevalence of LVDD and PAH in our study was in line with two earlier studies of IIM patients with Western-Northern European ethnicity[29 30], it appeared lower than the prevalence (42-77%) found in studies of IIM patients with East-Asian/Han-Chinese ethnicity.[26 31-33] While differences in diagnostic criteria and definitions of LVDD may explain some of the discrepancies[26 31-33], it is

possible that diastolic abnormalities suggest subclinical (peri)myocarditis in IIM patients with East-Asian/Han-Chinese ethnicity in particular.

The strengths of our study are the multimodality assessment of (peri)myocarditis, including novel diagnostic modalities and the use of the most recent consensus criteria for (peri)myocarditis.[6] The main limitations of our study are the following: 1) the relative small sample size; 2) prior treatment with immunosuppressants and diagnostic delay/missing data regarding CMR investigations in some of the patients, that may have led to underestimating the number of patients with a diagnosis of probable/definite (peri)myocarditis; and 3) the use of CMR and additional criteria for (peri)myocarditis instead of results from myocardial biopsies as the basis of the adjudication committee's assessment.[6] We used the criteria that were in accordance with consensus papers on diagnosis of myocarditis[4 6], but were devised for cancer therapies-associated myocarditis. We however favoured their use as they provided a clinically useful division into probabilities of the diagnosis. Finally, other diagnostic modalities were not studied in our study that may have had added value (e.g. technetium pyrophosphate scintigraphy and positron emission tomography).[6 22]

In conclusion, we show that routine multimodality screening for (peri)myocarditis in IIM at the time of diagnosis of IIM yields a considerable number of diagnoses of probable/definite (peri)myocarditis. A standardised screening strategy may however be more limited and consist of sequential testing with high-sensitivity cardiac troponins followed by CMR. As diagnostic uncertainty on the presence of (peri)myocarditis remained despite CMR and additional investigations in about a third of patients, our results are open for improvement and validation and are not a final algorithm for clinical use.

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Author contribution
JL, HW, RB, MJ, RP, WK, JR, YP, AA, SB, AK drafted and revised the manuscript, JL and AK drafted the initial study design, JL, RB, RP, WK, SB, AK analysed and interpreted the data, all authors accept responsibility for conduct of research and gave final approval.

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SUPPLEMENTAL MATERIAL

Supplemental table S1. Categories of Myocarditis

Definite Myocarditis

Any of the following:

1. Tissue pathology diagnostic of myocarditis (e.g., on biopsy or autopsy)
2. Cardiac magnetic resonance imaging (CMR) diagnostic of myocarditis, a clinical syndrome and one of following:
    a. Elevated biomarker of cardiac myonecrosis
    b. Electrocardiography (ECG) evidence of (peri)myocarditis
3. New wall motion abnormality on echocardiogram not explained by another diagnosis (e.g., acute coronary syndrome, stress induced cardiomyopathy, sepsis) and all of the following:
    a. Clinical syndrome consistent with myocarditis
    b. Elevated biomarker of cardiac myonecrosis
    c. ECG evidence of (peri)myocarditis
    d. Negative angiography or other testing to
    e. Exclude obstructive coronary disease

Probable Myocarditis

Any of the scenarios below that are not explained by another diagnosis (eg, acute coronary syndrome, trauma, stress induced cardiomyopathy):

1. CMR with findings diagnostic of myocarditis without any of the following:
    a. Clinical syndrome consistent with myocarditis
    b. Elevated biomarker of cardiac myonecrosis
    c. ECG evidence of (peri)myocarditis
2. Nonspecific CMR findings suggestive of myocarditis with any one or more of the following:
    a. Clinical syndrome consistent with myocarditis
    b. Elevated biomarker of cardiac myonecrosis
    c. ECG evidence of (peri)myocarditis
3. New wall motion abnormality on echocardiogram with a clinical syndrome consistent with myocarditis and either:
    a. Elevated biomarker of cardiac myonecrosis
    b. ECG evidence of (peri)myocarditis
4. A scenario meeting criteria for Possible Myocarditis with18 fluorodeoxyglucose positron emission tomography imaging showing patchy cardiac fluorodeoxyglucose uptake without another explanation

Possible myocarditis

Any of the scenarios below that are not explained by another diagnosis (e.g., acute coronary syndrome, trauma, stress induced cardiomyopathy):

1. Nonspecific CMR findings suggestive of myocarditis with none of the following:
    a. Clinical syndrome consistent with myocarditis
    b. Elevated biomarker of cardiac myonecrosis
    c. ECG evidence of (peri)myocarditis
2. New wall motion abnormality on echocardiogram and one of the following:
    a. Clinical syndrome consistent with myocarditis

   b. ECG evidence of (peri)myocarditis
3. New elevated biomarker (beyond baseline) and one of the following:
   a. Clinical syndrome consistent with myocarditis
   b. ECG evidence of (peri)myocarditis

4

Chapter 4

Optimization of diagnostic accuracy in idiopathic inflammatory myopathies (ADAPT study): a protocol for a prospective diagnostic accuracy study of multi-modality testing in patients suspected of a treatable idiopathic inflammatory myopathy

Hannah A.W. Walter[1], Renske G. Kamperman[1], Joost Raaphorst[1], Camiel Verhamme[1], Johannes H.T.M. Koelman[1], Wouter V. Potters[1], Robert Hemke[2], Frank F. Smithuis[2], Eleonora Aronica[3], Ester M.M. van Leeuwen[4], Paul A. Baars[4], Marianne de Visser[1], Ivo N. van Schaik[1, 6], Patrick M.M. Bossuyt[5], Anneke J. van der Kooi[1]

•

[1] DEPARTMENT OF NEUROLOGY AND CLINICAL NEUROPHYSIOLOGY, AMSTERDAM UNIVERSITY MEDICAL CENTRE, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF RADIOLOGY AND NUCLEAR MEDICINE, AMSTERDAM UNIVERSITY MEDICAL CENTRE, AMSTERDAM MOVEMENT SCIENCES, AMSTERDAM, THE NETHERLANDS
[3] DEPARTMENT OF (NEURO)PATHOLOGY, AMSTERDAM UNIVERSITY MEDICAL CENTRE, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[4] DEPARTMENT OF EXPERIMENTAL IMMUNOLOGY, AMSTERDAM INSTITUTE FOR INFECTION & IMMUNITY, AMSTERDAM UMC, AMSTERDAM, THE NETHERLANDS
[5] DEPARTMENT OF CLINICAL EPIDEMIOLOGY, BIOSTATISTICS AND BIOINFORMATICS, AMSTERDAM UNIVERSITY MEDICAL CENTRE, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[6] SPAARNE GASTHUIS, HAARLEM, THE NETHERLANDS

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ABSTRACT

Introduction: Idiopathic inflammatory myopathies (IIMs) excluding inclusion body myositis (IBM) are a group of heterogeneous auto-immune disorders characterised by subacute onset and progressive proximal muscle weakness which are frequently part of a multisystem auto-immune disorder. Reaching the diagnosis can be challenging, and no gold standard for the diagnosis of IIM exists. Diagnostic modalities include serum creatine kinase (sCK) activity, muscle imaging (magnetic resonance imaging (MRI) or ultrasound (US)), electromyography (EMG), myositis auto-antibody testing and muscle biopsy. Several diagnostic criteria have been developed for IIMs, varying in reported sensitivity and specificity.

Hypothesis: We hypothesize that an evidence-based diagnostic strategy, using fewer and preferably the least invasive diagnostic modalities, can achieve the accuracy of a complete panel of diagnostic tests, including MRI, US, EMG, myositis-specific auto-antibody testing and muscle biopsy.

Methods and analysis: The OptimizAtion of Diagnostic Accuracy in idioPathic inflammaTory myopathies (ADAPT) study is a prospective diagnostic accuracy study with an over-complete study design. One-hundred patients suspected of an idiopathic inflammatory myopathy excluding IBM will be included. A reference diagnosis will be assigned by an expert panel using all clinical information and all results of all ancillary tests available, including 6 months follow-up. Several predefined diagnostic strategies will be compared against the reference diagnosis to find the optimal diagnostic strategy.

Ethics and dissemination: Ethical approval was obtained from the medical ethics committee of the Academic Medical Centre, University of Amsterdam, the Netherlands (2019_814). The results will be distributed through conference presentations and peer-reviewed publications.

Trial registration number: Netherlands trial register (NL8764)

INTRODUCTION

Idiopathic inflammatory myopathies (IIMs), often called 'myositis', are a group of heterogeneous auto-immune disorders characterised by subacute-onset and often severe, progressive proximal muscle weakness. IIMs encompass four treatable subgroups: dermatomyositis (DM), antisynthetase syndrome (ASS), immune-mediated necrotizing myopathy (IMNM), and non-specific/overlap myositis (OM).[1] Since inclusion body myositis is not amenable to treatment it is not within the scope of this study. First line treatment usually consists of glucocorticoids. Besides the typical proximal muscle weakness, dysphagia is often present, and extra-muscular manifestations may occur in IIMs and may be the initial symptom, e.g., a skin rash, interstitial lung disease (ILD), connective tissue disease, or cardiomyopathy. The clinical symptoms and signs differ widely between patients at disease onset and reaching a correct diagnosis in a timely manner can be challenging.[2]

There is no gold standard for the diagnosis of IIM. Diagnostic modalities include standard laboratory testing (serum creatine kinase (sCK) activity, lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and aldolase), muscle imaging via magnetic resonance imaging (MRI) or ultrasound (US), electromyography (EMG), myositis auto-antibody testing, and muscle biopsy. Evaluations of a range of diagnostic strategies have resulted in divergent sensitivities and specificities for the individual diagnostic modalities.[1 3-6] Relatively new modalities, such as myositis-specific auto-antibody testing and ultrasound, seem promising.[7-9]

Although the diagnostic accuracy of some of the above-mentioned tests have been studied before[7 10-12], to the best of our knowledge, no previous study has examined a complete diagnostic panel for myositis. A prospective, comparative diagnostic accuracy study with an over-complete study design enables the evaluation of the diagnostic accuracy of individual items and procedures and of the incremental value of multi-test diagnostic strategies. We hypothesize that an evidence based diagnostic strategy, using fewer and preferably the least burdensome diagnostic modalities, can achieve the accuracy of the complete panel of diagnostic tests, which includes MRI, US, EMG, myositis-specific auto-antibody testing, and muscle biopsy.

Aim

The primary aim of this study (ADAPT - OptimizAtion of Diagnostic Accuracy in idioPathic inflammaTory myopathies) is to identify a diagnostic strategy with an optimal accuracy for patients suspected of an IIM who need treatment with glucocorticoids, by comparing the accuracy of a range of strategies against a panel-based reference diagnosis, based on all available information and follow-up data.

METHODS AND ANALYSIS

Study status

Recruitment of study participants started on June 16th, 2020. The expected end date of this study is September 2023, when all included patients will have completed their follow up visit. This project has been registered in the Netherlands Trial Register, with trial identification number NL8764.

Study design

The ADAPT study is a prospective, fully paired diagnostic accuracy study, with an over-complete diagnostic design for patients suspected of having IIM. This means that all consenting participants undergo standardized history taking, physical examination, standard laboratory testing (including sCK), muscle imaging by whole body muscle MRI and muscle US, EMG, myositis auto-antibody testing, and muscle biopsy. The clinical reference standard is the final diagnosis assigned by an expert panel with all clinical information available, including 6 months follow-up.

Participants

This study is a single-centre study. The Amsterdam University Medical Centre serves as a tertiary referral centre for IIM in the Netherlands. Potentially eligible patients are recruited according to the following in- and exclusion criteria. Eligible patients who refuse any of the diagnostic tests of the study protocol will not be included.

Inclusion criteria

To be eligible, a patient must be suspected of an IIM based on symptoms and signs:

- Symmetrical proximal muscle weakness causing a functional limitation that justifies treatment with high dose glucocorticoids*
- Onset of symptoms ≤24 months before inclusion
- In case of dermatomyositis with classical skin lesions: additional informed consent for muscle biopsy
- Age of 18 years and older

* Patients with a connective tissue disorder and/or cancer are eligible

Exclusion criteria

- Alternative cause for proximal muscle weakness, e.g. the use of myotoxic medication (with the exception of anti-HMGcoA reductase inhibitors), a positive family history for a hereditary neuromuscular disease, or known inflammatory or infectious causes for myopathy outside the spectrum of IIM (e.g. graft-versus host disease or sarcoidosis)
- A high suspicion of sporadic inclusion body myositis (sIBM) based on clinical symptoms, e.g. the combination of slow onset of asymmetrical, weakness of quadriceps and deep finger flexor muscles, dysphagia and age >50 years[2]
- High suspicion of a neurogenic disease, based on a neurological examination showing more severe distal weakness than proximal weakness, asymmetric weakness, distal muscle atrophy or fasciculations
- Follow-up up to 6 months not possible
- To avoid an effect of immunosuppressive treatment on results of diagnostic tests, patients with immunosuppressive treatment within the last three months prior to screening are excluded, with the exception of:
    - Oral prednisone ≤60mg/day since one week, without clinical response*
    - Oral prednisone ≤20mg/day since two weeks, without clinical response*
    - Steroid sparing agents (e.g. methotrexate, azathioprine, mycophenolate mofetil) when prescribed less than 4 weeks prior to screening, without clinical response*

- History of IIM
- Contraindication for MRI

* The presence or absence of a clinical response will be judged by the treating physician.

Ethics and informed consent procedure

The study protocol has been approved by the medical ethics committee of the Academic Medical Centre, Amsterdam, The Netherlands. Potentially eligible patients are informed about the study via a telephone call and study information is sent by (e)mail. A physical examination is performed by the treating physician A (see below) as a screening before the consent procedure.

Study structure

The study structure is presented in figure 1.

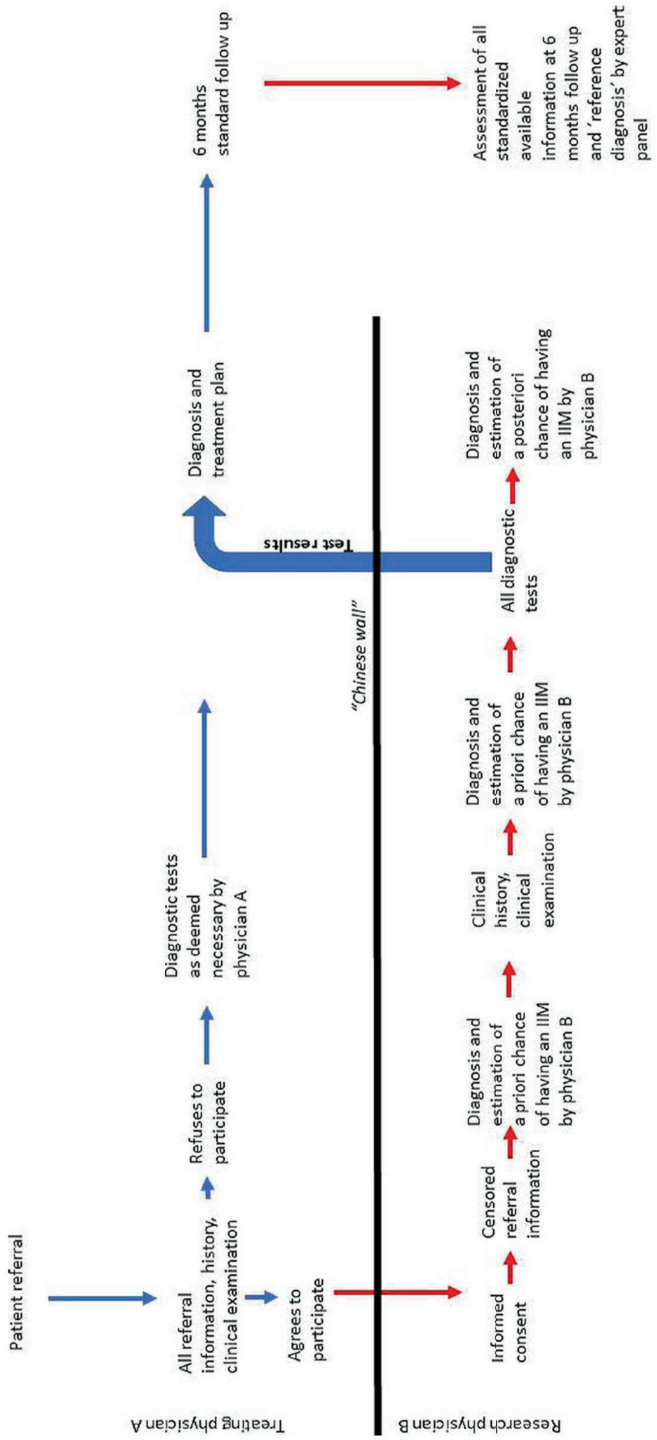

Figure 1. Study structure, describing the patient journey and roles of physician A and B and the expert panel
The 'Chinese wall' represents the blinding of research physician B from all information about the patient that is not related to the study as long as probability scores are given.

Study procedures

Upon inclusion the International Myositis Assessment and Clinical Studies (IMACS) outcome assessment tool[13] is filled out by the patient and treating physician, including all six core set measures: physician global activity, patient global activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire, muscle enzyme levels and extra muscular disease activity based on the Myositis Disease Activity Assessment Tool.

Blinding of physicians: All study-related neurological examinations are performed by research physician B, who is blinded from diagnostic results. The patient is diagnosed and treated, as part of regular care, by physician A. Both physicians are experienced neuromuscular specialists with expertise in IIM.[14]

Censoring of referral letter: referral letters are censored for physician B unto the following essential information: medical history, physical and neurological examination and standard laboratory investigations (electrolytes, sCK, LDH, ASAT, ALAT, aldolase).

Medical history taking and neurological examination: a standardized medical history is taken and structured neurological examination performed including signs of extra-muscular disease activity by physician B, i.e. assessment of cutaneous abnormalities, calcifications, signs of arthralgia or arthritis, dyspnoea, and Manual Muscle Testing (MMT13)[15]: neck flexors and extensors, and bilaterally: trapezius, deltoid, biceps brachii, gluteus maximus, gluteus medius, iliopsoas, hamstrings, quadriceps, wrist extensors, wrist flexors, ankle dorsiflexors and ankle plantar flexors, and additionally triceps brachii and deep finger flexors.

Diagnostic tests: the complete panel of diagnostic tests is performed according to the description below.

A study-related follow-up visit: after six months a study-related follow-up visit is used to collect the following data: disease course during the past six months i.e. change of symptoms and signs, occurrence of remission or relapse, use of immunosuppressive or immunomodulatory and other medication, final diagnosis according to current diagnostic criteria of the treating physician, alternative neuromuscular diagnosis and concomitant extra-muscular

manifestations (e.g. other auto-immune disorder, malignancy, cardiomyopathy, interstitial lung disease). The core set measures of the Total Improvement Score (TIS) of the IMACS will be collected again, which enables calculation of the TIS.[16]

Expert panel: All available clinical information and research data are provided in a standardized way to a panel of experts in the field of neuromuscular disorders: original referral letters, censored referral letters, medical history and neurological examination, results of all ancillary investigations, and the disease course during six months after diagnosis.

First, panel members evaluate cases individually, after which consensus is aimed for in a group discussion. The expert panel diagnosis for each patient serves as the clinical reference standard in evaluations of the accuracy and incremental accuracy of individual diagnostic tests and testing strategies. The panel will use criteria to achieve consistency and acceptable reproducibility.

Diagnostic tests

Whole body muscle MRI

Patients undergo a standardised 3.0 Tesla Whole body muscle magnetic resonance imaging (WB-MRI) (Philips, Best, The Netherlands) which includes water and fat imaging on T2-weighted 2-point Dixon axial 2D scans. Axial planes through shoulder region, upper limbs, hip region, mid upper leg and mid lower leg region are performed. Additional coronal T2 mDixon images of the thighs are performed to allocate the biopsy location.

Ultrasound

Ultrasound examination (US) is performed by an experienced clinical neurophysiologist, using a Esaote MyLabTwice ultrasound scanner with an 8–14 MHz broadband linear transducer with a 53-mm footprint and an axial resolution of around 0.2 mm (Esaote SpA, Genoa, Italy). Nine muscles are examined bilaterally: deltoid, biceps brachii, flexor carpi radialis, flexor carpi ulnaris, flexor digitorum profundus, rectus femoris, vastus lateralis, tibialis anterior and gastrocnemius muscles. The neurophysiologist analyses every single muscle semi-quantitatively using the four-point Heckmatt grading scale[17], and visually by examination of the echo intensity, calcifications and focal abnormalities.

Electromyography

Needle-Electromyography (EMG) is performed by an experienced clinical neurophysiologist, being a different person than the US evaluator. Ten muscles are tested unilaterally: deltoid, biceps brachii, flexor carpi radialis, flexor digitorum profundus, iliopsoas, vastus medialis, gluteus maximus, tibialis anterior and gastrocnemius (lateral head), and one paraspinal muscle at level L3.[18 19] EMG is performed after muscle imaging, in order to avoid misinterpretation of damage due to needle insertion for inflammatory abnormalities.

Myositis specific and myositis associated antibodies (MSAs and MAAs)

Antibodies are analysed in serum using the EUROline myositis 16 Ag. line-blot assay and the EUROline ANA Profile 5 line-blot assay of Euroimmun (Lübeck, Germany). Antibodies against HMGCR are analysed with a quantitative ELISA (Inova, San Diego, CA, USA). The presence of the following MSA's will be detected: antibodies against SRP, EJ, OJ, Mi-2α, Mi-2β, TIF1-γ, MDA5, NXP2, SAE1, PL-12, PL-7, Jo-1 and HMGCR, and the following MAA's: antibodies against Ku, RNP (70, A and C), PM/Scl-75 and PM/Scl-100 and anti-Ro52.[20] Antibodies against HMGCR are analysed with a quantitative ELISA (Inova, San Diego, CA, USA). The presence of an antibody is scored negative (-), weakly positive (+), positive (++) and strongly positive (+++) by an experienced immunologist.

Antinuclear antibody testing is performed simultaneously and the results of nuclear and cytoplasmatic Hep2 indirect immunefluorescence staining (Euroimmun) will be used as a verification of the presence of autoantibodies, where applicable.

Muscle biopsy

The optimal biopsy site is based on the presence of oedema on muscle MRI, as indicated by the radiologist and the treating physician. If no oedema is present, the biopsy is taken from a clinically weak muscle. The biopsy is taken according to recommended standards for muscle biopsies.[21]

Evaluations of diagnostic tests

The evaluating clinical neurophysiologist (n=2), radiologist (n=2), immunologist (n=2) and pathologist (n=1) are blinded from the contents of the censored referral letter, results of medical history and neurological examination, and results of the other diagnostic modalities.

Participants are kindly asked not to speak about any known results to the research physician and evaluators of the diagnostic tests. The diagnostic tests are evaluated using standard methods used in clinical practice. For MRI, the imaging assessment is performed by two musculoskeletal radiologists who should reach consensus. The test result - the probability of an IIM based on the diagnostic test - is expressed on a 5-point Likert scale (low to high probability) by the evaluator (figure 2). For myositis auto-antibodies, the test results are given as follows: Certainly not: all antibodies negative; Probably not: any positivity of Ro52 and/or MAA+; Uncertain: MSA +, Probably yes: MAA ++ or +++; Certainly yes: MSA ++ or +++. For the muscle biopsy, an experienced neuropathologist, specialized in the field of muscle diseases, decides whether the findings are compatible with an IIM according to international criteria.[22 23]

Probability of IIM diagnosis

In different phases of the study, research physician B assigns a probability of an IIM diagnosis. In addition, evaluators of the diagnostic tests and the expert panel assign a probability of an IIM diagnosis. These phases are explained below.

Phase 1: Probability before additional testing

Step 1: Physician B assigns the a priori probability of a diagnosis of IIM on a 5-point Likert scale based on the censored referral letter.

Step 2: Physician B assigns a second a priori probability of a diagnosis of IIM on a 5-point Likert scale based on the information of the censored referral letter, his/her standardized medical history and neurological examination, and laboratory tests.

Phase 2: Probability of additional diagnostic testing (figure 3).

Step 3: Each evaluator of a diagnostic test evaluates whether the findings of their particular diagnostic modality are compatible with an IIM, and assigns a probability score on a 5-point Likert scale.

Step 4: Physician B assigns the a posteriori probability of an IIM diagnosis on a 5-point Likert scale, based on all available information of Steps 1 to 3 (censored referral letter, standardized medical history, neurological examination, laboratory results and results of each evaluator of a diagnostic test).

Phase 3: Probability by expert panel (reference diagnosis)

Step 5: The expert panel first assigns its probability of a diagnosis of IIM on a 5-point Likert scale, and, second, whether the available information allows for a more specific (sub)diagnosis (DM, ASS, IMNM and OM). The 5-point Likert scale gives the reference panel the opportunity to express uncertainty.

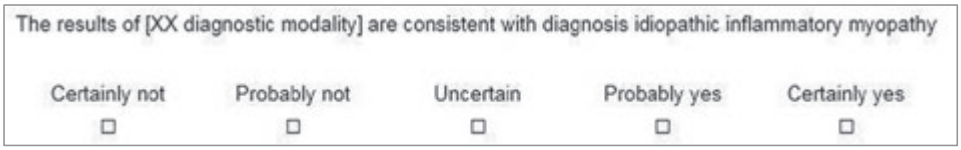

Figure 2. Form for every diagnostic modality filled in by the evaluator of the diagnostic test

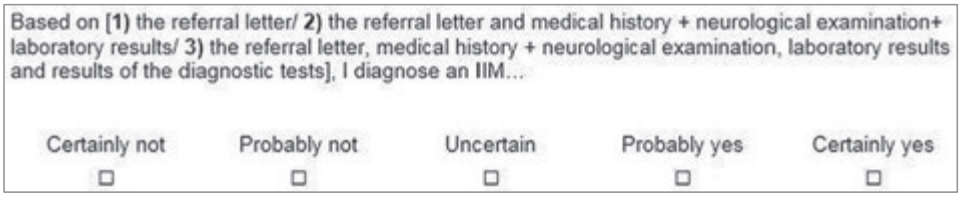

Figure 3. Form after diagnostic testing filled in by Physician B

Patient burden

Patient burden is evaluated using a questionnaire. For each diagnostic modality, study participants are invited to rate the experienced burden on a four-point Likert scale, anchored at 4= very burdensome, 3= fairly burdensome, 2= somewhat burdensome, or 1= no burden at all. These data will be used to compare the burden of different combinations of diagnostic tests.

Sample size

We expect to include two patients per month in this study and aim to recruit one-hundred patients, of whom 60% is expected to have an idiopathic inflammatory myopathy that needs glucocorticoid treatment. Two previous studies substantiate this expectation with a mean 'IIM' percentage of 62% and 'no IIM' percentage of 37%.[12 24] This sample size would allow us

to provide a confidence interval from 80% to 96% around an expected sensitivity of 90% in assigning a correct IIM diagnosis, and from 87% to 100% around an expected specificity of 97%.[25]

Statistical analysis

In the statistical analysis, the focus will be on the incremental accuracy of diagnostic tests over the censored referral letter, standardised medical history, physical examination and basic laboratory findings. First, all probability scores on the 5-point Likert scales will be dichotomised: options 'certainly not', 'probably not' and 'uncertain' will be categorised as 'No IIM'. Options 'probably yes' and 'certainly yes' will be categorised as 'IIM'. Estimates of diagnostic accuracy - sensitivity, specificity and positive and negative predictive values – will be calculated for the following:

Diagnostic accuracy of medical history, neurological examination, basic laboratory findings and diagnostic tests: for every single patient the a priori and a posteriori probability diagnosis of Physician B will be compared against the final diagnosis assigned by the expert panel. The difference in diagnostic accuracy between a priori and a posteriori probability will show the incremental accuracy of medical history, neurological examination and basic laboratory findings, and diagnostic tests relative to the information in the referral letter.

Diagnostic accuracy of the single diagnostic tests: the diagnostic accuracy of the single test modalities will be calculated by comparing the results of the individual diagnostic test (scores assigned by the evaluator) against the final diagnosis assigned by the expert panel. We will use the McNemar test[26] to compare the sensitivities and specificities between single diagnostic tests. Since whole-body muscle MRI may not be routinely available in all hospitals, a sub-analysis using the MRI of the thighs only will be performed.

Diagnostic accuracy of diagnostic strategies: The accuracy of diagnostic strategies, based on combinations of diagnostic tests, will be calculated. For every diagnostic strategy, estimates of sensitivity, specificity, and predictive values will be calculated. We will start by comparing strategies built with the following items: medical history (H_x), physical examination (Physical Ex), laboratory results (Lab), MRI, ultrasound (US), EMG, antibodies (Ab), muscle biopsy (Muscle B_x) *

1) [H_x, Physical Ex, Lab]
2) [H_x, Physical Ex, Lab] + Ab-pos
3) [H_x, Physical Ex, Lab] + Ab-pos + MRI
4) [H_x, Physical Ex, Lab] + Ab-pos + US
5) [H_x, Physical Ex, Lab] + Ab-pos + EMG

6) [H_x, Physical Ex, Lab] + Ab-neg + [MRI+ B_x]
7) [H_x, Physical Ex, Lab] + Ab-neg + US + [MRI+ B_x]
8) [H_x, Physical Ex, Lab] + Ab-neg + EMG + [MRI+ B_x]
9) [H_x, Physical Ex, Lab] + Ab-neg + EMG + US + [MRI+ B_x]

* It will be taken into account that the muscle biopsy is dependent on imaging, since the biopsy location is MRI-guided.

Comparing diagnostic strategies: The guiding principle in the analysis will be that we will compare diagnostic strategies in terms of their accuracy and cumulative burden. The burden related to procedures will be based on the mean reported burden in the questionnaires. The total burden of a diagnostic strategy will be evaluated as the cumulative burden score of the different tests. We will rank the strategies in terms of their sensitivity and list the corresponding specificity, predictive values, and patient burden with 95% confidence intervals. Dominated strategies, i.e. strategies with a higher burden without providing better sensitivity or specificity, will then be eliminated from the ranking, if differences are statistically significant. In addition, we will explore the use of decision curves, based on net benefit analysis, to highlight the comparison of the remaining diagnostic strategies.[27]

Missing data: Inconclusive diagnostic test results will be treated as negative in the calculation of the diagnostic accuracy. Results are considered inconclusive if no probability diagnosis is made by the evaluator. If two or more diagnostic tests of the same patient are missing, the expert panel might decide that a reference diagnosis cannot be assigned to that particular patient, which will result in exclusion from the analysis. We anticipate very few missing data due to structured planning of the diagnostic tests.

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Author contribution
A.W., J.R., C.V., P.M.B., M.V., I.S. and A.K. drafted the initial study design. A.W., R.K., J.R. and A.K. conduct the study procedures and data acquisition. C.V., J.K., W.P., R.H. F.S., E.A., E.L. and P.A.B. evaluated the diagnostic modalities. A.W, J.R. and A.K. drafted the manuscript which was critically revised for important intellectual content by all other authors. All authors read and approved the final manuscript before submission.

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Conflicts of interest
A.W., R.K., C.V., J.K., W.V., R.H., F.S., E.A., E.L. P.A.B., and P.M.B. report no competing interests.

I.S. chaired a steering committee for a CSL-Behring study investigating the safety and efficacy of SCIg in CIDP and received departmental honoraria for serving on scientific advisory boards for CSL-Behring and Kedrion. He received departmental research support from The Netherlands Organisation for Scientific Research, and from the Dutch Prinses Beatrix Spierfonds. All lecturing and consulting fees for I.S were donated to the Stichting Klinische Neurologie, a local foundation that supports research in the field of neurological disorders. He served on the editorial board of the Cochrane Neuromuscular Disease Group, was a member of the organising committee of the Inflammatory Neuropathy Consortium (INC), a standing committee of the Peripheral Nerve Society and was a member of the Scientific Board of the Kreuth III meeting on the optimal use of plasma-derived medicinal products, especially coagulation factors and normal immunoglobulins organised under the auspices of the European Directorate for the Quality of Medicines & HealthCare (EDQM). A.K. received departmental honoraria for serving on a scientific advisory board for ArgenX. J.R. received departmental research support from the Dutch Prinses Beatrix Spierfonds, Dutch ALS foundation and Sanquin Plasma Products. M.V. is a member of the Data Monitoring Committee of Novartis Pharma AG and chair of the Independent Data Monitoring Committee of Dynacure.

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Chapter 5

B cell receptor profiling before and after IVIg monotherapy in newly diagnosed idiopathic inflammatory myopathies

Dornatien C. Anang[1,2,3]*, Hannah A.W. Walter[4]*, Johan Lim[4], Ilse Niewold[1,3], Linda van der Weele[1,2], Eleonora Aronica[5], Filip Eftimov[4], Joost Raaphorst[4], Barbera D.C. van Schaik[6], Antoine H. C. van Kampen[6], Anneke J. van der Kooi[4], Niek de Vries[1,2]

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[1] DEPARTMENT OF RHEUMATOLOGY AND CLINICAL IMMUNOLOGY, AMSTERDAM RHEUMATOLOGY AND IMMUNOLOGY CENTER, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF EXPERIMENTAL IMMUNOLOGY, AMSTERDAM INFECTION AND IMMUNITY INSTITUTE, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[3] DEPARTMENT OF GENOME ANALYSIS, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, NETHERLANDS
[4] DEPARTMENT OF NEUROLOGY, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[5] DEPARTMENT OF (NEURO)PATHOLOGY, AMSTERDAM NEUROSCIENCE, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[6] BIOINFORMATICS LABORATORY, DEPARTMENT OF EPIDEMIOLOGY AND DATA SCIENCE, AMSTERDAM PUBLIC HEALTH INSTITUTE, AMSTERDAM INFECTION AND IMMUNITY INSTITUTE, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS

* SHARED FIRST AUTHORSHIP

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ABSTRACT

Objective: To unravel B cell receptor (BcR) characteristics in muscle tissues and peripheral blood and gain more insight into B cell receptor (BcR) repertoire changes in peripheral blood in idiopathic inflammatory myopathies (IIMs), and study how this correlates to the clinical response to intravenous immunoglobulin (IVIg).

Methods: Nineteen treatment naïve patients with newly diagnosed IIM were prospectively treated with IVIg monotherapy. RNA-based BcR repertoire sequencing was performed in muscle biopsies collected before, and in peripheral blood (PB) collected before and nine weeks after IVIg treatment. Results were correlated to patients' clinical improvement based on the total improvement score (TIS).

Results: Prior to IVIg treatment, BcR clones found in muscle tissue could be retrieved in peripheral blood. Nine weeks after IVIg treatment, new patient-specific dominant BcR clones appeared in peripheral blood while pre-treatment dominant BcR clones disappeared. The cumulative frequency of all dominant BcR clones before treatment was significantly higher in individuals who responded to IVIg compared to those who did not respond to IVIg, and correlated with a higher CK. During follow up, a decrease in the cumulative frequency of all dominant clones correlated with a higher TIS.

Conclusion: In treatment naïve patients with newly diagnosed IIM, muscle tissue and peripheral blood share expanded BcR clones. In our study a higher cumulative frequency of dominant BcR clones in blood before treatment was associated with a higher CK and better treatment response, suggesting that response to IVIg may depend on the composition of the pre-treatment BcR repertoire.

INTRODUCTION

Idiopathic inflammatory myopathies (IIM), often referred to as 'myositis', are a heterogeneous group of auto-immune disorders characterized by subacute, often severe progressive proximal muscle weakness. Several treatable subtypes can be distinguished: i.e., dermatomyositis (DM), antisynthetase syndrome (ASS), immune-mediated necrotizing myopathy (IMNM), non-specific/overlap myositis (NM/OM), and polymyositis (PM).[1 2] Polymyositis is a contested entity, which is currently considered a diagnosis after exclusion of all other forms of myositis. Here, first line treatment consists of high dose corticosteroids, but in more than half of the patients long-term treatment with additional immunosuppressive therapy is needed.[3 4] However, despite combined medical treatment, the majority of patients (70%) have a chronic or polyphasic disease course and develop significant residual disability and reduced quality of life.[5] Consequently, there is a clear need for a better understanding of the disease pathogenesis, for better treatment strategies and a better understanding of their mechanism of action.

In recent years, pooled human IgG purified from healthy donors[6], Intravenous immunoglobulins (IVIg), are increasingly used in the treatment of IIMs.[7-9] IVIg has been reported to bind and neutralize autoantibodies, pro-inflammatory cytokines such as TNF-alpha [10], and the B cell activation factor (BAFF).[11-13] Furthermore, IVIg was shown to increase the number of B cells and plasmablasts in peripheral blood of Guillain Barré syndrome (GBS) patients, and can directly affect the B cell repertoire [14-16]. This is of interest since B cells may play a pivotal role in IIMs[17 18]: muscle biopsies of myositis patients show plasma cells[19-21], 60 to 70% of IIM patients are positive for myositis specific and myositis associated antibodies[22], and B-cell directed therapies such as rituximab are effective in the treatment of myositis.[23-26]

Although B cells may play a key role in IIMs, and IVIg can influence the B cell repertoire, little information is available on modulation by IVIg of the B cell response at the clonal level in IIM and other auto-immune diseases, and whether differences in the effect of this modulation explain the large inter individual variability in clinical response to IVIg.[27-29] To fill this gap we used RNA-based next generation sequencing to study the B cell receptor (BcR) repertoire in baseline muscle tissue and in peripheral blood before and 9 weeks after IVIg treatment in treatment-naive myositis patients. The results presented here provide new insights into the possible role of B cells in the disease pathogenesis and the mechanism of action of IVIg in peripheral blood over time.

METHODS

Ethical statement

All patients signed informed consent prior to inclusion in the study. This study was conducted with approval of the local medical research ethics committee of the Academic Medical Centre, Amsterdam, and in accordance with the declaration of Helsinki.

Patients and study design

Adult, treatment-naive patients with newly diagnosed, biopsy-proven IIM were included in a 9 week phase-2 open-label study that investigated intravenous immunoglobulins (IVIg) as first-line treatment in IIM patients.[27] IVIg monotherapy was started with 2g/kg at baseline, followed by two subsequent doses of 1g/kg at week three and week six. Patients received no glucocorticoids or other immunosuppressive treatment.

Clinical assessment

Clinical and laboratory examination was performed at two time points: at baseline before treatment, and at follow up after treatment at 9 weeks, or earlier in case of preliminary ending of protocol. Clinical examination consisted of all core set measures (CSM) of the IMACS (supplemental materials and methods), and results in a Total Improvement Score (TIS). TIS ≥40 was defined as response to treatment.[30]

Collection and processing of muscle tissues and blood samples

Muscle biopsies were taken upon diagnosis before treatment (baseline) in all patients. For collection of muscle biopsies, the optimal biopsy location was based on the presence of edema on muscle imaging (MRI or ultrasound). If no edema was present, the biopsy was taken from a clinically weak muscle. Biopsies were taken according to recommended standards for muscle biopsies.[31] Muscle biopsies were stored at -80°C until RNA isolation. Peripheral blood was drawn from all patients at two time points: prior to treatment (baseline) and at week 9, after three cycles of IVIg treatment, or earlier in case of preliminary ending of the protocol (follow up) in PAXgene blood RNA tubes (PreAnalytiX, Breda, The Netherlands). Blood samples were all stored at -80°C until RNA isolation.

RNA isolation and Next generation sequencing of the BcR repertoire

RNA isolation from peripheral blood was carried out using PAXgene blood miRNA isolation kit (Qiagen, Hilden, Germany). RNA was isolated from stored muscle biopsies after homogenization with the MagNA Lyser (Roche) as extensively described in supplementary materials and methods. Amplification of the BcR was carried out as previously described[32 33] and further reported in supplementary materials and methods.

Bioinformatics generation and analysis of BcR repertoires

The obtained sequencing reads were analyzed with an in-house developed pipeline for repertoire analysis "RESEDA" (REpertoire SEquencing Data Analysis, https://bitbucket.org/barbera/reseda) which has been previously described extensively[34] and is further described in online materials and methods. Clones with a frequency greater than or equal to 0.5% of the total repertoire were labelled dominant or highly expanded clones (HECs).[33 34]

Clustering related clones

Clones were grouped on the CDR3 amino acid sequence using in-house developed R scripts. In brief, the Hamming distance between clones was calculated. An approach similar to [35] was taken to calculate a dynamic threshold for grouping clones together as described in the supplementary materials and methods.

Statistical analysis

After testing for normality with D'Agostino and Pearson omnibus test, data are presented as mean and SD for normally distributed data or median with IQR and analysed using the Kruskall Wallis or Mann Whitney test for non-normally distributed data. Correlations were calculated with Spearman's Rank Correlation Coefficient. All statistical analyses were performed using GraphPad Prism Software (version 8.0, GraphPad Software, Inc. La Jolla, CA). P-values <0.05 were considered statistically significant.

RESULTS

Patients

Twenty newly diagnosed IIM patients participated in the IMMEDIATE study, and outcomes have been reported previously.[27] One patient was excluded earlier from analysis (1) due to inability to reach the threshold of improvement on the TIS due to a ceiling effect, thus leaving 19 patients for

analysis. Seven (37%) patients ended the protocol preliminary, after a median of 6 weeks, last observation carried forward, as reported in the IMMEDIATE.[27] Eight (42%) patients had DM, five (26%) had NM/OM, five (26%) had an IMNM and one patient (5%) had ASS. All patients had proximal muscle weakness at inclusion. Muscle biopsies were taken from the vastus lateralis muscle in 16 patients, from the triceps in two patients and from the deltoid in one patient. All biopsied muscles showed edema on magnetic resonance imaging T2-weighed Dixon scans. Patient characteristics are summarized in **Table 1 and Supplementary Table S2 and S3**.

Table 1. Baseline characteristics of the 19 included patients.

Characteristic	**Patients (n=19)**
Age at onset in years, median (IQR)	59 (37 – 69)
Months between start of symptoms until diagnosis, median (IQR)	5 (3-6)
Gender, females, n (%)	12 (63)
Connective tissue disorder, n (%)[¤]	3 (16)
Cancer, n (%)	1 (5)
Serum CK, median (IQR), U/L	1199 (179–6500)
Myositis-specific and myositis associated antibodies, n (%)	
Anti-HMGCR	3 (16)
Anti-NXP2	3 (16)
Anti-Jo1	1 (5)
Anti-MDA5	1 (5)
Anti-SRP	1 (5)
Anti-TIF1gamma	1 (5)
Myositis associated antibodies (MAA) only	2 (11)
Seronegative	7 (37)
Responders, TIS ≥40 at 9 weeks, n (%)	8 (42)

QR; interquartile range, CK; creatine kinase, U/L; units per litre. [¤] n=1 mixed connective tissue disease, n=1 Sjögren's syndrome, n=1 systemic sclerosis

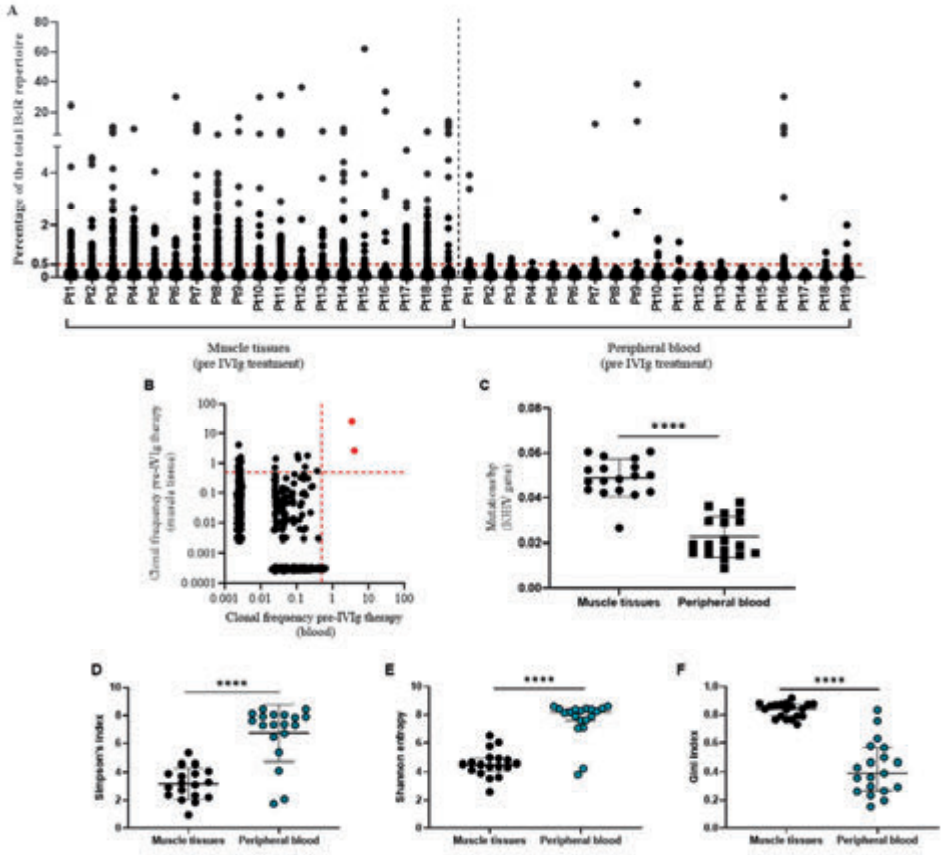

Figure 1: Identification of BcR clones in muscle tissues and peripheral blood before IVIg treatment **(A)** Scatter plot of the BcR repertoire in muscle tissues and peripheral blood of IIM patients prior to IVIg treatment. Each dot represents a unique BcR clone. **(B)** Representative example of CDR3 clonal overlap between peripheral blood and muscle tissue pre-IVIg treatment, shown for patient one (Pt1). Each dot represents a unique BcR clone, and its frequency in the analyzed repertoire is depicted on the x (blood pre-IVIg treatment) and y (muscle tissue pre-IVIg treatment) axes as percentage of total UMIs. The dotted lines on each axis indicate the 0.5% cut-off for dominant BcR clones. BcR clones in red are clones which are dominant in both samples. **(C)** Mutation load (expressed as mutations / base pair(bp)) of BcR clones detected in muscle tissues and peripheral blood prior to IVIg treatment. (D-F) Simpson's index, Shannon entropy and Gini index to evaluate the diversity of the BcR repertoires in peripheral blood and muscle tissues at baseline.

Dominant BcR clones are present in muscle tissue and peripheral blood of myositis patients prior to IVIg treatment

To get a better understanding of B cell involvement in IIMs, we started by investigating the hypothesis that in IIMs, muscle biopsies and peripheral blood harbour expanded BcR clones. We sequenced the BcR heavy chain repertoires in muscle tissue and peripheral blood obtained in all patients prior to IVIg treatment and detected multiple dominant BcR clones in all 19 muscle tissues sequenced and in 14 of the 19 peripheral blood samples sequenced **(Figure 1A)**. Since (dominant) BcR clones were detected in both muscle tissue and peripheral blood prior to IVIg treatment, we next investigated whether the (dominant) BcR clones present in muscle tissues were the same (dominant) BcR clones present in peripheral blood. In 18 of the 19 patients, BcR clones found in muscle tissue could be detected in peripheral blood. In three of these patients, BcR clones which were dominant (clones with a frequency ≥ 0.5% of the total BcR repertoire) in muscle tissues were also dominant in peripheral blood of those patients **(Figure 1B** and Supplementary Table S1**)**. We next investigated whether BcR clones found in both muscle tissues and peripheral blood shared similar characteristics. From each patient, we performed a clustering analysis at the CDR3 level of the top 100 most expanded clones shared between muscle tissues and peripheral blood. Indeed, some of these BcR clones in each patient clustered together based on CDR3 amino acid similarity (Supplementary Figure 1A). Additional analysis on these shared clones showed a preferential usage of certain V-J gene combinations with IGHV4-31, IGHV3-23, IGHV3-3 and IGHV3-74 the most used V genes in muscle tissues, IGHV1-2 and IGHV3-3 the most used V genes in blood and JH4 the most used J gene segment in both blood and muscle tissue (Supplementary Figure 1B and 1C). We next investigated whether the different myositis subtypes showed different BcR clonal repertoires (the number and impact of dominant BcR clones) as well as other BcR repertoire features such as CDR3 length, CDR3 charge and VJ gene usage. Subgroup analysis based on antibody type (myositis specific antibodies (MSA), myositis associated antibodies (MAA) and seronegative) (Supplementary figure 2) or myositis subtype, even in the largest group of eight DM patients, did not show significant differences (data not shown). The total number of clones retrieved from each antibody subgroup is further listed in Supplementary Table S2.

To further explore the phenotypic composition of the BcR repertoire in muscle tissues and peripheral blood prior to IVIg treatment, we analysed the mutation load of the BcR heavy chain variable region (IGHV) as an indication of the maturation status of the BcR repertoire. A

high mutation load indicates that the repertoire is dominated by mature BcR clones (i.e., memory and plasma blasts/cells), while a low mutation load indicates that the BcR repertoire is mainly composed of immature BcR clones (naïve). When we compared the mutation load of the BcR repertoire obtained from muscle tissues to that of peripheral blood, the BcR repertoire in muscle tissues had a significantly higher mutation load compared to the pretreatment BcR repertoire obtained from peripheral blood ($p < 0.0001$) **(Figure 1C).** Finally, we compared the diversities of the BcR repertoires obtained from peripheral blood and muscle tissues at baseline using the Simpson's index, Shannon entropy and the Gini index (these indices are further explained in supplementary materials and methods). We observed that the peripheral blood repertoires were more diverse when compared to the repertoires in muscle tissues (**Figure 1D-1F**).

Taken together, our analyses in this limited number of patients show that muscle tissue and peripheral blood of IIM patients harbour dominant BcR clones. In addition, the BcR repertoire of muscle tissues is mostly composed of highly mutated BcR clones when compared to peripheral blood.

The dynamics of BCR clonality in peripheral blood after IVIg treatment

To explore the effects of IVIg treatment on the BcR repertoire, we compared the number and cumulative frequency (impact) of dominant BcR clones in peripheral blood before and after 9 weeks of IVIg treatment. At the time points, the number of dominant BcR clones **(Figure 2A)** and the impact of dominant BcR clones **(Figure 2B)** did not differ. After subgrouping based on the type of antibody, this analysis also did not show significant differences (Supplementary Figure 2A-F). BcR repertoire features like CDR3 length, CDR3 charge, V and J gene usage and repertoire diversity did not differ between both time points (Supplementary Figure 3A-C and 3F-H). However, comparing dominant clones at baseline with those present 9 weeks after IVIg treatment we observed that only one patient had a shared dominant clone between both time points (Supplementary Figure 3D). In all other patients, dominant BcR clones present 9 weeks after treatment were completely different from the dominant clones present before treatment **(Figure 2C)**. Additionally, CDR3 amino acid overlap plots between different individuals showed that these newly formed dominant clones at week 9 were patient specific

as no two patients shared dominant clones both before and after IVIg treatment (Supplementary Figure 3E).

We further explored whether the dominant clones at week 9 were *de novo* or were in any way related to clones present prior to IVIg treatment. From each patient, the CDR3 amino acid sequences of all dominant clones retrieved at week 9 were compared with the CDR3 amino acid sequences of all clones present at baseline in a clustering analysis. Our analysis revealed that, dominant BcR clones at follow-up were completely different from the BcR clones pre-treatment, as no clusters were observed between clones present prior to IVIg treatment and the newly formed dominant BcR clones after IVIg **(Figure 2D).**

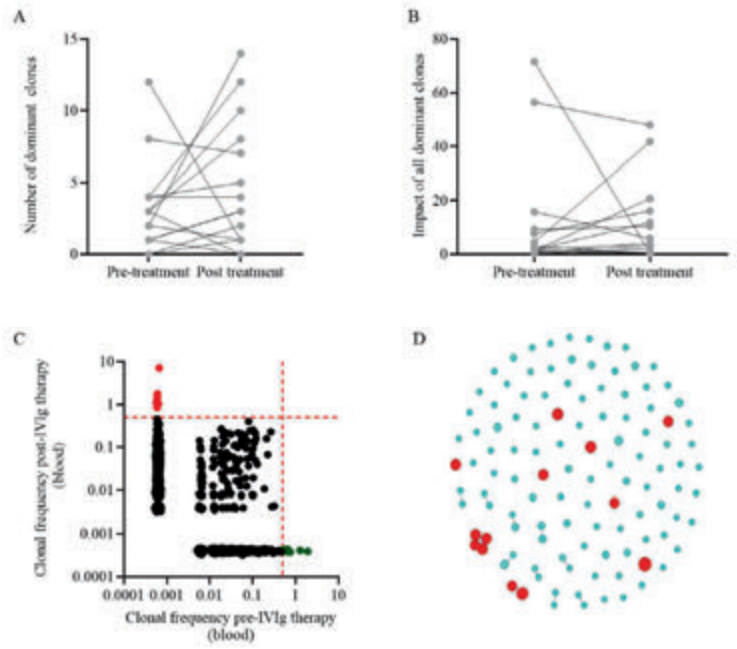

Figure 2: BcR repertoire characteristics in peripheral blood before and after IVIg treatment For each patient panel **(A)** shows the number of dominant BcR clones, while panel **(B)** shows the combined impact of all dominant BcR clones on the BcR repertoire before and 9 weeks after IVIg treatment. **(C)** Representative CDR3 overlap plot before (X-axis, dominant clones in green) and after (Y-axis, dominant clones in red) IVIg treatment for one patient. Each dot represents a unique BcR clone, its frequency being calculated as percentage of total UMI-corrected reads. The dotted red lines on each axis indicate the 0.5% cut-off for dominant BcR clones. **(D)** A representative example of a CDR3 amino acid cluster analysis in 1 patient is shown. In red are newly formed dominant BcR clones 9 weeks after IVIg treatment. Clones present at baseline (in blue) show no clustering with the newly formed red clones. For clarity only the top 100 clones at baseline are shown.

Patients with a high impact of dominant BcR clones in peripheral blood at baseline respond better to IVIg treatment

We subsequently analysed whether BcR repertoire characteristics were associated with treatment response. The number of dominant clones before IVIg treatment was not different between responders and non-responders (response defined as TIS score ≥ 40) **(Figure 3A)**. Of interest, the impact of all dominant clones (cumulative frequency) before IVIg treatment was significantly higher in responders compared to non-responders **(**$p<0.05$; **Figure 3B)**. Amongst the six patients without dominant clones at baseline, three were responders (Supplementary

Table S2). Additionally, in comparison to the other responders, we did not see any differences in the two responders who had the highest impact of dominant clones at baseline in terms of clinical characteristics (Supplementary Table S2) or BcR features such as CDR3 length, CDR3 charge and V and J gene usage (data not shown). The two patients with the highest impact of all dominant clones before treatment (figure 3B) are two patients with immune mediated necrotizing myopathy (IMNM), one with HMGCR antibodies and the other patient was seronegative.

In muscle biopsies, the impact of dominant clones did not show a correlation with response. Nine weeks after treatment, we did not observe this difference as the impact of all dominant BcR clones was comparable between responders and non-responders **(Figure 3C).** In conclusion, based on a limited set of patients (N=19), we observed a significantly higher impact of dominant BcR clones in blood before start of therapy in responders compared to non-responders to IVIg therapy.

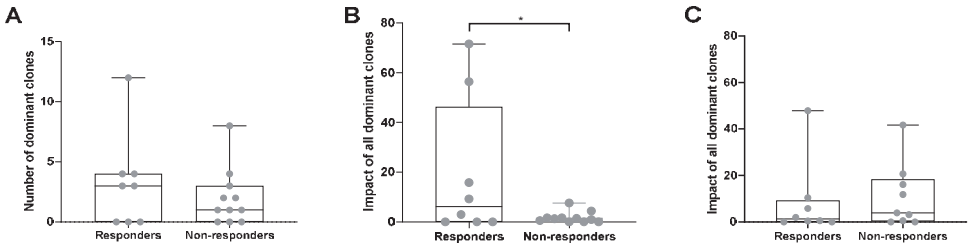

Figure 3: BcR repertoire in peripheral blood before and after IVIg treatment in responders and non-responders in peripheral blood

(A) The number of dominant BcR clones and **(B)** the impact of all dominant BcR clones before IVIg treatment. **(C)** the impact of all dominant BcR clones after IVIg treatment. *p<0.05. Box plots show the median and 25th and 75th interquartile, error bars show the range. Single dots in grey represent values for each patient.

Correlation of BCR clonality with disease activity and response

Next, we assessed whether BcR repertoire features correlated with various markers of disease activity or therapy response. At baseline the impact of the dominant clones in blood correlated with increased CK in blood (Figure 4A; r = 0.51; p = 0.02, 95% CI [0.08 - 0.78]). This was also reflected in a non-significant trend to association of CK with the number of dominant clones

in peripheral blood (Supplementary Figure 4B). Here, the number of dominant clones but not the impact of these clones also showed a significant inverse association with baseline MMT (Supplementary Figure 4A; r=-0.46, p=0.04, 95% CI [-0.76 - 0.008]). In muscle tissues, we did observe a non-significant (r=0.36; p=0.08, 95% CI [-0.16 - 0.70]). trend in correlation between baseline MMT and impact of dominant clones (Supplementary Figure 5). There was no significant correlation between the number and impact of dominant clones in muscle tissues at baseline with MMT, CK (Supplementary figure 4E&F).

After IVIg treatment, in blood a decrease in the *impact* of dominant clones correlated with an increase in MMT (Figure 4C; r = 0.53; p=0.02, 95% CI [-0.8 - -0.12]) and non-significantly with a better TIS response (Figure 4B; r = 0.45; p=0. 06, 95% CI [-0.77 - 0.05]). The *number* of dominant clones in blood did not correlate with CK, MMT or TIS (Supplementary Figure 4C&D).

In patients who shared dominant clones between muscle tissues and blood (n=3), no significant correlations between the number and impact of shared dominant clones (blood-muscle) and TIS, MMT or CK were demonstrated (data not shown). There was no significant correlation between the number and impact of dominant clones in muscle tissues at baseline clinical response at follow-up (data not shown).

In conclusion, the impact of dominant BcR clones in blood correlated with higher CK levels at baseline. After IVIg, a decrease in the impact of dominant clones in blood correlated with higher total improvement score (TIS) as well as better muscle strength (MMT).

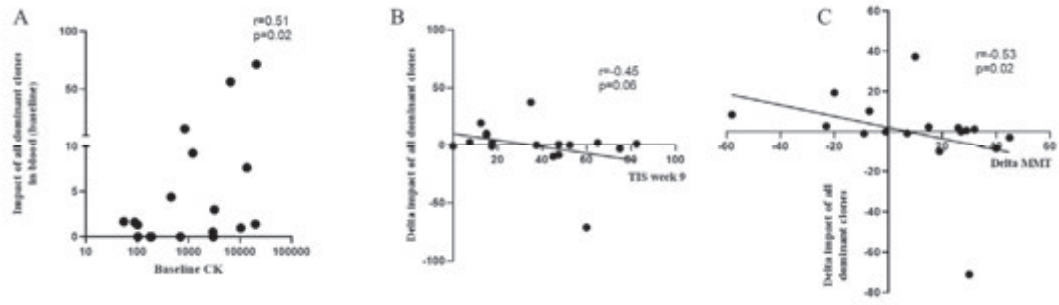

Figure 4. Correlation of BcR repertoire characteristics before and after treatment with markers of disease activity and therapy response.

(A) The impact of all dominant BcR clones in blood at baseline versus CK levels at baseline. Correlation of the difference in impact of all dominant BcR clones in blood (week 9 minus week 0) with **(B)** the total improvement score (TIS) and **(C)** the improvement in manual muscle testing (MMT). p and r values are shown for each curve.

DISCUSSION

In this prospective exploratory study, we identified expanded BcR clones in muscle tissues of IIM patients prior to treatment, which confirms a report on treatment naive dermatomyositis and polymyositis patients [18]. In addition, our study shows a possible relation between BcR clonality in peripheral blood and response to treatment, which was not described in the previous report because it focused on muscle tissue. We also observed expanded BcR clones in peripheral blood of IIM patients, and some expanded BcR clones present in muscle tissues could be found in peripheral blood. To the best of our knowledge, we report for the first time the presence of B cells carrying the same BcR signatures in muscle tissue and peripheral blood of treatable myositis patients. This finding suggests that B cells have the ability to migrate between peripheral blood and muscle tissues of myositis patients, as observed before with T cells in inclusion body myositis.[36] It is worth noting that some dominant BcR clones were muscle restricted as they were not detected in peripheral blood. This raises an intriguing question as to whether these muscle restricted BcR clones express high affinity homing receptors which enable their firm attachment to muscle tissues, or whether they even proliferate and differentiate locally. Future work addressing this question may lead to a better understanding of the phenotype of B cells in muscle tissues of myositis patients. Additionally, whether tissue-restricted or overlapping BcR clones are disease-associated and whether they produce myositis-related antibodies warrants further investigation. Since the pathogenesis of myositis is different for each antibody, we investigated whether different myositis subtypes would show different BcR clonality patterns or repertoire features. We did not find any significant differences between the different myositis subtypes in terms of BcR clonality or BcR repertoire features. A possible explanation could be the limited number of patients in our pilot study.

The analysis of the BcR repertoire in muscle tissue revealed the presence of highly mutated BcR clones when compared to BcR clones retrieved from peripheral blood, indicating the presence of matured cells of the B-cell lineage in muscle tissue. This is in line with previous literature[18] and expected since organized lymphoid structures are present in tissues of myositis patients[37 38], which to contain matured (and thus mutated) plasma and memory B cell subsets that may contribute to autoantibody production.

We also described the preferential usage of certain V-J gene rearrangements in the BcR clones present in both muscle tissues and peripheral blood. Preferential V gene usage by B cell clones has been described in various autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) as well as in myositis.[39-41] The preferential usage of certain V genes was described before in a cohort of 12 myositis patients [42]. Several reasons including antigen structure as well as antigen availability have been put forward as plausible reasons for this observed preferential usage of V genes in autoimmune conditions.[43] It is therefore tempting to speculate that this observation in our study is due to a selective response to specific antigens.

Another finding from our study was the presence of completely different dominant BcR clones before and after IVIg treatment. Similar findings have been observed in Guillain Barré syndrome patients treated with IVIg.[44] Their results suggest that IVIg can induce a *de novo* B cell response which can be observed after treatment. These results are contrary to another study that investigated the effects of IVIg on the B cell repertoire in patients undergoing desensitization therapy before transplantation and reported no major changes in the BcR repertoire after IVIg treatment.[45] Several reasons including dose as well as time of sampling could account for these differences. However, the authors did not describe whether BcR clones present before IVIg treatment were present after IVIg treatment.

Finally, an intriguing question is whether the B cell repertoire both before and after IVIg treatment is associated with clinical benefits in myositis patients. In our study, we found that patients who had a high impact (cumulative frequency) of dominant BcR clones before treatment responded better to IVIg treatment. Of note, the impact of dominant BcR clones in blood also correlated with higher CK levels at baseline, and a decrease in the impact of dominant clones in blood correlated with higher total improvement score (TIS). This might suggest that patients exhibiting a dominant B-cell response in the pre-treatment repertoire do respond better on IVIg. However, our data should be interpreted with care as the number of patients included in our study is limited. A study in Guillain Barré syndrome patients reported an association between IVIg-induced plasmablasts after treatment and clinical recovery.[44] We did not find such a correlation between IVIg-induced dominant B cell clones after treatment and clinical benefit. Further research with appropriate controls, is needed to confirm these early observations.

Our study has some limitations. First, the relatively low number of patients did not allow subgroup analysis for different myositis subtypes. Therefore, our observations need additional confirmation in larger patient cohorts that allow subgroup analysis. Secondly, since we did not make use of conventional flow cytometry in our study, our analysis yields little information on the phenotype of these dominant BcR clones. Thirdly, the lack of a control group precludes us from making firm conclusions on whether the effects on the BcR repertoire seen in this study are totally due to IVIg or simply due to the passage of time. Finally, our BcR repertoire technique is not able to determine the specificity and pathogenicity of these dominant BcR clones present both before and after IVIg treatment as well as in muscle tissues, e.g. if the clones are related to the formation of myositis related antibodies or are directly involved in the disease pathogenesis. As a result, extensive approaches such as single cell sequencing coupled with recombinant antibody expressions as well as cell stimulation assays are needed to fully unravel the specificity and pathogenicity of these dominant clones.

In conclusion, we have shown that BcR clones present in muscle tissues can be retrieved in peripheral blood of IIM patients. Although not associated with treatment response, in peripheral blood, new patient specific dominant BcR clones were formed during IVIg treatment and, pre-treatment dominant BcR clones disappeared after treatment. Finally, the high impact of dominant B cell clones before treatment is associated with a better clinical response to IVIg, suggesting that response to IVIg might depend on the pre-treatment BcR repertoire. Future work tailored towards a better understanding of the pre-treatment BcR repertoire in myositis patients might result in the identification of biomarkers that could predict eventual response to IVIg treatment.

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Author contribution
Study conception and design; AK, NdV, JL. Data acquisition; DCA, HW, JL, IN. Analysis and interpretation of data; DCA, HW, EA, BvS, AvK, JR, LvdW, FE, NdV, AK. All analyses were performed by DA & HW and independently by LvdW. All authors revised the article for important intellectual content and all authors have read and approved the final version of the manuscript.

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Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTAL MATERIAL

Supplementary materials and methods

Clinical assessment

The six core set measures of the IMACS score used for clinical examination included: : the patient global activity assessment (PGA), Physician global activity assessment (PhGA), Health Assessment Questionnaire (HAQ), extra muscular disease activity based on the Myositis Disease Activity Assessment Tool (MDAAT), muscle enzymes and muscle strength testing according to manual muscle testing (MMT 13) in which a score of 10 reflects maximal strength in the individual muscle, and the total score per patient ranged from 0-260.[1] At follow up, a change score (follow up–baseline) was calculated for MMT and CK. Clinical improvement after treatment was measured by the total improvement score (TIS) ranging from 0-100, with higher scores reflecting more improvement [1], and a score ≥40 reflecting clinically significant response to IVIg treatment.

RNA isolation and Next generation sequencing of the BcR repertoire

Following RNA isolation from peripheral blood, muscle biopsies (stored in -80°C) were homogenized by spinning twice with 900µl of RLT buffer (1% B-mercaptoethanol) and ceramic beats in a MagNA Lyser (Roche) at 6500rpm for 30 seconds with cooling on ice for 1 minute in-between. The cell lysate was incubated with 60µl of Proteinase K and 240µl RNase free water for 10 minutes at 55°C on a shaking heat block. RNA was extracted from the resulting cell lysate using the RNeasy Mini kit (Qiagen, Hilden, Germany), Complementary DNA of BcR heavy chain molecules was synthesized using a BcR heavy chain Joining reverse primer tagged with a 9 random nucleotide UMI and a consensus sequence, followed by Exonuclease I treatment (Thermo Fisher Scientific, Breda, The Netherlands) to remove left over primers. This was followed by a PCR with forward primers covering the BcRh Variable genes and a reverse primer binding to the consensus sequence previously introduced in the specific-cDNA and tagged with an 8 bp patient identifier (MID, Molecular Identifier). Obtained amplicons were purified using two rounds of AMPure XP beads clean-up (Beckman Coulter, Woerden, The Netherlands), quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific), dual-indexed with i5 and i7 adapters (Nextera XT Index Kit v2) and sequenced using the Illumina Miseq Kit v3 2 x 300 bp technology according to the manufacturer's manual (Illumina, San Diego, California, USA).

Bioinformatics generation and analysis of BcR repertoires

The generation of BcR repertoires from the RESEDA pipeline (REpertoire SEquencing Data Analysis, https://bitbucket.org/barbera/reseda) was carried out using the following steps: 1) pairwise assembly of the paired-end reads using PEAR[2], 2) identification of the 8 bp MID, 3) identification of the complement determining region 3 (CDR3), 4) alignment to the IMGT database [3] to obtain the Variable and Joining gene assignment, 5) removal of reads with low quality bases (Q score < 30) in the CDR3, 6) clustering of reads in clones based on 100% amino acidic CDR3 identity, 7) UMI-based correction of clonal frequencies and 8) contamination check between samples from different individuals. The final list of clones obtained from the RESEDA pipeline were analyzed with in-house developed R scripts using R version 4.1 [4] using R studio.[5] The frequency of each clone was calculated as percentage of the total number of reads with unique UMIs obtained from sequencing of that sample. Clones with a frequency greater than or equal to 0.5% of the total repertoire were labelled dominant or highly expanded clones (HECs).[6 7] The impact of a clone was calculated as its UMI-corrected frequency in the repertoire, and the impact of a group of clones as their cumulative frequency.

Clustering related clones

A dynamic threshold was determined that was used to cluster clones. For every sample a density function was calculated over the Hamming distance matrix. This resulted in a bimodal distribution. The minimum between the two peaks was taken as a threshold with a minimum value of 3 amino acids. Clones with a Hamming distance below the dynamic threshold were grouped together to form a cluster and therefore considered to be related to each other.

Evaluation of diversity

To evaluate the diversity between the repertoires, the Simpson's index, Shannon entropy and the Gini index were used. While the Simpson's index and Shannon entropy are able to measure the diversity in the BcR repertoires with a larger score indicating a higher diversity, the Gini index is able to measure the heterogeneity of different clones in a sample; it is bound between 0 and 1. A Gini index of 0 indicates that all the clones in the samples have the same frequency (i.e., all clones have equal number of clustered reads), whereas an index of 1 indicates that the reads in the sample are more clustered towards individual or specific clones.

SUPPLEMENTARY REFERENCES

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Supplementary tables

Supplementary Table 1: CDR3 amino acid sequences of BcR clones dominant in both muscle tissues and peripheral blood prior to IVIg treatment.

CDR3 AA sequence	Patient	Clone size in muscle tissue (%)	Clone size in blood (%)	IGHV gene	JH gene
CVKDLGDDYGANPRRFDYWGQGTLVT	PT1	2.72	3.9	3-64	4
CARILARSVFPFDYWGQGTLVT	PT1	24.6	3.5	4-31	4
CARAPPFRYVLRFLDDPHYFDYWGQGTLVT	PT9	6.8	35.6	1-2	4
CAGSEYSSSWFPFRGPRPPLTNWGQGTLVT	PT9	0.5	12.9	3-3	4
CTRASSGWYVGNNWFDPWGQGTLVT	PT12	1.2	1.5	3-49	5

Supplementary Table S2: BcR clonality at baseline for the different patient subgroups

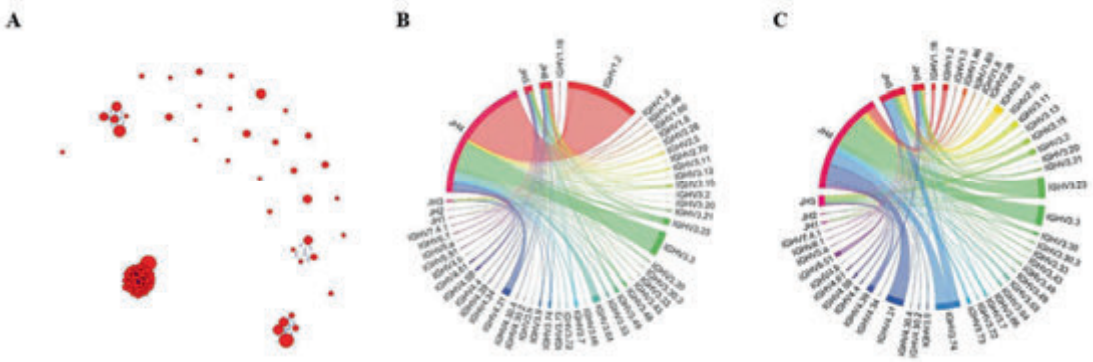
Characteristic	Myositis specific antibody (MSA) (n=11)	Myositis associated antibody (MAA) (n=5)	Seronegative (n=3)
Number of clones blood (baseline) median (IQR)	4402 (3462-5071)	4643 (3140-5524)	5111 (1699-5690)
Impact of dominant clones blood (baseline) median (IQR)	1.4 (0-7.63)	1.7 (0.5-10.1)	0.0 (0-56.4)
Number of clones muscle (baseline) median (IQR)	516 (436-936)	442 (343.5-616.0)	431 (374-495)
Impact of dominant clones muscle (baseline) median (IQR)	58.6 (41.6-66.9)	73.8 (63.2-79.5)	69.2 (64.0-70.7)

Supplementary table S3: Clinical characteristics of responders and non-responders

Characteristic	Responders (n=8)	Non responders (n=11)
Age at onset in years, median (IQR)	62 (39 – 70)	56 (37 – 67)
Months between start of symptoms until diagnosis, median (IQR)	4 (1.5 – 7)	5 (4 – 6)
Gender, females, n (%)	5 (63)	7 (64)
Connective tissue disorder, n (%)	1 (13)	2 (18)
Cancer, n (%)	1 (13)	2 (18)
Serum CK, times normal value of CK (mean, SD)[¤]	22 (31)	20 (32)
Responders, TIS ≥40 at 9 weeks, n (%)	8 (42)	11 (58)
Number of dominant clones blood (baseline) median (IQR)	3 (0-4)	1 (0-3)
Impact of dominant clones blood (baseline) median (IQR)	9.2 (0-46.43)	1.2 (0.0-1.7)
Number of dominant clones muscle (baseline) median (IQR)	67.2 (60.1-73.48)	65.1 (45.5-78.3)
Impact of dominant clones muscle (baseline) median (IQR)	69.9 (59.9-73.0)	61.4 (45.5-76.4)

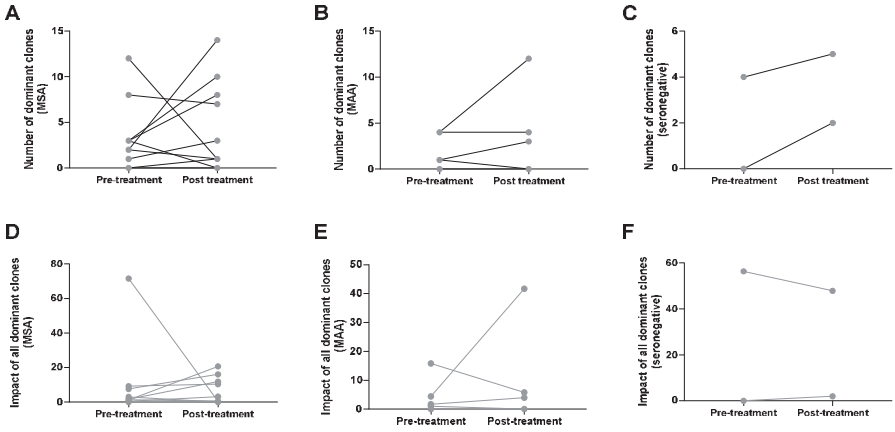
IQR interquartile range; CK creatine kinase; [¤] according to a CK reference value <217U/L.

Supplementary Figures



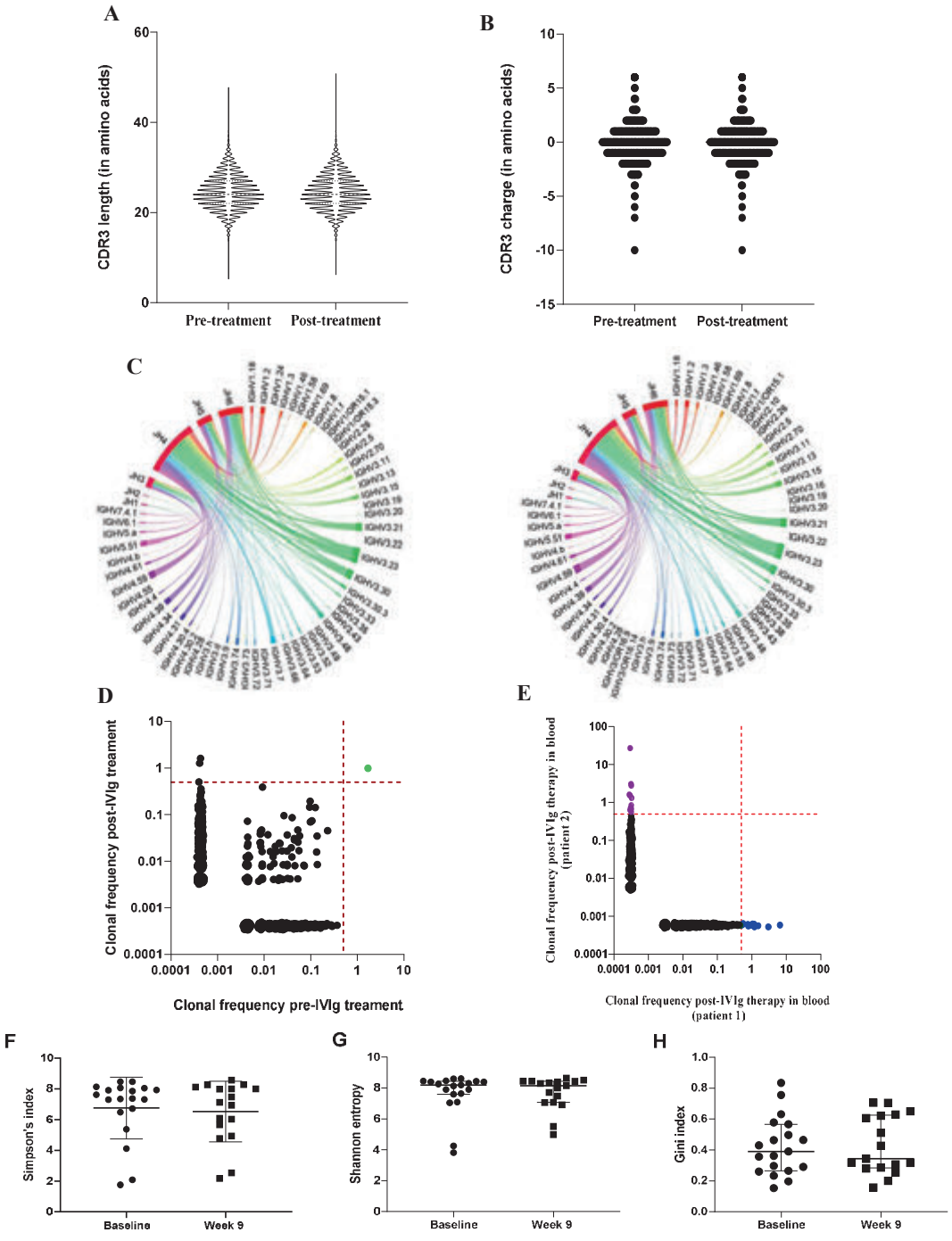
Supplementary figure 1: Characteristics of BcR clones present in both muscle tissues and peripheral blood prior to IVIg treatment.

(A) Representative DR3 network analysis of top 100 most expanded clones present in both peripheral blood and muscle tissues prior to IVIg treatment. Data is shown for one patient. V and J gene usage in **(B)** peripheral blood and **(C)** muscle tissues for BcR clones present in both peripheral blood and muscle tissues prior to IVIg treatment. Data is shown for all 19 patients.



Supplementary figure 2: BcR repertoire characteristics in peripheral blood before and after IVIg treatment.

For each patient panel **(A&D)** shows the number (upper row) and impact of dominant BcR clones (lower row) in patients with myositis specific antibodies (MSA). **(B&E)** shows the number and impact of all dominant BcR clones in patients with myositis associated antibodies (MAA) and, **(C&F)** shows the number and impact of all dominant BcR clones in patients who were seronegative.



Supplementary figure 3: Characteristics of the BcR repertoire in peripheral blood prior to and 9 weeks after IVIg treatment.

Overall **(A)** CDR3 length and **(B)** CDR3 charge of BcR clones detected in peripheral blood before and 9 weeks after IVIg treatment. Data is shown for all 19 patients. **(C)** V and J gene usage for BcR clones present before (left panel) and 9 weeks (right panel) after IVIg treatment. Data is shown for all 19 patients. **(D)** CDR3 overlap plot is shown for 1 patient in which the same dominant BcR clone was present before and 9 weeks after IVIg treatment. Each symbol represents a unique BcR clone, and its frequency in the analyzed repertoire is depicted on the x-axis (blood pre-IVIg treatment) and y-axis (blood post-IVIg treatment) as percentage of total reads. The dotted red lines on each axis indicate the 0.5% cut-off for dominant BcR clones. Dominant BcR clone present before and after IVIg treatment is colored in green. **(E)** Representative CDR3 overlap plot for two 2 different patients 9 weeks after IVIg treatment. Colored clones in each patient are newly formed dominant BcR clones. (F-G) Simpson's index, Shannon entropy and Gini index to evaluate the diversity of the BcR repertoires pre and post IvIg treatment.

6

Chapter 6

Assessment of disability in idiopathic inflammatory myopathy: a call for linearity

Minoesch Min[1]*, Hannah A.W. Walter[1]*, Johan Lim[1], Filip Eftimov[1], Camiel Verhamme[1], Marianne de Visser[1], Ivo N. van Schaik[1,3], Rohit Aggarwal[4], Rob J. de Haan[2], Anneke J. van der Kooi[1], Joost Raaphorst[1], and the Dutch Myositis Network

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[1] DEPARTMENT OF NEUROLOGY, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM NEUROSCIENCE, AMSTERDAM, THE NETHERLANDS
[2] DEPARTMENT OF CLINICAL EPIDEMIOLOGY, BIOSTATISTICS AND BIOINFORMATICS, AMSTERDAM UMC, UNIVERSITY OF AMSTERDAM, AMSTERDAM, THE NETHERLANDS
[3] SPAARNE GASTHUIS, HAARLEM, THE NETHERLANDS
[4] DEPARTMENT OF RHEUMATOLOGY AND CLINICAL IMMUNOLOGY, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE, PITTSBURGH, PA, USA

*SHARED FIRST AUTHORSHIP

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ABSTRACT

Objectives: To evaluate the clinimetric properties of the Academic Medical Centre Disability Score (ALDS) in patients with idiopathic inflammatory myopathy (IIM).

Method: We used prospectively collected data of IIM patients who completed a phase-2 study with first-line IVIg monotherapy. The ALDS is a patient reported questionnaire which contains 25 items relevant for disability in myositis. ALDS and all core set measures (CSMs) for myositis (including Health Assessment Questionnaire-Disability Index (HAQ-DI)) were evaluated at baseline and 9 weeks follow-up. In addition, the 2016 ACR/EULAR myositis response criteria outcome called Total Improvement Score (TIS) was evaluated at 9 weeks. We examined floor/ceiling effects, reliability and construct validity of the ALDS. To examine known-group validity, ALDS change scores over time were compared with TIS and physician impression of clinical response (PICR).

Results: Nineteen patients with IIM (median age 59 years, 12 (63%) female) were enrolled. At baseline, ALDS showed a median score of 65.4 (IQR 58.2–73.5), good Cronbach's alpha (α=0.84) and a small ceiling effect (11%). Construct validity was confirmed by moderate to strong correlations between ALDS and HAQ-DI (r_s=-0.57 (baseline); -0.86 (follow-up)). ALDS change score correlated with TIS (r_s=0.70), discriminated between responders and non-responders (TIS≥40; p=0.001), between groups based on PICR (p=0.03), and detected deterioration.

Conclusion: The ALDS showed promising clinimetric properties and detected relevant changes in disability in patients with myositis.

INTRODUCTION

Assessment of treatment effects in patients with idiopathic inflammatory myopathy (IIM) is challenging.[1 2] Recently, the ACR and EULAR approved a validated response criteria called Total Improvement Score (TIS) to assess treatment effect in IIM patients. TIS is a composite measure combining disability, different measures of disease and patient's and physician's impressions.[3 4] Despite the increasing use of TIS in clinical practice and research, it is important to develop validated outcome measures that specifically represent (single) domains. Functioning, captured by outcome measures that assess disability, is one of the most important outcome measures from patients' perspective.[1 5]

Disability in the TIS composite score is measured by the Health Assessment Questionnaire Disability Index (HAQ-DI) which has been adopted from rheumatoid arthritis, and has not been fully validated in myositis. The HAQ-DI is an ordinal scale which has some methodological shortcomings in terms of clinimetrics.[6]

Linear scales (as opposed to ordinal scales) are based on Item Response Theory with one of the advantages that similar change scores on different points on a scale represent similar changes in function. The Academic Medical Center Linear Disability Score (ALDS) is a linear scale and enables the use of a subset of items to obtain a detailed picture of disability. ALDS has been validated in patients with stroke, Parkinson's disease and rheumatoid arthritis.[7-10]

We aimed to evaluate the clinimetric properties of the ALDS in IIM patients.

METHODS

Patients

We included adult patients with newly diagnosed, treatment naive, biopsy-proven IIM with a disease duration of less than 9 months and a minimal disability of at least 10% loss on manual muscle testing (MMT)-score. Patients were also included in a phase-2 open-label study investigating intravenous immunoglobulins (IVIg) as first-line monotherapy (IMMEDIATE study).[11]

Patient data

Data were obtained at baseline and after 9 weeks of treatment and included demographic and disease variables [11], ALDS questionnaire, core set measures (CSMs) and EQ5D (a generic

quality of life measure).[12] The study was conducted in accordance with the Declaration of Helsinki and the ethical committee of the Amsterdam Medical Centre approved the study protocol and. All patients gave written informed consent.

The CSMs include the Physician Global Activity, Patient Global Activity, MMT, HAQ-DI and Muscle enzyme and Extramuscular Activity. The TIS is obtained by adding up all the weighted individual improvement scores of the six core measurements and ranges from zero to 100.[3] The physician impression of clinical response (PICR), assigned by the treating physician, was based on all available information except TIS, and included the categories slightly, moderately, and markedly improved and, slightly, moderately, and markedly deteriorated.

ALDS Questionnaire

The ALDS is a generic item bank of 73 ADL-items ordered from simple to complex activities. A panel of three physicians with myositis expertise and a patient (myositis representative of the Dutch Patient Association of Neuromuscular Diseases) selected 25 relevant items (Supplementary Data S1). Patients rated their ability to carry out activities. Answer options were: 1) “yes”, 2) “yes with difficulty”, 3) “no” and 4) “do not know”. To calculate ALDS scores, 1 and 2 were both scored as “yes”. [7 13] Original units of the ALDS scale are (logistic) regression coefficients, expressed in logits. [7] To facilitate interpretation of results, logit scores are linearly transformed into ALDS values between 0 and 90, with lower scores indicating more disability.

Clinimetric evaluation of the ALDS

We used baseline and follow-up data to examine clinimetric properties of the ALDS:

a) Floor and ceiling effects were calculated by the number (%) of patients with minimal and maximal ALDS scores.
b) Reliability was expressed in terms of homogeneity, referring to the statistical coherence of scale items (baseline data).
c) Construct validity (referring to whether ALDS measures the intended construct) was examined by correlating ALDS (change) scores with the different CSMs, TIS and EQ5D; correlation coefficients were considered weak ($r \leq 0.30$), moderate ($r = 0.40\text{-}0.60$) or strong ($r \geq 0.70$).[14]

d) Known-group validity was calculated using a change score (follow-up *minus* baseline ALDS score). We compared ALDS change scores:
   1) Between responders and non-responders based on TIS≥40 (at least moderate improvement) and between patients with and without minimal improvement (TIS≥20).[3] For these comparisons effect sizes (Hedges' g) were calculated using logit sores.
   2) Between groups based on TIS scores: no (<20), minimal (20-39), moderate (40-59) and major improvement (≥60).[3]
   3) Between different levels of physician impression of clinical response (PICR): slightly, moderately, and markedly improved; and slightly, moderately, and markedly deteriorated. For analyses, we used four categories (categories "slightly" and "moderately" were combined).

Statistical analysis

Patient characteristics and outcome measurements were summarized using descriptive statistics.

ALDS scores were calculated using previously published algorithms [7 13]. Missing items or "do not know" responses were discarded.[13] We used original ALDS logits for analyses and linearly transformed ALDS scores for presentation.

Reliability of the ALDS was expressed as Cronbach's α ($\geq$ 0.80 indicates good homogeneity).[15] Associations between ALDS (change) scores and CSMs (change) as well as TIS scores were expressed as Spearman correlation coefficients (r_s). Between-group differences of ALDS baseline and change scores were analyzed using Mann-Whitney-U-test or Kruskal Wallis test, where appropriate. When the Kruskal-Wallis test showed statistically significant score differences across groups, we performed post-hoc pairwise comparisons. A p-value <0.05 was considered statistically significant. In view of the explorative nature of this study, we did not correct for multiple comparisons.[16]

RESULTS

Patient characteristics

Nineteen patients with IIM were included (63% female; median age 59 years (IQR 37-69); median duration between first symptoms and diagnosis was 5 months (IQR 3 – 6). Eight (42%) patients had dermatomyositis (DM), six (32%) had immune mediated necrotizing myopathy (IMNM), four (21%) had non-specific or overlap myositis (NM/OM) and one (5%) had

antisynthetase syndrome (ASS). Ten (53%) patients had myositis specific antibodies (MSA; MDA-5 (n=1), TIF-1γ (n=1), NXP-2 (n=3), SRP (n=1), Jo-1 (n=1) and HMGCR (n=3)), two (11%) patients had myositis associated antibodies (MAA; Ku (n=1), U1RNP (n=1)) and six (32%) patients were seronegative. Three (16%) patients had a concomittant connective tissue disorder (mixed connective tissue disease, Sjögren's syndrome and systemic sclerosis) and one patient with DM was diagnosed with ovarian cancer three weeks after inclusion.

Clinimetric evaluation of the ALDS

At baseline ALDS showed no floor effect; two patients had a maximum score (ceiling=11%). At follow-up no patient had a minimum score and seven patients achieved the maximum score (37%). Reliability of ALDS at baseline was considered good (Cronbach's α = 0.84). The correlation between ALDS and HAQ-DI were moderate to strong (r_s =-0.57 (baseline) and -0.86 (follow up); $p<0.05$; table 1). In addition, patient and physician global impressions showed moderate correlation with ALDS and correlation was lower with less related constructs such as CK and extra-muscular activity (EMA). Muscle strength (MMT) had low correlation at baseline, at follow up there was a statistically significant moderate correlation with ALDS. The association between ALDS change scores and change in several relevant CSMs such as HAQ-DI, MMT, patient and physician global impressions, as well as the EQ5D, was moderately strong. Moreover, ALDS change correlated strongly with TIS (r_s=0.70, $p<0.01$). For comparison of clinimetric properties between the ALDS and the HAQ-DI, we show correlations between HAQ-DI and the CSMs, TIS and EQ-5D, respectively in Supplementary Table S2.

Table 1. Construct validity of the ALDS: Test scores and Spearman's correlation coefficients of ALDS, CSMs, TIS and EQ5D.

Outcome measurement	Test score (baseline)	Spearman's correlation with ALDS (baseline)	Test score (Follow-up[a])	Spearman's correlation with ALDS (follow-up)	Spearman's correlation of change scores with ALDS change score
ALDS	65.4 (58.2 – 73.4)	-	75.6 (65.1 – 89.1) [b]	-	-
PhGA	3.8 (3.2 – 4.0)	-0.46*	2.3 (1.0 – 4.0) [b]	-0.71**	-0.60**
PGA	6.1 (5.3 – 7.6)	-0.49*	4.6 (2.0 – 6.6) [b]	-0.43*	-0.64**
MMT	211 (185 – 225)	0.28	227 (191 – 241)	0.69**	0.61**
HAQ-DI	2.0 (1.5 – 2.5)	-0.57*	1.6 (0.8 – 2.1) [b]	-0.86**	-0.77**
CK	1199 (179 – 6500)	-0.20	196 (83 – 3877) [b]	-0.45	-0.29
EMA	2.2 (0.6 – 3.0)	-0.11	1.0 (0.3 – 2.3) [b]	0.10	-0.17
TIS			35 (15 – 53)	0.54*	0.70**
EQ5D	0.45 (0.41 – 0.57)	0.52*	0.60 (0.43 – 0.78)	0.78**	0.68**

Score values are presented as median (IQR); Spearman's correlation coefficients of ALDS logits with TIS core set measures *Significant at the 0.05 level. **Significant at the 0.01 level. [a]Follow-up = nine weeks (or premature ending of participation in the study); [b]Significantly different compared to baseline ($p<0.05$; Wilcoxon signed rank test). ALDS: Academic medical center Linear Disability Score; CK: Creatine kinase; PhGA: Physician Global Assessment; PGA: Patient Global Assessment; MMT: Manual Muscle Testing; EMA: Extramuscular Activity; HAQ-DI: Health Assessment Questionnaire-Disability Index; TIS Total Improvement Score, EQ5D (index); a generic quality of life measure.

Known-group validity: comparison with responders according to TIS

ALDS change scores discriminated between responders (based on a TIS≥40; n=8) and non-responders (n=11): within-group change scores were 18.7 and 0.0 in responders and non-responders, respectively ($p<0.01$; Hedges' g effect size -1.82; figure 1A). Baseline ALDS scores did not differ ($p=0.93$). ALDS change scores also differed between patients with (n=10) and without (n=9) *minimal* improvement (TIS≥20; within-group change scores 18.5 vs. 0.0; $p<0.05$; Hedges' g effect size -1.12).

Known-group validity: ALDS in relation to improvement on the TIS

ALDS change scores differed between groups based on TIS-categories: no, minimal, moderate and major improvement. Within-group change scores were 0.0, -3.2, 19.6 and 17.7, respectively ($p<0.01$; figure 1B); baseline ALDS scores did not differ ($p=0.19$). Post-hoc analysis showed differences between "no improvement" vs "major improvement" ($p=0.03$) and "no improvement" vs "moderate improvement" ($p<0.01$).

Known-group validity: ALDS in relation to physician impression of clinical response (PICR)

Within group ALDS change scores differed between groups based on PICR: slightly/moderately deteriorated (-0.9), markedly deteriorated (-48.3), slightly/moderately improved (9.2) and markedly improved (18.7; $p=0.03$; figure 1C). Baseline ALDS scores did not differ ($p=0.28$). Post-hoc analysis showed a difference between "markedly improved" and "slightly/moderately worse ($p=0.01$).

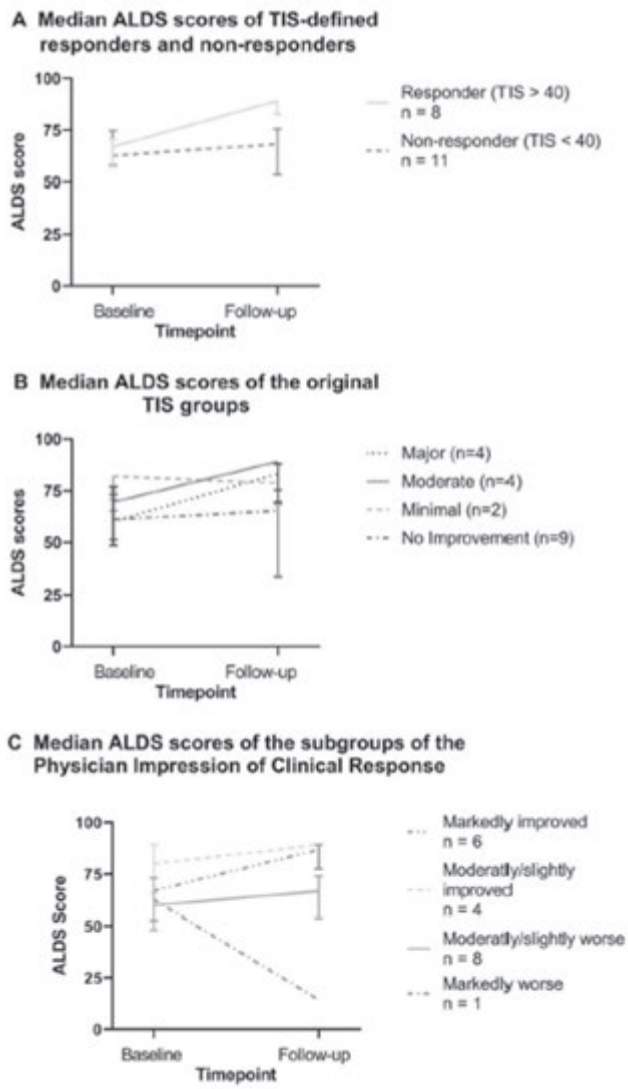

Figure 1: Known group validity of ALDS

A. Median and IQR of ALDS scores of TIS-defined responders and non-responders.

B. Median and IQR of ALDS scores of original TIS groups.

C. Median and IQR of ALDS scores of the subgroups of the Physician impression of clinical response.

Data are median and IQR; follow-up = after nine weeks of IVIg monotherapy or premature ending of participation of the study. Note that data points in the graphs are based on median ALDS scores and numbers in the result section represent median(s) of individual change scores

DISCUSSION

We examined clinimetric properties of a generic linear disability scale in IIM patients treated with monotherapy IVIg. Findings were compared with Total Improvement Score (TIS), individual core set measures (CSMs) and EQ5D. ALDS showed promising clinimetric properties: good reliability, no floor effect and a small ceiling effect; moderate to high correlations with HAQ-DI (a measure of disability) and lower correlations with less related constructs. ALDS showed known-group validity and discriminated between different levels of clinical improvement based on TIS and a physician's impression. The moderate to high correlations with a measure of quality of life confirm the importance of disability for patients with myositis. The ceiling effect at baseline was larger as expected, possibly due to little disability in some patients.[9 10] We chose 25 ALDS-items with divergent difficulty levels and excluded most difficult items (e.g. vacuum a flight of stairs), which could have contributed to the ceiling effect. Another explanation is related to answer options: patients answered many difficult items as "yes with difficulty", which were analyzed as "yes" (according to the scoring algorithm of ALDS).

Despite some ceiling effect, ALDS provides unique and additional information as compared to HAQ-DI and TIS. Firstly, the linear design of ALDS improves clinical interpretation of scores, as opposed to ordinal scales (HAQ-DI) of which identical score differences may not represent identical clinical changes at another point on the scale.[17]

Secondly, ALDS score is a unidimensional and interpretable outcome, opposed to TIS which is a composite of (changes of) CSMs, making it easier to interpret as a supplementary outcome in myositis clinical trials. ALDS also provides a meaningful baseline value, which facilitates interpretation of clinical trial results (variation in baseline disability between groups).

Thirdly, ALDS detects deterioration. This is relevant in clinical practice and clinical trials, both when a pharmaceutical compound is compared to standard of care and when dependency on maintenance treatment needs to be ascertained before an intervention.[18]

Limitations of this study include a small sample size and use of a fixed-length ALDS. In future research, different sets of ALDS items for individual patients may be used, without losing the ability to compare scores.[2 19] Further, ALDS could be transformed into a computerized adaptive test, with difficulty levels being automatically adapted, after each response.[7 13]

In conclusion, our pilot study shows promising clinimetric properties of the ALDS in myositis, which warrants further investigation.

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Authorship contribution

All authors contributed to the conception or design of the work, or the acquisition, analysis, or interpretation of data. M.M., A.W. A.K. and J.R. wrote the initial draft and all authors revised it critically for important intellectual content and gave final approval of this version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Collaborators: The Dutch Myositis Network collaborated to this paper and provided and cared for study patients and gave final approval of this version to be published. On behalf of the Dutch Myositis Network: Christiaan G.J. Saris, Department of Neurology, Radboud UMC, Donders Institute for Brain Cognition and Behaviour, Nijmegen, The Netherlands; Esther Brusse, Department of Neurology, Erasmus UMC, Rotterdam, The Netherlands; Jessica E. Hoogendijk, Department of Neurology, Brain Centre Rudolf Magnus, UMC Utrecht, Utrecht, The Netherlands.

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Conflicts of interest

None declared. All co-authors declared there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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SUPPLEMENTAL MATERIAL

Supplemental table S1: Selected items of the ALDS

	Can you…	Yes	Yes, but it is difficult	No	I don't know
1	… carry a bag of shopping upstairs?	☐	☐	☐	☐
2	… fetch groceries for a few days?	☐	☐	☐	☐
3	… walk for more than 15 minutes?	☐	☐	☐	☐
4	… walk 150 meters up a hill or over a high bridge?	☐	☐	☐	☐
5	… stand for 10 minutes?	☐	☐	☐	☐
6	… hang out and take in a load of washing?	☐	☐	☐	☐
7	… get something out of a high cupboard?	☐	☐	☐	☐
8	… walk up a flight of 10 to 15 stairs?	☐	☐	☐	☐
9	… walk for a maximum of 15 minutes?	☐	☐	☐	☐
10	… fetch a few things from the shop?	☐	☐	☐	☐
11	… have a shower and wash your hair?	☐	☐	☐	☐
12	… put on and take off lace-up shoes?	☐	☐	☐	☐
13	… get up from and sit down back on a low chair?	☐	☐	☐	☐
14	… pick something up from the floor?	☐	☐	☐	☐
15	… get in and out of a car?	☐	☐	☐	☐
16	… clear the table after a meal?	☐	☐	☐	☐
17	… eat a meal at the table?	☐	☐	☐	☐
18	… put on and take off socks and slip-on shoes or slippers?	☐	☐	☐	☐
19	… put long trousers on and take them off?	☐	☐	☐	☐
20	… move from lying in to sitting on the edge of a bed?	☐	☐	☐	☐
21	… put on and take off a coat?	☐	☐	☐	☐
22	… go to a bathroom sink and wash your face and hands?	☐	☐	☐	☐
23	… get out of bed and sit in a (wheel)chair and vice versa?	☐	☐	☐	☐
24	… go to the toilet?	☐	☐	☐	☐
25	… put on and take off a vest or T-shirt without buttons?	☐	☐	☐	☐

Supplemental Table S2: Spearman's correlation coefficients of the HAQ-DI and ALDS, other Core Set Measures, Total Improvement Score and EQ5D.

Outcome measurement	Spearman's correlation with HAQ-DI (baseline)	Spearman's correlation with HAQ-DI (follow-up)	Spearman's correlation of change scores with HAQ-DI change score
ALDS	-0.57*	-0.86**	-0.77*
PhGA	0.70**	0.85**	0.71**
PGA	0.44	0.62**	0.65**
MMT	-0.43	-0.80**	-0.62**
CK	0.26	-0.20	0.30
EMA	-0.00	0.03	0.18
TIS		-0.69**	0.71**
EQ5D	-0.83**	-0.86**	0.74**

*Significant at the 0.05 level. **Significant at the 0.01 level. ALDS: Academic medical center Linear Disability Score; CK: Creatine kinase; PhGA: Physician Global Assessment; PGA: Patient Global Assessment; MMT: Manual Muscle Testing; EMA: Extramuscular Activity; HAQ-DI: Health Assessment Questionnaire-Disability Index; TIS Total Improvement Score, EQ5D (Index): a generic quality of life measure.

Chapter 7

General discussion and directions for future research

GENERAL DISCUSSION AND DIRECTIONS FOR FUTURE RESEARCH

In this thesis, we described a study protocol and the results of different studies with the aim to optimize the diagnostic strategy and follow-up for patients with treatable idiopathic inflammatory myopathies (IIM). We evaluated existing diagnostic procedures used in daily practice and explored the characteristics of B cells receptors in muscle tissue and blood IIM. Furthermore, we explored the clinimetric properties of a generic disability measure during the first phase of the IIM disease course.

When a diagnosis of IIM is considered for a patient with subacute proximal weakness, a structural history and physical examination usually precede ancillary **diagnostic tests**. Diagnostic tests are used to confirm the diagnosis and to exclude other differential diagnostic possibilities.[1] An accurate and fast diagnosis is important, because early treatment leads to a better functional outcome.[2] There is no gold standard for diagnosing IIM. The available ancillary diagnostic tests all have their own test characteristics, local availability, costs and patient burden. This has resulted in various diagnostic test protocols and classification criteria used worldwide. It is likely that because of this practice-variation not all patients are diagnosed in the most efficient way and/or with a minimal of burden. When a diagnosis of IIM has been assigned, patients with IIM receive treatment. In this phase the **follow-up** can be structured by questionnaires and imaging, addressing patients' as well as doctors' needs.

Classification criteria: an evolution due to the development of diagnostic tests

The terms diagnostic and classification criteria are often interchangeably used, albeit they do have a different purpose.[3] Diagnostic criteria are used in clinical practise as an aid in the diagnostic work up to identify patients with IIM. Subsequently, for patients with a high likelihood of IIM, classification criteria help to subdivide these patients into distinct subtypes and to guide to a specific treatment and follow-up. Several diagnostic and classification criteria, with varying sensitivity and specificity, have been proposed over five decades.[4-6] In 1975, Bohan and Peter published criteria as a diagnostic guidance for clinicians, and classified IIM into two groups, i.e. dermatomyositis and polymyositis. Their criteria included clinical characteristics such as cutaneous changes, laboratory abnormalities, electromyography and muscle biopsy, and excluded other myopathic conditions such as muscular dystrophies. Inclusion body myositis (IBM) was identified as a distinct subtype in

the late 1970s[7], but only in 1991 the Bohan and Peter criteria were modified to distinguish three subtypes: dermatomyositis, polymyositis and IBM.[8] The occurrence of myositis as an overlap syndrome with other auto-immune diseases was mentioned in 1991, but not classified as a distinct subtype. In the early 1990s it was recognized that patients with anti-histidyl-transfer RNA synthetase (anti-Jo1) antibodies have distinct clinical features, leading to the diagnosis of antisynthetase syndrome (ASS).[9] In 1995, new classification criteria were put forward including the myositis specific antibody (MSA) anti-Jo1 for the first time.[10] Classification criteria changed as new diagnostic tests emerged, such as muscle imaging. In 1997, an important publication proposed a modification of the classification criteria from 1995 by adding two elements: muscle magnetic resonance imaging (MRI) and other MSAs; PL7, PL12, OJ, EJ, anti-Mi2 and anti-SRP.[11] In 2003, new diagnostic criteria were published by Dalakas who added more specific pathological characteristics, as well as criteria for amyopathic dermatomyositis (DM). Antibodies, however, were not included.[12]

One of the first descriptions of IIM without inflammatory cell infiltrates, showing cell necrosis, cell regeneration and with an association to cancer, dated from 1965.[13] Clear pathological characteristics of this subtype were described in 2003.[14] The European Neuromuscular Center (ENMC) criteria[15] incorporated this subtype as immune mediated necrotizing myopathy (IMNM) in 2004. In 2005 new classification criteria based on the presence of an antisynthetase or a myositis associated antibody (MAA) were published, and introduced overlap myositis (OM) as a new diagnostic entity.[16] Comparison of the six classification criteria described above showed that the Dalakas criteria (2003) had the highest diagnostic accuracy (sensitivity 0.77, specificity 0.99), followed by the ENMC criteria (sensitivity 0.71, specificity 0.82).[6]
The EULAR/ACR criteria[17] published in 2017 incorporated only (amyopathic) DM, polymyositis (PM) and IBM, leaving out ASS, IMNM, and OM. MRI was not integrated due to a lack of MRI positive cases during the validation of their criteria. Of all MSAs, only anti-Jo1 was incorporated into these criteria.
Although it was shown that a diagnostic accuracy above 80% could be reached without a muscle biopsy[17 18], muscle biopsy remains important for the classification of IIM, according to the EULAR/ACR criteria.[19]
An innovative classification system was proposed in 2018[20], based on clinical, biological, serological, and pathological data and resulted in four classification clusters: DM, IBM, IMNM

and ASS. The described classification algorithm showed that myositis specific antibodies play a key role in estimating the connection to a cluster: the classification algorithm with histology data showed an accuracy of 81% versus 84% for the classification tree without histology data. It was noted that that muscle biopsy remains warranted in ambiguous cases or in the absence of an MSA.

An overview of the clinical presentation, MSAs and MAAs, and muscle pathology of all thus far identified IIM subtypes i.e. DM, ASS, IMNM, OM, PM and IBM was published in 2018.[21] This is an important step towards new consensus classification criteria including all, currently known, IIM subtypes.

What should be the aim for diagnostic criteria?

We should endeavour an accurate and fast diagnosis, followed by a specified treatment. To be developed new diagnostic criteria should focus on the potential of non-invasive diagnostic tests. This would make muscle biopsy only a necessity for a select group of dubious cases instead of a mandatory test for nearly all subtypes. Incorporation of ASS, OM and IMNM as distinct subtypes into evidence-based and internationally accepted classification criteria would be a step towards giving the optimal (and specific) treatment to the right patient groups, and to enhance clinical relevance in trials.

The diagnostic value of ancillary investigations

Imaging

We conducted a prospective cohort study on muscle imaging by comparing whole body muscle MRI (WB-MRI) and muscle ultrasound (US), to evaluate the ability to detect muscle abnormalities compatible with IIM in adult patients upon diagnosis (**Chapter 2**). One of our important findings was that quantitative ultrasound assessment, using imaging software to measure the grayscale of individual muscles and compare it to reference values, performed poorly (sensitivity 28%). Therefore, we recommend not to use quantitative US at diagnosis with the currently available software.[22] WB-MRI was most sensitive for detecting muscle abnormalities compatible with IIM (sensitivity 100%), followed by semi-quantitative or qualitative ultrasound (sensitivity 89% in both). Apparently, muscle oedema was too subtle to be detected by US and a computer assisted programme, while it was possible to detect muscle oedema as ‘shine through’ appearance or ‘see-through echogenicity increase’ by the

human eye with semi-quantitative and qualitative US. Interestingly, in patients with juvenile dermatomyositis quantitative muscle US (using the same software) was a valuable test as it showed an increased echo-intensity in 71% of the patients at diagnosis.[23] Why quantitative US showed different results in children remains unclear, but may be related to more fatty infiltration due to a longer disease duration at diagnosis. A limitation of our study which only included adults, was the lack of a control group, making it impossible to calculate the specificity of US and MRI as a diagnostic test at diagnosis.

While we evaluated quantitative, semi-quantitative and qualitative grey-scale analysis in US, others studied US elastography.[24 25] The latter is a relatively new technique measuring muscle stiffness. Two different methods may be applied: strain elastography (STE) and shear wave elastography (SWE). Although these methods use a slightly different technique, they both visualize muscle stiffness with a colour code, after manually assigning a region of interest. Both methods have shown their diagnostic potential in IIM, with a similar level of sensitivity as compared to the values obtained with semi-quantitative and qualitative US in our study.

Worldwide, MRI remains the most sensitive and widely used method to visualize muscle abnormalities in IIM patients, and showed the highest sensitivity in our study (**Chapter 2**).[26] Besides muscle oedema, fascial oedema was found in about one-third of the muscle regions at diagnosis. Our study additionally showed that sub-clinical muscle oedema was found in almost half of the distal muscles in the lower limbs, it remains unclear if this is disease related or is associated with mechanical stress.[27] The high sensitivity for (subclinical) muscle and subcutaneous oedema has been described in previous studies,[28 29] and others described diverging MRI patterns in several IIM subtypes, indicating that MRI may be useful to classify IIM subtypes.[30 31] Fascial oedema may be present early after disease onset in dermatomyositis[32], and was shown to be an independent associated risk factor for the development of rapid progressive interstitial lung disease in dermatomyositis.[33]

Another imaging method that can be used to detect muscle inflammation and extra-muscular disease activity in one scan is 18F-fluorodeoxyglucose positron emission tomography (PET) combined with computed tomography (CT) or MRI.[34 35] It has the capacity to 1) quantify muscle disease activity, 2) measure disease activity in the lungs (ILD), 3) screen for cancer[36], and 4) screen for myocarditis.[37]

In conclusion, several imaging techniques have potential in the diagnostic process of IIM. It is important to realise that superiority does not only depend on imaging properties, but also on availability, costs, patient preferences and observer experience. Future imaging techniques that answer several objectives should get priority to lower patient burden and costs and increase diagnostic efficiency.

Screening for cardiac involvement

Clinically apparent cardiac involvement was reported in 9% of IIM patients in the Euromyositis registry[38], and arrhythmias or cardiac failure in patients with IIM results in a mortality rate of 4%, mainly if left untreated.[39] Epidemiological knowledge and awareness for IIM associated (peri)myocarditis is increasing.[40 41] The gold standard for myocarditis is the histological proof from a endocardial biopsy, which is rarely performed due to its invasiveness. There is a need for practical diagnostic tests and cut-off points to guide the diagnostic process for cardiac involvement in IIM.[42]

In **chapter 3** we investigated the diagnostic value of laboratory biomarkers, electrocardiography, echocardiography, and cardiac magnetic resonance imaging (CMR) for the diagnosis (peri)myocarditis in a cohort of 34 newly diagnosed IIM patients**.** We diagnosed (peri)myocarditis in nearly one-fifth of the patients, of whom one patient required pacemaker implantation because of conduction abnormalities. We proposed a diagnostic strategy for (peri)myocarditis. Troponin T with a cut off value <113ng/L, followed by Troponin I with a cut off value of >35ng/L can be used as gatekeeper for a CMR. As described in previous reports[43-45], Troponin I was more specific for (peri)myocarditis, and Troponin T yielded a higher sensitivity. However, elevated troponin levels (T and I) can be found in patients with skeletal muscle pathology, but without ischemic or inflammatory cardiac aetiology.[44-47] Our proposed gatekeeper would have helped to renounce from further ancillary tests in nearly all IIM patients within these studies that included troponin levels.[45 46] For clinicians, the uncertainty whether troponin levels are actually related to cardiac involvement in IIM, is a concern. It leads to unnecessary percutaneous cardiac interventions, with a high patient burden and high health care costs as a result. Our study provides a guidance for clinicians by providing cut off values for troponin T and I, although further validation is necessary.

The reason why Troponin T (and to a lesser extent Troponin I) may be elevated in muscular disorders without (peri)myocarditis is not yet fully understood.[48] One of the proposed causes

is the expression of cardiac troponine T by skeletal muscle in response to injury that is immunoreactive with the antibodies used in assays for troponine T.[49] Others hypothesize that an elevated level of troponin T reflects subclinical acute or non-acute heart disease.[50] We could not confirm this in our cohort, since patients without myocarditis according to CMR showed a median troponin T level of 50 ng/L, range 31-205.

CMR is considered the most accurate diagnostic test in the diagnosis of (peri)myocarditis. Characteristics of cardiac dysfunction in DM, IMNM and PM, and patterns of myocardial damage between these three IIM subtypes have been described.[51] [52] Another interesting diagnostic modality for (peri)myocarditis is FDG-PET CT, which has shown to be a feasible modality to detect myocarditis with high diagnostic accuracy.[37] The diagnostic and prognostic value of FDG-PET CT in comparison to CMR in patients with a clinical suspicion on myocarditis is currently being investigated.[53] [54]

Until now it remains unclear whether IIM patients with concomitant (peri)myocarditis require more intensive immunosuppressive treatment as compared to the standard glucocorticoids and steroid sparing agent.

Combining diagnostic tests to increase diagnostic accuracy

In **chapter 4,** we described the rationale and study design of the ADAPT study. We hypothesize that an evidence-based diagnostic strategy, using fewer and preferably the least invasive diagnostic modalities, can achieve the accuracy of a complete panel of diagnostic tests, including WB-MRI, US, EMG, myositis-specific auto-antibody testing and muscle biopsy. The rationale of the ADAPT study, as outlined in chapter 4, is the fact that no gold standard for the diagnosis of IIM exists, which resulted in the development of many diagnostic strategies with divergent sensitivities and specificities, as described above.[6] [12] [15-17] With the development of (relatively) new diagnostic modalities[20] [55], there is a need for a diagnostic study including the complete panel of diagnostic tests. The ADAPT study focusses on the diagnosis of patients with IIM who should be treated with glucocorticoids. Recruitment is ongoing at the time of writing this discussion (74 out of the targeted 100 patients signed informed consent as per October 5, 2022). With the help of many enthusiastic physicians at different hospitals in The Netherlands (The Dutch Myositis Network) we hope to finish recruitment by September 2023. With the results of this study, we expect to be able to draw an optimal diagnostic strategy for patients with clinically suspected IIM, that should be treated with prednisone, soon

thereafter. Since patients with clinically suspected IIM are included in the study some patients were diagnosed as 'IIM mimics', such as a RYR1 mutation, IBM, vasculitis, chronic inflammatory demyelinating polyneuropathy, Lambert-Eaton myasthenic syndrome and amyloidosis.

Several retrospective studies addressing diagnostic accuracy of different combinations of diagnostic test in IIMs have been published recently. A study from the USA showed that EMG, MRI, and skin biopsy may be just as useful as muscle biopsy in contributing to the diagnosis of DM.[56] Interestingly, only patients suspected of a DM were included, for which international guidelines, among which the Dutch and German guideline, already state that this diagnosis can be assigned without a muscle biopsy, when a patient shows characteristic skin lesions and muscle weakness.[17] [57] [58] This study highlights the differences in diagnostic approach worldwide, and underlines the need for more intercontinental collaboration. This is the main reason why we include DM patients in the ADAPT study and obtain a muscle biopsy in this group (with separate informed consent for the biopsy). Others evaluated the diagnostic yield of EMG in detecting abnormalities on muscle biopsy (showing a sensitivity of 87% and specificity of 65% for an abnormal muscle biopsy)[59], and a retrospective diagnostic protocol evaluated the diagnostic value of IIM related laboratory tests, WB-MRI, EMG, myositis related antibodies and muscle biopsy for patients with solitary dysphagia and IIM according to the EULAR/ACR criteria.[60] The studies described above were all conducted retrospectively, evaluated the diagnostic value of a limited number of diagnostic tests, and therefore demonstrate the persisting need for prospective diagnostic accuracy studies combining a more complete panel of diagnostic tests. We hope our findings contribute to a European (EAN/Euro-NMD) guideline in the future.

B cells: a new and engaging field in IIM

As described in chapter 5, the hypothesis that B cells may play a pivotal role in the pathogenesis of IIM is supported by the presence of auto-antibodies, presence of B-cells or plasma cells in muscle biopsies of IIM patients and response to B-cell depleting treatment. The development of accessible, high throughput sequencing technologies has opened doors to evaluate the relation between the B cell receptor (BcR) repertoire of patients' tissue (e.g. muscle) or peripheral blood (PB) and treatment. In **chapter 5** we utilized next generation sequencing to characterize the BcR heavy chain repertoires of muscle tissue and peripheral blood of IIM patients at baseline before treatment with intravenous immunoglobulins (IVIg) as well as after treatment. We found that B cell receptor clones found in muscle tissue were also present in the peripheral blood. In some patients the dominant clones, (defined as a total frequency >0.5% of the total repertoire) in the muscle tissue, could be retrieved in the peripheral blood. Additional analysis of V-J gene combinations of the B cell receptor clones showed preferential use of certain V-J gene combinations. We found a preferential use of V3 gene segments in blood in our IIM cohort but found no preference of V-segments in amongst the IIM subtypes. The preferential usage of V3 gene segments was described before in a cohort of IIM patients.[61] They found other preference for V-segments in DM patients compared to PM patients. We found that patients with a high impact of dominant BcR clones in blood responded better to IVIg treatment, suggesting that a certain B cell clonal repertoire may have prognostic significance in relation to immunosuppressive treatment. Limitations of our study were the lack of a control group, the inability to stratify for IIM or antibody subgroups due to low patient numbers, and the inability to show the cell phenotype of the dominant BcR clones (our methods identified the receptors only). Further research, including different immunosuppressive compounds and a control group consisting at least of PB samples of healthy controls (as muscle tissue of healthy people is hard to obtain), is necessary to confirm our observations. In future studies, it would be interesting to stratify for different IIM subtypes or V-J gene combinations (necessitating larger patient numbers). Secondly, as we found that patients with a high impact of dominant clones responded better to therapy, it would be of interest to further identify these dominant clones based on cell type with flow cytometry.

Identification of the cell type of B cell subsets has been done by others. Flow cytometry of blood samples in patients with anti-Jo1 antisynthetase syndrome showed that the majority of anti-Jo1 binding B cells were skewed towards distinct B cell subsets: a higher percentage of CD21 cells related to disease severity, compared to non-anti-Jo1 IIM and healthy donors.[62] Immune endotypes have been studied through detailed immunophenotyping of T, B and myeloid cells, by mass Cytometry by time of flight (CyTOF) in patients with IIM.[63] Shared immunologic features across all IIM patients were found, among which decreased memory B cells as well as two distinct immune endotypes with possible clinical correlations, i.e. disease severity and pro-fibrotic phenotype. These studies suggest that the presence of certain cell types in peripheral blood may be related to an IIM subtype and this may be a potential field for future therapies. Limited patient numbers and potential confounders such as previous immunosuppressive therapy complicate the formulation of strong conclusions. However, these studies support the idea that different B cell subsets may be correlated with distinct IIM subtypes and concomitant treatment response and are an intriguing potential area for future research.

Currently, B cells and their receptors of IIM patients are investigated in more detail by Cytometry by time of flight (CyTOF) (data not yet published).

Follow up of patients with IIM: clinimetrics and imaging

Management of patients during follow up starts by asking ourselves: What does the patient need and what does the doctor need? Where doctors (often) focus on bodily parameters to evaluate disease activity, patients may have different needs to be evaluated. Ideally, doctors use parameters that accurately and precisely reflect disease activity and discriminate from disease damage. These different needs are endorsed in the International Classification of Functioning, Disability and Health (ICF), which is a framework and classification system to organise information on functioning and disability, and was published by the World Health Organisation in 2001.[64] The three components of the ICF reflect different domains: A) 'functioning' reflects physiological functions of the body system or disease activity, and is closely related to doctors' needs when treating a patient. B) 'disability' or activity reflects the execution of a task or action, and C) 'health' or participation reflects problems an individual may experience in life situations.[65]

Clinimetrics

For IIM, the International Myositis Assessment and Clinical Studies Group (IMACS) is the internationally accepted and validated measurement tool for follow-up in clinical trials.[66] The IMACS is composed of six separate scales, the so-called core set measures (CSMs) leading to the Total Improvement Score (TIS), which is often used as primary outcome measurement in clinical trials.[67] The CSMs comprise different parameters reflecting the heterogeneity of this disease: 1) muscle enzymes: CK, ALAT, ASAT, LDH, aldolase and creatinine; disease activity: 2) Patient Global Activity (PGA) and 3) Physician Global Activity (PhGA), 4) Extra muscular disease activity, 5) Functional outcome: Health Assessment Questionnaire – disability index (HAQ-DI), and 6) muscle strength: Manual Muscle Testing (MMT). Below we discuss other possible parameters for the follow-up of IIM.

As its name reveals, the IMACS TIS focusses on improvement, and cannot be used for disease flares or remission.[68] One might argue that this is a limitation of the TIS. Clinimetrically, the TIS is a complex scale that combines different constructs such as disease activity and disability in a composite measurement. By doing this, a TIS does not give insight into the particularly improving domain: is it due to less disease activity, or is the patient becoming more skilful and experiences less impairment? Secondly, the TIS is a non-linear scale, and rates each of the CSMs differently to come to the eventual score. Albeit, the weighed scores have been developed using clinical trial validation and by consensus by experts in the field.[68] Thirdly, a patient requires a certain level (>10%) of muscle weakness (the CSM with the highest weight within the TIS) to be able to show at least moderate improvement. Other outcome measurements that would evaluate disease activity or disability on a less complex scale are therefore desirable.

Within the IMACS, disability (domain B) is assessed by the HAQ-DI. The HAQ-DI is a generic scale which assesses dysfunction of proximal and distal extremities. The HAQ-DI has frequently been used in trials for rheumatic inflammatory diseases in which it shows clinical validity (treatment vs. placebo)[69] and construct validity[70], The scale also has some disadvantages, such as floor effects. The HAQ-DI was originally developed for rheumatoid arthritis, and the clinical and construct validity for IIM patients is unclear.[71] We evaluated in **chapter 5** the clinimetric properties of the Amsterdam Linear Disability Scale (ALDS) within patients with IIMs. This scale evaluates physical function and evaluates the same ICF domain as the HAQ-DI (B - disability). The ALDS is a linear and generic scale. It has been tested

thoroughly, and showed good construct validity, test-retest reliability and internal consistency.[72-74] The ALDS measures disability as the only construct and is therefore more transparent compared to the TIS. Our study showed that the ALDS has promising clinimetric properties: it detected relevant changes in disability, discriminated between responders and non-responders to treatment with IVIg, and detected deterioration. Because disability (or physical function) is one of the key domains assigned by patients, the ALDS may be an interesting measurement tool to use for the follow-up of IIM patients.

Evaluation of the patients' needs during follow up has resulted in patient reported outcome measures (PROMS). PROMS focus on participation in daily living (domain C). To emphasize patient-prioritized outcomes, the IMACS and the Outcome Measures in Rheumatology (OMERACT) group have recommended the use of PROMS in clinical and observational studies. The OMERACT group has an impressive reach: they are active on three continents and receive response from patients as well as caregivers and health care providers. A qualitative study of this group revealed that the following range of symptoms are important for IIM patients: pain, physical tightness/stiffness, fatigue, disease effect on emotional life and relationships, and treatment-related side effects.[75] As such, key domains have been identified, and amongst them fatigue, pain and physical function have been selected as mandatory in all trials.[76]

Imaging

We evaluated if quantitative, semi-quantitative and qualitative US and MRI were able to detect change during follow-up after approximately three months treatment with intravenous immunoglobulins (**chapter 2**). We showed that semi-quantitative and qualitative US were able to detect improvement after this relatively short period of time. Other studies evaluating responsiveness of US to disease activity are scarce. Moreover, standardization of US protocols is needed to improve reproducibility and reliability to make it a generally usable and validated tool for follow-up.[77]

Although MRI showed a non-statistically significant decrease of oedema over time in our study, probably due to a relatively short follow-up period, previous studies showed a significant decrease of muscle oedema on MRI after 2-9 months of treatment.[78-80] Muscle MRI is generally accepted as being helpful in assessing and monitoring disease activity and damage.[31] Aiming to increase the accuracy of follow-up, quantitative MRI assessment has raised increased clinical interest.[81] Several quantification techniques have been investigated,

among which quantitative MRI using T2, fat fraction measurement, and diffusion tensor imaging (DTI) quantified by the apparent diffusion coefficient (ADC).[26 82] Future research is necessary to confirm the diagnostic value of these quantitative MRI techniques. One concern in quantitative imaging assessment (ultrasound and MRI both) is assigning the region of interest, which is time consuming since it is assigned manually, and therefore less suitable for clinical practice. Free online tools, for the analysis of quantitative MRI data are available, but need validation for the application to IIM.[83-85] Furthermore, artificial intelligence software will help to allow for easier quantitative MRI assessment.

Future outcome measures should address both patients' doctors' needs, can ideally be filled in online and easily transformed into a visible result in the outpatient clinic during a consultation.

DIRECTIONS FOR FUTURE RESEARCH

Diagnosis

There is the need for a diagnostic algorithm that includes the least invasive (combination of) diagnostic tests. The results of our ADAPT study will direct to such a diagnostic algorithm but will need external validation in the future. The ADAPT study focusses on establishing the diagnosis of "IIM which should be treated with corticosteroids". Because of the relatively small sample size there will be a limited power for subgroup analysis, necessitating expansion of the number of included patients.

Classification

New classification criteria, incorporating all treatable IIM subtypes will be essential to keep moving forward. To establish incorporation of all IIM subtypes, larger patient cohorts are needed, which is hard to achieve in a rare disease. Technological advances and (inter)national working groups are promising tools to achieve this: patient cohorts as complete as possible should be collected on a national level through an electronic patient record-based patient registry. The latter is one of the goals of the Dutch Myositis Network. The existing MYonet (former EuroMyositis) registry is a promising initiative helping to connect myositis research woldwide.[38]

Follow-up

Ideally, follow-up should be personalised. According to the ICF model, elements for follow-up

should focus on three domains: disease activity, disability/activity and participation. Optimizing quantitative imaging techniques to obtain accurate evaluation of imaging procedures will contribute to get a precise follow-up and may lead to earlier (and personalised) tapering of medication. New potential biomarkers will be helpful, such as specific metabolites in serum and urine who are exclusively important amongst different IIM subtypes.[86] Follow-up does not always need to be physical at the outpatient clinic, since telemedicine is now widely available, and patient reported tests assessing disease activity are being validated.[87] The ALDS, of which we evaluated the clinimetric potential, may be a useful tool in telemedicine to evaluate disability. Of course, the possibility for e-health is dependent on the patients' digital skills. This 'patient-dependent approach' requires creativity from doctors, as well as further digitalization of patient records, and a willingness from insurance companies to finance teleconsultation. At this moment a well validated 'participation' scale is not available for IIM patients. Development of such an outcome parameter, as developed for myotonic dystrophy patients[88], that may be based on the ALDS, would be of additional value in patient-centred longitudinal follow-up in clinical trials. The Canadian Occupational Performance Measure (COMP) is a participation scale that identifies patients' self-rated performance in self-care, productivity and leisure. It has been used in other neuromuscular diseases[89], stroke[90] and Parkinson's disease.[91] The COMP may be an interesting questionnaire for IIM patients to measure participation in future clinical trials, in a personalised perspective.

In respect to all possibilities discussed above, I believe that future myositis patients will be 1) diagnosed with a higher accuracy, 2) diagnosed with less invasive tests and, 3) will undergo a more personal and patient-centred follow-up.

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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. doi: 10.1002/acr.20532 [published Online First: 2012/05/25]

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Appendices

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Dankwoord

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SUMMARY

Chapter 1 is the introduction of this thesis in which idiopathic inflammatory myopathies (IIM) and the distinct subtypes are described. In short, IIM are a group of rare, auto immune mediated myopathies, commonly referred to as 'myositis'. Most subtypes are treatable, except for inclusion body myositis (IBM). IBM is for that reason not within the scope of this thesis. Clinically, IIM are characterised by subacute proximal muscle weakness which usually develops within weeks to months. Depending on the subtype, multiple extra muscular organs may be involved. Several treatable subtypes are distinguished: dermatomyositis, affecting not only the muscles but also the skin; antisynthetase syndrome, characterized by the trias of myositis, arthritis and interstitial lung disease; non-specific myositis or overlap-myositis, in which myositis is accompanied by another connective tissue disease such as rheumatoid arthritis, systemic sclerosis; and immune mediated necrotizing myopathy, characterised by often severe muscle weakness which may be associated with statin use. Polymyositis, the last treatable subtype, has been one of the most diagnosed subtypes for a long time, but is nowadays a contested entity which can be diagnosed when all other subtypes are excluded. Several subtypes are associated with characteristic auto-antibodies; e.g. MI2, TIF1-γ, MDA5, SAE and NXP2 in dermatomyositis, Jo-1, PL-7, PL-12, EJ and OJ in antisynthetase syndrome, SSa, SSb, Ro52, U1-nRNP, PM-Scl and Ku in non-specific or overlap myositis, and HMGCR and SRP in immune mediated necrotizing myopathy, albeit patients may be seronegative. The presence of an auto-antibody often suggests the co-existence of specific extra muscular disease activity. For instance, patients with antisynthetase related antibodies or anti-MDA5 often have an interstitial lung disease, and TIF1gamma suggests a paraneoplastic syndrome.

Furthermore, possible emergencies and the required treatment in IIM are outlined in this chapter. The most frequently occurring emergencies are respiratory failure, cardiac failure, extensive skinulcers and severe dysphagia. Dyspnoea and respiratory failure may appear when inflammation of the respiratory muscles causes weakness and subsequent hypoventilation but may also be caused by inflammation of the lung interstitium, so called interstitial lung disease. It requires early recognition and quick and rigorous treatment to prevent irreversible lung damage. Cardiac involvement in IIM is mostly related to the myocardium and may result in myocarditis. It may evolve into cardiac failure, and cardiac rhytm disturbances. Myocarditis requires systemic therapy, and interdisciplinary treatment with a cardiologist to prevent

cardiac overload. Severe ulcers of the skin are mostly seen in dermatomyositis with MDA5 antibodies. In MDA5 DM ulcers are typically located at the palmar side of the hands and elbows but may be located on other body parts as well. Ulcers may become infected but are nevertheless treated with immunosuppressant(s). Dysphagia affects IIM patients frequently and develops as the result of inflammation of pharynx and upper oesophageal sphincter as these consist of skeletal muscle. Dysphagia increases the risk of aspiration pneumonia and death, is an indication to reinforce treatment, and one needs to consider a nasogastric feeding-tube until the swallowing function has improved.

The overarching aim of this manuscript was to evaluate the diagnostic value of currently used tests at diagnosis and their value during the follow-up of patients. In **chapter 2** we focussed on the diagnostic value of currently available imaging modalities. In a prospective pilot study, we aimed to compare the ability of muscle ultrasound and muscle MRI to detect muscle changes in newly diagnosed IIM patients, and to evaluate their ability to detect changes over time after treatment with intravenous immunoglobulins (IVIg). Biopsy proven, treatment naïve, IIM patients were included, and all underwent muscle ultrasound and whole-body muscle MRI (WB-MRI) at two time points: upon diagnosis at baseline and at follow-up after nine weeks of IVIg treatment. Eighteen IIM patients with different IIM subtypes were included. At baseline, comparison of quantitative, semi-quantitative and qualitative ultrasound and semi-quantitative MRI detected muscle abnormalities in 28%, 89%, 89% and 100% of the patients respectively. At follow-up, semi-quantitative and qualitative ultrasound detected a statistically significant change over time in muscle abnormalities. We concluded that WB-MRI is most sensitive to detect muscle abnormalities at diagnosis, semi-quantitative and qualitative ultrasound are a reasonable alternative, and quantitative ultrasound is not useful when evaluating muscle abnormalities in adult IIM patients at diagnosis. Change over time after treatment was better detected by semi-quantitative and qualitative ultrasound compared to WB-MRI.

In **chapter 3** we focussed on possible diagnostic tests for (peri)myocarditis in IIM patients. We evaluated a multimodality screening strategy with the hypotheses that firstly, it would identify patients with active (peri)myocarditis and, secondly, it would devise a screening strategy for (peri)myocarditis. In this cross-sectional study newly diagnosed IIM patients were included, and all were evaluated by a cardiologist for the presence of clinical signs and symptoms of

(peri)myocarditis. All patients underwent laboratory investigations including troponin T, Troponin I, NT-pro BNP, and anti-mitochondrial antibodies, and ECG, echocardiography, and cardiac magnetic resonance imaging (CMR). For the statistical analysis we used classification and regression tree (CART) analysis. Using current consensus criteria for (peri)myocarditis, patients were diagnosed with definite, probable, possible, or no (peri)myocarditis. Thirty-four patients were included. We found definite (peri)myocarditis in 18%, probable in 6%, possible in 32% and no (peri)myocarditis in 44% of the patients. CART analysis showed that CMR was the most sensitive diagnostic modality, identifying all patients with definite or probable (peri)myocarditis. Furthermore, CART analysis showed that both cardiac troponins were useful as a diagnostic gatekeeper for CMR. Troponin T with a cut-off value of <113ng/L was the optimal value to rule out (peri)myocarditis with a sensitivity of 88% and specificity of 67%. Troponin I with a cut-off value of >35ng/L was the optimal value to rule in (peri)myocarditis with a sensitivity of 63% and specificity of 100%. On the contrary, neither NT-pro BNP, anti-mitochondrial antibodies, ECG, nor echocardiography had an added value in distinguishing between patients with definite or probable (peri)myocarditis or without (peri)myocarditis. We concluded that routine multimodality screening yields a considerable number of patients with definite or probable (peri)myocarditis. A diagnostic strategy could consist of cardiac troponin T and I followed by CMR.

In **chapter 4** the study protocol of the ADAPT study is described. The study is designed to show the diagnostic value of several individual diagnostic tests, and the incremental value of combinations of different diagnostic tests in diagnostic strategies. We hypothesized that an evidence-based diagnostic strategy, using fewer and preferably the least invasive diagnostic modalities, can achieve the accuracy of a complete panel of diagnostic tests. The panel of diagnostic modalities includes: muscle imaging (magnetic resonance imaging or ultrasound), electromyography, myositis auto-antibody testing and muscle biopsy. The study is conducted according to the principles of a prospective diagnostic accuracy study with an over-complete study design; one-hundred patients suspected of an IIM will be included, and all will undergo the over-complete diagnostic study design. A reference diagnosis will be assigned by an expert panel, which will be a surrogate gold-standard. Several predefined diagnostic strategies will be compared against the reference diagnosis to find the optimal diagnostic strategy. At the

moment of writing this summary, data collection is ongoing, and over two-thirds of the intended number of patients are included in the study.

In **chapter 5** we explored a preliminary diagnostic field in IIM, in a laboratory setting. B cells may play an important role in the pathogenesis of IIM since muscle biopsies of IIM patients show plasma cells, the majority of IIM patients are positive for myositis related antibodies, and B cell directed therapies are effective in the treatment of IIM. We investigated the B cell receptor (BcR) repertoire in muscle biopsies at baseline before treatment, and in peripheral blood samples both at baseline and at follow-up after IVIg treatment. Additionally, we studied the correlation of the BcR repertoire with clinical response to treatment. Clones with a frequency greater than or equal to 0.5% of the total repertoire were labelled dominant clones. The impact of a clone was calculated as its frequency in the repertoire, and the impact of a group of clones as their cumulative frequency (total impact). Nineteen patients with biopsy proven IIM were included. First, we found that (dominant) BcR clones in muscle tissue can be retrieved in peripheral blood. Secondly, we found that the impact of all dominant BcR clones before treatment was higher in patients that responded to IVIg treatment compared to the patients who did not respond to IVIg. Finally, we found that decrease in the total impact of dominant clones over time in peripheral blood at follow-up correlated with a higher clinical improvement, as measured by the Total Improvement Score (TIS) (an internationally validated outcome measure). We concluded that response to IVIg may depend on the composition of the BcR repertoire of a patient before treatment.

In **chapter 6** we focussed on functional outcome, which is one of the most important outcome measures from patients' perspective. We explored the clinimetric properties of a functional disability scale, the Academic Medical Centre Disabiltiy Score (ALDS) in patients with IIM. The clinimetric properties of the ALDS were compared against the Health Assessment Questionnaire Disability Index (HAQ-DI), which is one of the core set measures in the internationally validated TIS. Nineteen IIM patients with at least 10% loss of muscle strength according to the manual muscle testing (MMT) were included, and data were obtained before treatment and after nine weeks of treatment. We confirmed construct validity by moderate to strong correlations between the ALDS and HAQ-DI (rs= -0.57 (baseline); -0.86 (follow-up). Secondly, the ALDS change score (ALDS score at follow-up – ALDS score at baseline) correlated with the TIS (rs=0.70), discriminated between responders and non-responders to treatment

and detected deterioration. We concluded that the ALDS has promising clinimetric properties and detected relevant changes in disability in patients with IIM.

In **Chapter 7** the findings of this thesis are discussed and future directives are put forward.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 is de introductie van deze thesis, waarin idiopathische inflammatoire myopathieën (IIM) en haar afzonderlijke subtypen worden beschreven. In het kort zijn IIM een groep zeldzame, auto immuun gemedieerde spierziekten, en wordt het in de volksmond ook wel 'myositis' genoemd. De meeste subtypen zijn behandelbaar, behalve inclusion body myositis (IBM). Om die reden valt IBM niet onder het bereik van deze thesis. Klinisch wordt IIM gekarakteriseerd door subacute, proximale spierzwakte, dat zich gewoonlijk binnen weken tot maanden manifesteert. Afhankelijk van het subtype kunnen meerdere extra-musculaire organen betrokken zijn. De verschillende behandelbare subtypen zijn: dermatomyositis, waarbij niet alleen de spieren maar ook de huid is aangedaan; antisynthetase syndroom, dat omschreven wordt door de trias myositis, arthritis en interstitiële longziekte; niet-specifieke of overlapmyositis, waarbij myositis samen voorkomt met een andere bindweefselziekte zoals rheumatoide artritis of systemische sclerose; en immuun gemedieerde necrotiserende myopathie, dat zich kenmerkt door vaak ernstige spierzwakte en een mogelijke associatie met statine gebruik. Polymyositis, het laatste behandelbare subtype, was lange tijd een van de meest gediagnosticeerde subtypen, maar is tegenwoordig een betwiste aandoening, die gediagnosticeerd kan worden indien alle andere subtypes uitgesloten zijn. Verscheidene subtypen zijn geassocieerd met karakteristieke auto-antilichamen: namelijk MI2, TIF1-γ, MDA5, SAE en NXP2 met dermatomyositis, Jo-1, PL-7, PL-12, EJ en OJ met antisynthetase syndrome, SSa, SSb, Ro52, U1-nRNP, PM-Scl en Ku met niet-specifieke of overlapmyositis, en HMGCR en SRP met immuun gemedieerde necrotiserende myopathie, alhoewel patiënten ook seronegatief kunnen zijn. De aanwezigheid van een auto-antilichaam is vaak gelinkt aan de aanwezigheid van specifieke extra-musculaire ziekteactiviteit. Patiënten met antisynthetase gerelateerde antilichamen of anti-MDA5 hebben bijvoorbeeld vaak een interstitiële longziekte, en TIG1gamma suggereert een paraneoplastisch syndroom.

Daarnaast worden mogelijke spoedgevallen en de benodigde behandeling bij IIM uiteen gezet in dit hoofdstuk. De meest voorkomende spoedgevallen zijn respiratoir falen, hartfalen, uitgebreide huidulcera en ernstige dysfagie. Dyspnoe en respiratoir falen kan voorkomen indien inflammatie de ademhalingsspieren verzwakt en vervolgens hypoventilatie

veroorzaakt, maar inflammatie van het long interstitium, ook wel interstitiële longziekte, kan eveneens de onderliggende oorzaak zijn. Dit vergt vroege herkenning, en snelle en agressieve behandeling om onomkeerbare longschade te voorkomen. Cardiale betrokkenheid bij IIM is vaak gerelateerd aan het myocard, en kan myocarditis tot gevolg hebben. Dit kan leiden tot hartfalen en hartritme afwijkingen. Myocarditis vereist systemische behandeling en interdisciplinaire behandeling met een cardioloog om overvulling te voorkomen. Ernstige huidulcera worden meestal gezien bij dermatomyositis in combinatie met MDA5 antilichamen. Kenmerkend voor MDA5 DM is dat de ulcera op de handpalmen en ellebogen gelokaliseerd zijn, maar ook andere plekken kunnen zijn aangedaan. Ulcera kunnen geïnfecteerd raken, maar worden desalniettemin behandeld met immunosuppressiva. Dysfagie komt regelmatig voor als gevolg van inflammatie van de farynx en de bovenste slokdarm sfincter, gezien deze bestaan uit skelet spier. Dysfagie verhoogt de kans op een aspiratie pneumonie en overlijden, is een indicatie om de behandeling te intensiveren, en een neus-maagsonde dient overwogen te worden totdat de slikfunctie is verbeterd.

Het overkoepelende doel van dit manuscript was de diagnostische waarde van de huidige gebruikte testen bij diagnose, en hun waarde gedurende follow-up van patiënten te evalueren. In **hoofdstuk 2** is gefocust op de waarde van de huidige beeldvormende technieken. We hadden als doel om het vermogen van spierecho en spier MRI om spier veranderingen bij diagnose en na behandeling met intraveneuze immuunglobulinen (IVIg) te detecteren, door middel van een prospectieve pilot studie. Biopt bewezen, niet behandelde, IIM patiënten werden geïncludeerd, en ondergingen allen spierecho en een spier MRI van het hele lichaam (WB-MRI) op twee momenten: bij diagnose op baseline en bij follow-up na negen weken behandeling met IVIg. Achttien IIM patiënten met verschillende subtypen werden geïncludeerd. Vergelijking van kwantitatieve, semi-kwantitatieve en kwalitatieve echo en semi-kwantitatieve MRI toonde spier afwijkingen in respectievelijk 28%, 89%, 89% en 100% van de patiënten in de baseline. Bij de follow-up detecteerden semi-kwantitatieve en kwalitatieve echo een statistische significante verandering over tijd in spierafwijkingen. We concludeerden dat WB-MR het meest sensitief is bij het detecteren van spierafwijkingen bij diagnose, semi-kwantitatieve en kwalitatieve echo zijn een redelijk alternatief, en kwantitatieve echo is niet bruikbaar om te spierafwijkingen in volwassen IIM patiënten te

beoordelen. Verandering over tijd na behandeling werd beter gedetecteerd door semi-kwantitatieve en kwalitatieve echo in vergelijking tot WB-MRI.

In **hoofdstuk 3** hebben we gefocust op mogelijke diagnostische testen naar (peri)myocarditis bij IIM patiënten. We hebben een multimodaliteit screening strategie geëvalueerd met de hypothese dat: 1) deze strategie patiënten met actieve (peri)myocarditis identificeert, en 2) dit een screeningstrategie zou vormen voor (peri)myocarditis. Nieuw gediagnosticeerde IIM patiënten werden geïncludeerd in deze cross-sectionele studie, en zij werden allen beoordeeld door een cardioloog op de aanwezigheid van klinische tekenen of symptomen van (peri)myocarditis. Alle patiënten ondergingen laboratorium bepalingen, waaronder troponine T, troponine I, NT-pro BNP en anti-mitochondriale antilichamen, en een ECG, echocardiografie, en *cardiale magnetic resonance imaging* (CMR). Bij de statistische analyse is gebruik gemaakt van *classification and regression tree* (CART) analyse. Gebruik makende van de huidige consensus criteria voor (peri)myocarditis, werden patiënten gediagnosticeerd met zekere, waarschijnlijke, mogelijke, of geen (peri)myocarditis. Vierendertig patiënten werden geïncludeerd. We vonden zekere (peri)myocarditis in 18%, waarschijnlijke in 6%, mogelijke in 32% en geen (peri)myocarditis in 44% van de patiënten. CART analyse toonde dat CMR de meest sensitieve diagnostische methode was, die alle patiënten met zekere of waarschijnlijk (peri)myocarditis detecteerde. Daarnaast toonde CART analyse dat beide cardiale troponines bruikbaar waren als een diagnostische poortwachter voor CMR. Troponine T was met een drempelwaarde van <113ng/L de optimale waarde om (peri)myocarditis uit te sluiten met een sensitiviteit van 88% en een specificiteit van 67%. Troponine I was met een drempelwaarde van >35ng/L de optimale waarde om (peri)myocarditis in te sluiten, met een sensitiviteit van 63% en een specificiteit van 100%. Anderzijds, NT-pro BNP, anti-mitochondriele antilichamen, ECG, noch echocardiografie hadden een toegevoegde waarde om patiënten met zekere of waarschijnlijke (peri)myocarditis te onderscheiden van geen (peri)myocarditis. Wij concludeerden dat routine multimodaliteit screening een aanzienlijk aantal patiënten met zekere of waarschijnlijke (peri)myocarditis oplevert. Een diagnostische strategie zou kunnen bestaan uit cardiale troponine T en I gevolgd door CMR.

In **hoofdstuk 4** wordt het studie protocol van de ADAPT studie beschreven. De studie is ontworpen om de diagnostische waarde van verschillende individuele diagnostische testen,

en de toegevoegde waarde van combinaties van verschillende diagnostische testen in diagnostische strategieën aan te tonen. De hypothese hierbij is dat een *evidence based* diagnostische strategie, die minder en bij voorkeur de minst invasieve diagnostische modaliteiten gebruikt, de accuratesse van een compleet panel aan diagnostische testen kan evenaren. Het panel van diagnostische modaliteiten bestaat uit: beeldvorming van de spier (MRI en echo), electromyografie, myositis auto-antilichamen en een spierbiopt. De studie wordt uitgevoerd volgens de principes van een prospectieve diagnostische accuratesse studie met een overcompleet studie design; honderd patiënten met een klinische verdenking op een IIM zullen worden geïncludeerd, en allen zullen het overcomplete diagnostische studie design ondergaan. Een referentie diagnose wordt toegewezen door een expert panel, wat zal fungeren als een surrogaat goudstandaard. Een aantal vooraf gestelde diagnostische strategieën zal vergeleken worden met de referentie diagnose om te optimale diagnostische strategie te vinden. De datacollectie is gaande op het moment van schrijven van deze samenvatting, en ruim twee-derde van het beoogde aantal patiënten is geïncludeerd in de studie.

In **hoofdstuk 5** onderzochten we een verkennend diagnostisch terrein in IIM. B cellen zouden een belangrijke rol in de pathogenese van IIM kunnen spelen, gezien spierbiopten van IIM patiënten plasmacellen tonen, de meerderheid van de IIM patiënten antilichamen heeft tegen myositis gerelateerde antistoffen, en B cel gerichte therapie effectief is in de behandeling van IIM. We onderzochten het B cel receptor (BcR) repertoire in spierbiopten op baseline, vooraf aan behandeling met IVIg, en in perifeer bloed monsters zowel op baseline als bij follow-up na behandeling met IVIg. Daarnaast bestudeerden we de correlatie tussen het BcR repertoire en de klinische reactie op behandeling. Klonen met een frequentie groter of gelijk aan 0,5% van het totale repertoire werden gemarkeerd als dominante klonen. De impact van een kloon werd bepaald door het aantal keer dat deze kloon voorkwam in het repertoire, en de impact van een groep van klonen de *cumulative* frequentie (totale impact). Negentien patiënten met een middels spierbiopt bewezen IIM werden geïncludeerd. Allereerst constateerden we dat (dominante) BcR klonen in spierweefsel teruggevonden werden in het perifere bloed. Als tweede constateerden we dat de impact van alle dominante BcR klonen vooraf aan behandeling hoger was in patiënten die goed reageerden op behandeling met IVIg in vergelijking met de patiënten die niet reageerden op behandeling met IVIg. Als laatste

constateerden we dat de afname van de totale impact van dominante klonen bij follow-up correleerde met een grotere klinische verbetering, die gemeten werd met de *Total Improvement Score* (TIS) (een internationaal gevalideerde uitkomst maat). We concludeerden dat reactie op behandeling met IVIg samen zou kunnen hangen met de samenstelling van het BcR repertoire van een patiënt vooraf aan behandeling.

In hoofdstuk 6 focusten we op functionele uitkomst, wat een van de meest belangrijke uitkomst maten is vanuit patiënten perspectief. We exploreerden de klinimetrische eigenschappen van een functionele onbekwaamheid schaal, de Academic Medical Centre Disability Score (ALDS) in patiënten met IIM. De klinimetrische eigenschappen van de ALDS werden vergeleken met de Health Assessment Questionnaire Disability Index (HAQ-DI), wat een van de *core set measures* van de internationaal gevalideerde TIS is. Negentien IIM patiënten met minimaal 10% spierkrachtverlies volgens de *manual muscle testing* (MMT) werden geïncludeerd, en de data werden verkregen vooraf aan behandeling en na negen weken behandeling. We bevestigden construct validiteit met matige tot sterke correlaties tussen de ALDS en de HAQ-DI ((rs= -0,57 (baseline); -0,86 (follow-up)). Als tweede correleert ALDS-verander-score met de TIS (rs=0,70), onderscheidt de ALDS-verander-score (ALDS-score bij follow-up – ALDS-score bij baseline) patiënten die wel en niet reageerden op behandeling, en detecteerde de ALDS-verander-score achteruitgang. We concludeerden dat de ALDS veelbelovende klinimetrische eigenschappen heeft, en relevante veranderingen detecteert in onvermogen bij patiënten met IIM.

In **hoofdstuk 7** worden de bevindingen van deze thesis bediscussieerd en aanbevelingen voor toekomstig onderzoek worden aangedragen.

CONTRIBUTING AUTHORS AND AFFILIATIONS

Author	Title	Affiliation
Aggarwal, R.	MD, PhD	Department of Rheumatology and Clinical Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
Amin, A.S.	MD, PhD	Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands
Anang, D.C.	Msc.	Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, Amsterdam UMC, University of Amsterdam, The Netherlands Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, The Netherlands Department of Genome analysis, Amsterdam UMC, University of Amsterdam, Netherlands
Aronica, E.	MD, PhD	Department of (Neuro)Pathology, Amsterdam University Medical Centre, The Netherlands
Baars, P.A.	PhD	Department of Experimental Immunology, Amsterdam institute for Infection & Immunity, Amsterdam UMC, The Netherlands
Boekholdt, M.	MD, PhD	Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands
Bossuyt, P.M.M.	PhD	Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Amsterdam University Medical Centre, The Netherlands
de Bruin-Bon, R.A.C.M.		Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands
de Haan, R.J.	PhD	Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Amsterdam University Medical Centre, University of Amsterdam, The Netherlands
de Harder, J.M.	MD, PhD	Department of Radiology and Nuclear medicine, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
de Visser, M.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
de Vries, N.	MD, PhD	Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, Amsterdam UMC, University of Amsterdam, The Netherlands Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, The Netherlands
Eftimov, F.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Glaubtiz, S.	MD	University medical centre Göttingen, Germany
Hemke, R.	MD, PhD	Department of Radiology and Nuclear medicine, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Jarings, M.C.		Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands
Kamperman, R.G.	MD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Koelman, J.H.T.M.	MD, PhD	Department of Neurology and Neurophysiology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Kok, W.E.M.	MD, PhD	Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands

Lim, J.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Min, M.	MD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Niewold, I.	BSc.	Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, Amsterdam UMC, University of Amsterdam, The Netherlands Department of Genome analysis, Amsterdam UMC, University of Amsterdam, Netherlands
Pinto, Y.M.	MD, PhD	Department of Cardiology, Amsterdam UMC, University of Amsterdam, The Netherlands
Planken, R.N.	MD, PhD	Department of Radiology and Nuclear Medicine, Amsterdam UMC, University of Amsterdam, The Netherlands
Potters, W.V.	PhD	Department of Neurology and Neurophysiology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Raaphorst, J.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Saris, C.G.J.	MD, PhD	Department of Neurology, Radboud UMC, Radboud University, Nijmegen, The Netherlands
Schmidt, J.	MD, PhD	University medical centre Göttingen, Germany
Smithuis, F.F.	MD	Department of Radiology and Nuclear medicine, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
van der Kooi, A.J.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
van der Weele, L.	MD	Department of Rheumatology and Clinical Immunology, Amsterdam Rheumatology and Immunology Center, Amsterdam UMC, University of Amsterdam, The Netherlands Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, The Netherlands
van Kampen, A.H.C.	PhD	Bioinformatics Laboratory, Department of Epidemiology and Data science, Amsterdam Public Health Institute, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
van Leeuwen, E.M.M.	PhD	Department of Experimental Immunology, Amsterdam institute for Infection & Immunity, Amsterdam UMC, The Netherlands
van Schaik, B.D.C.	BSc.	Bioinformatics Laboratory, Department of Epidemiology and Data science, Amsterdam Public Health Institute, Amsterdam Infection and Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
van Schaik, I.N.	MD, PhD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands Spaarne Gasthuis, Haarlem, The Netherlands
Verhamme, C.	MD, PhD	Department of Neurology and Neurophysiology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands
Walter, A.W.	MD	Department of Neurology, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands

Group authors

Dutch Myositis Network		
Author	**Title**	**Affiliation**
Brusse, E.	MD, PhD	Department of Neurology, Erasmus UMC, Rotterdam, The Netherlands
Hoogendijk, J.E.	MD, PhD	Department of Neurology, Brain Centre Rudolf Magnus, UMC Utrecht, Utrecht, The Netherlands
Saris, C.G.J.	MD, PhD	Department of Neurology, Radboud UMC, Radboud University, Nijmegen, The Netherlands

LIST OF ABBREVIATIONS

Abbreviation	Meaning
ADAPT	optimizAtion of Diagnostic Accuracy in idioPathic inflammaTory myopathies
ALAT	alanine aminotransferase
ALDS	Academic Medical Centre Disability Score
AMA	anti-mitochondrial autoantibodies
ANOVA	Analysis of variance
ARD	acute respiratory distress
ASAT	aspartate aminotransferase
ASS	antisynthetase syndrome
BAFF	B cell activation factor
BcR	B cell receptor
CART	Constructing Classification and Regression Tree
CK	creatine kinase
CMR	cardiac magnetic resonance imaging
CMR	cardiac magnetic resonance imaging
CSM	core set measures
CTD	connective tissue disease
CVD	cardiovascular disease
CVRF	cardiovascular risk factors
DM	dermatomyositis
ECV	extracellular volume
EI	echo-intensity
EMA	Extra Muscular Activity
EMG	electromyography
ENMC	European Neuromuscular Centre
FE	Fascial oedema
FT	Fascial thickness
GBS	Guillain Barré syndrome
HAQ-DI	Health Assessment Questionnaire Disability Index
hs-TnI	high sensitive troponine I
hs-TnT	high sensitive troponine T
IBM	inclusion body myositis
IIM	idiopathic inflammatory myopathy
ILD	interstitial lung disease
IMACS	International Myositis Assessment and Clinical Studies
IRT	Item Response Theory
IVIg	intravenous immunoglobulin
LDH	lactate dehydrogenase
LGE	late gadolinium enhancement
LVDD	left ventricular diastolic dysfunction
MAA	myositis associated antibody
MAP	Myositis Activity Profile

MMT	manual muscle testing
MRI	magnetic resonance imaging
MSA	myositis specific antibody
MT	Muscle thickness
NM/OM	non-specific/ overlap myositis
OM	overlap myositis
PAH	pulmonary artery hypertension
PB	peripheral blood
PGA	Patient Global Activity
PhGA	Physician Global Activity
PICR	physician impression of clinical response
PM	polymyositis
PROM	patient reported outcome measure
RA	rheumatoid arthritis
ROI	range of interest
sCK	serum creatine kinase
SLE	systemic lupus erythematosus
TIS	total improvement score
US	ultrasound
WB-MRI	whole body magnetic resonance imaging

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**Shared first and last authorship*

PHD PORTFOLIO

Name PhD student: Hannah Walter
PhD period: August 2019 – August 2022
Name PhD supervisor: prof. dr. I.N. van Schaik
Name copromotors: dr. A.J. van der Kooi and dr. J. Raaphorst

	Year	Workload (ECTS)
General courses		
- e-BROK, AMC Graduate School, AMC	2020	1.5
Specific courses		
-Advanced Immunology course, Vu Amstel Academie	2020	2.9
- Basiscursus spierechografie, Sint Maartenskliniek en Radboud UMC, Nijmegen	2019	0.5
Seminars, workshops and master classes		
-Research seminars on department (every other week)	2019-2021	3.0
-Weekly neuromuscular pathology meeting	2019 - 2021	5.0
-Weekly Clinical Immunology and Rheumatology meeting	2019 - 2021	1.0
Presentations and posters		
- Poster presentation 'B cell receptor profiling in new onset myositis', Global Conference of Myositis, Prague	2022	0.5
- Oral presentation 'Imaging in myositis: MRI versus ultrasonography', Belgisch-Nederlandse Neuromusculaire studieclub and research seminar on department	2021	0.5
- Poster presentation 'Imaging in myositis: MRI versus ultrasonography', 16th International Congress on Neuromuscular Diseases, Valencia (virtual) and Nederlandse Vereniging voor Neurologie	2020, 2021	1.5
- Poster presentation 'Cardiac screening for myocarditis in myositis patients', 16th International Congress on Neuromuscular Diseases, Valencia (virtual)	2021	0.5
-Oral presentation 'Diagnostic accuracy in myositis: the ADAPT study', Belgisch-Nederlandse Neuromusculaire studieclub and research seminar on department	2020	0.5
- Poster presentation 'Diagnostic accuracy in myositis: the ADAPT study', Wetenschapsdagen Nederlandse Vereniging voor Neurologie (virtual), Global Conference of Myositis, Prague	2020, 2022	0.5
(Inter)national conferences		
- Belgisch Nederlandse studieclub	2021	0.5
-Myositis Netwerk Nederland conference	2019, 2020	0.2
-Spierziekten dag	2019, 2020	0.5
-Symposium Neuromusculaire Ziekten	2019	0.2
-NVN Wetenschapsdagen	2019, 2020	1.0
-16th International Congress on Neuromuscular Diseases, Valencia (online)	2020	0.5
-International Congress on Neuromuscular Imaging	2021	0.5

-Global Conference On Myositis, Prague	2022	1.5
Lecturing		
-Lecture about myositis and lecture with live patiënt	2021	1.0
- Instruction on muscle strength testing	2020, 2021	1.5
-Lecture myositis to rheumatology residents	2020	0.5
Supervising		
- Supervision of bachelor thesis of medical student 'The histological difference between MDA5 dermatomyositis and cutaneous Lupus Erythematodes'	2021	2.0

ABOUT THE AUTHOR

Anne Willemijn Walter, better known as Hannah, was born on March 12, 1991 in Nijmegen. At the age of three she moved with together with her parents and younger brother to Oss. Her interest in neurology was awakened in the third grade of high school, by her professor in biology Pierre Schrauwen. She graduated from the Titus Brandsma Lyceum in 2009 and followed her heart which meant first discover Australia before starting a study.
Coming back in 2010, she started studying psychobiology at the University of Amsterdam, but switched to medicine after finding out that she missed interaction with people while doing laboratory work. In her free time she was active in student rowing association Nereus, and became the cox for lightweight man 2012, and was member of woman *dispuut* Nox. She first started cooperating in research on the topic: spousal burden in oesophageal carcinoma, under supervision of dr. Nadia Haj Mohammed and prof. Hanneke van Laarhoven in the second year of her study. After finishing the bachelor degree, she travelled with a study friend to Sri Lanka and started with a research project on constipation in toddlers and children at the University of Kelaniya in Sri Lanka under the passionate supervision of prof. Shaman Rajindrajith, his wife prof. Naranga Devanaranaya, and prof. Marc Benninga. Inspired by the mentality of doctors in the non-Western world, she went to the University of Manipal in India for a clerkship social medicine. She finished her master degree with a research internship on advanced care planning in degenerative neurological diseases supervised by Em. prof. Marianne de Visser at the neurology department of the Academic Medical Centre in Amsterdam, where she got to know PhD student Johan Lim and dr. Anneke van der Kooi.
She graduated medical school in 2018 and started working as a resident not in training at the neurology department in the Noordwest Ziekenhuisgroep in Alkmaar. Encouraged by dr. Bjorn van Geel, neurologist in Alkmaar, to continue in the Neurological field she received an invitation from Johan Lim to follow up his PhD position on idiopathic inflammatory myopathies. Hannah started her PhD project in 2019 under supervision of dr. Anneke van der Kooi, dr. Joost Raaphorst and prof. dr. Ivo van Schaik. During her PhD she launched the ADAPT study, was involved in the enrolment of the Dutch Myositis Database and initiated the Myositis Fatigue study. From January 2022 she started working as a resident in training at the neurology department of the Amsterdam UMC. She lives happily with her partner Dico in Amsterdam.

DANKWOORD

Hier is het dan, mijn proefschrift. Het is nu daadwerkelijk af! Het is een proces geweest waarbij ik aan het begin nog niet wist hoe het resultaat eruit zou gaan zien, gaandeweg is het iets geworden waar ik trots op ben. Mijn naam staat op de kaft, maar dit boekje had ik niet kunnen schrijven zonder de samenwerking en hulp van velen. Hen wil ik graag bedanken.

Allereerst wil ik alle myositis patiënten bedanken die mee hebben willen werken aan het wetenschappelijke onderzoek waaruit dit boekje is voortgekomen. Dat zijn de patiënten die mee hebben gedaan aan de IMMEDIATE studie, en ook de patiënten die op dit moment mee doen met de ADAPT studie (waarvan het studieprotocol een onderdeel is van dit proefschrift, en resultaten volgen). Ik vind het een enorme blijk van vertrouwen dat jullie ondanks ziekzijn, de wetenschap een stapje verder hebben willen helpen door mee te doen aan deze studies. Hier heb ik alleen maar lof en ontzag voor: heel veel dank.

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Beste Ivo, wanneer ik verzandde in verschillende richtingen waarop we een bepaalde methodiek moesten uitvoeren, of hoe de resultaten te analyseren, dan was jij degene die me terugriep naar de basis. Je wist altijd feilloos te verwoorden waarom een bepaalde optie duidelijk beter was dan een andere. Onze meetings, die wegens covid vaak digitaal waren, vond ik inspirerend. Ze gaven me altijd weer nieuwe energie doordat ze een concrete conclusie opleverden, die een duidelijke richting gaven hoe verder te werken. Je feedback kwam altijd snel retour, wat ik als ontzettend fijn en betrokken heb ervaren!

Anneke en Joost, in een van de eerste weken van mijn aanstelling zei Filip: ‘ik ben benieuwd hoe gedragsexperiment van der Kooi-Raaphorst gaat uitpakken’. Inmiddels heb ik het antwoord: heel erg goed. Jullie vulden elkaar goed aan en waren daardoor samen een zeer betrokken en fijn team.

Lieve Anneke, ik wens elke patiënt een dokter zoals jou toe. Je maakt je echt hard voor de patiënt, en dat is ook een van de dingen die ik van je geleerd heb. Je maakte altijd tijd voor onze wekelijkse afspraak op vrijdag hebt, toegezien op de rode draad en voortgang tijdens mijn PhD traject, en hebt me geleerd dat agenda beheer de hoeksteen is voor voortgang. Je bent super attent, toen ik vóór de vaccinaties covid had was jij degene die met een bos bloemen voor de deur stond. Dankjewel!

Beste Joost, er zijn er maar weinig die zo’n goed gevoel voor tekst en taal hebben als jij. Tja, ik moest wel even wennen dat stukken altijd flink rood retour kwamen, maar je hebt me hierdoor bijgebracht hoe de lezer mee te nemen in een tekst, en dat mooie zinnen nou eenmaal belangrijk zijn. Je bent een kunstenaar in het initiëren van nieuwe ideeën, vaak met een humoristische of ludieke actie, waardoor de myositis wereld heel sprankelend werd en het stoffige NMZ-imago als sneeuw voor de zon verdween. Afgelopen juni hebben we de dansvloer in Praag onveilig gemaakt met veel myositis onderzoekers, ik heb ervan genoten!

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Beste Niek, het B cel project is was voor mij het moeilijkste project van dit boekje, waar ik best wel tegenop heb gezien. We hebben samen met Dornatien op donderdagen uren door de data gespit, en was verrast door de hoeveelheid tijd die je in je drukke agenda kon vrijmaken. Ik wil je bedanken voor alle tijd en uitleg die je in me hebt geïnvesteerd.

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een soort NMZ-ouders voor me. De NMZ-etentjes waren altijd memorabel en maakten dat ik me snel thuis voelde in de NMZ-squat groep.

Dear Dornatien, a journey it has been. I am very excited and proud that we managed to publish the B cell paper. I never thought we could have so many discussions about something 'physical' as an antibody. And I think I will never meet such a hardworking guy again that will start almost every explanation with 'basically' (LOL). Thank you for your never ending positivity, it was a pleasure working together with you!

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Lieve Josje, ouwe Brabander, en paranimf. Hoe zijn we toch samen in Alkmaar en vervolgens in het harde Amsterdam beland? Van wijntjes filosoferend over baby's bij bar Lempica tot samen in opleiding bij de neurologie in het Aumc. Er is nog heel veel meer veranderd (laat ik daar maar niet over uitweiden), maar ik ben blij met zo'n Brabantse in de buurt die me wél verstaat na een weekend in het Zuiden.

Alle andere onderzoekers uit 'de Grote Kamer': Rosanne, Sander, Ilse, Twan, Robin, Iris, Leon, Jeffrey, Lotte, Melanie, Hanna, Koos, Laura, Mariëlle, Yarit en Eileen. Er heeft een grote 'knip' gezeten in onze tijd op H2-235 door de covid pandemie. Maar ik kijk terug op een mega leuke tijd met jullie. Ik wil jullie bedanken voor alle gezelligheid, tea-trains, lunchpauzes, borrels, foto's en ja ook opruim sessies. De vrijdagen waren een uitdaging met 12 gevulde bureau's in één kamer. Lunch moest altijd klokslag 12uur (stelletje neuroten die we ook eigenlijk zijn). Het experiment met de koffie machine in de kamer heeft de ballotage helaas niet doorstaan. (Waar is dat ding eigenlijk?) Sander, het was behoorlijk wennen toen je de kliniek in ging en het bureau naast me ineens leeg was. Je 10:30 croissantjes en 15:30 cola's hebben me door de dode momenten getrokken. Laura Kummer, je was een heel gezellige nieuwe buurvrouw, ik ben blij dat je Sanquin tijdelijk heb ingeruild voor de grote kamer. Leon, we moeten het toch nog eens hebben over water drinken uit een mok. Twan, de grote leider pronkt nog altijd aan het plafond en blijft een mooie herinnering aan jouw communistische lezingen als intermezzo tijdens de onderzoeksdagen. Melanie, je bent met afstand de stoerste uit de kamer. Koos, ik hoop dat je achtervolging zich blijft voortzetten. Jeffrey, onze discussies over tiny houses en huwelijken waren een welkome afwisseling.

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(deels) tegelijkertijd te promoveren. Het werd nog even spannend, maar gelukkig was je eerder klaar dan ik. Bedankt voor je kritische vragen over mijn methoden; ik ben bang dat ik je nooit helemaal kan uitleggen waarom in de geneeskunde elke hoofdstuk een andere methodiek en onderwerp beslaat. Mama, ik zal onthouden dat onderzoek altijd tot iets praktisch moet leiden, hiervoor doen we het immers.

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