Studies of immunopathogenic mechanisms and treatment of chronic, inflammatory myopathies, myositis

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STUDIES OF IMMUNOPATHOGENIC
MECHANISMS AND TREATMENT OF
CHRONIC, INFLAMMATORY MYOPATHIES,
MYOSITIS

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M.D

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To my patients with myositis with thanks and with the hope of being of some help

And

To my family with love
**ABSTRACT**

Polymyositis (PM), dermatomyositis (DM) and sporadic inclusion body myositis (sIBM) are chronic inflammatory myopathies which are characterized by muscle weakness, fatigue and extra-muscular involvement. Mononuclear inflammatory cells are typically found in muscle tissue. Treatment is based on glucocorticoids together with other immunosuppressive agents. Despite favorable response most patients with PM and DM develop sustained muscle weakness and some patients do not respond at all, including sIBM. The explanation for this is unknown.

**Aims:** The overall aim was to achieve increased understanding of disease mechanisms in myositis by evaluating therapeutic effects on muscle performance and variables in muscle tissue of pharmacological (high dose IVIg treatment and TNF-blockade, infliximab) and of non-pharmacological treatment, endurance and resistance training.

**Patients and methods:** Clinical outcome measures (disease activity and muscle performance), laboratory tests and muscle biopsies were performed before and after the interventions. Muscle biopsies were investigated for signs of inflammation, fibrosis and muscle fiber characteristics. Patients with chronic, refractory myositis were treated with high dose IVIg for 12 weeks (study I, n=13) or with infliximab for 16 weeks (study II, n=12). In studies III and IV patients with chronic, stable and low active inflammatory DM or PM were applied to endurance training for 12 weeks (study III, n=8) or resistance training for 7 weeks (study IV, n=8).

**Results:** After high dose IVIg treatment muscle performance improved in 3 of 13 patients. In two patients there were signs of decreased inflammation in muscle biopsies, but there was no correlation between improved muscle performance and changes of inflammatory markers in muscle tissue. There was still a pronounced expression of inflammatory markers in muscle tissue after treatment. After infliximab treatment 3 patients had decreased, 2 had increased and 5 had unchanged disease activity. Four stopped prematurely due to adverse events. Muscle performance did not improve in any patient. Signs of active inflammation by magnetic resonance imaging were apparent before treatment in two completers and after treatment in five cases. There were minor changes in inflammatory markers in muscle biopsies, but they were still present after treatment with infliximab in all patients.

Before training a low proportion of oxidative, type I fibers (mean 32±10%) and an increased proportion of intermediate type IIC fibers (3±3%) was apparent in muscle biopsies compared to in healthy individuals. After endurance training type I fibers had increased significantly (42±13%) and type IIC fibers were reduced to 1±1%. Improved muscle performance correlated to an increased proportion of type I fibers and increased type II fiber area. After resistance training improved muscle performance was associated with downregulation of several genes signalling for inflammation and fibrosis. Gene transcripts involved in metabolic regulation were also modified.

**Conclusion:** Treatment with high dose IVIg or infliximab had limited clinical effects and there was no consistent decrease of inflammatory molecules expressed in muscle tissue. With infliximab treatment some patients even worsened, arguing against TNF as a key molecule in the underlying disease mechanism in chronic, treatment-resistant PM, DM and sIBM. In contrast, endurance as well as resistance training had positive effects on muscle performance and this was associated with changes in muscle characteristics and molecular expression in muscle, supporting the notion that non-immune mechanisms may have a role in causing muscle weakness.

**Keywords:** Idiopathic inflammatory myopathies, polymyositis, dermatomyositis, IVIg, infliximab treatment, physical training, muscle biopsy

Wisdom gives you the power

Knowledge keeps your heart young for ever

(Hakim Abolghasem Ferdosi, Persian poet fr 940)
LIST OF PUBLICATIONS

This thesis is based on the following papers which will be referred to by their Roman numerals:

   Limited effects of high-dos intravenous immunoglobulin (IVIg) treatment on molecular expression in muscle tissue of patients with inflammatory myopathies
   Annals of Rheumatic Disease 2007, 66 (10): 1276-83

II. Dastmalchi M., Grundtman C., Alexanderson H., Einarsdottir H., Barbasso Helmers S., Elvin K., Nennesmo I., Lundberg I.E.
    A high incidence of disease flares in an open study of infliximab in patients with refractory inflammatory myopathies
    Submitted

III. Dastmalchi M., Alexanderson H., Loell I., Ståhlberg M., Borg K., Lundberg I. E., Esbjörnsson M
    Effect of physical training on the proportion of slow-twitch type I muscle fibers, a novel nonimmune-mediated mechanism for muscle impairment in polymyositis or dermatomyositis
    Arthritis & Rheumatism (Arthritis Care & Research) 2007, 57(7): 1303-1310

    Resistance exercise reduces the expression of inflammation and fibrosis associated genes in autoimmune myositis patients
    Submitted
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<tr>
<td>aaRS</td>
<td>aminoacyl-tRNA synthetases</td>
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<tr>
<td>ALAT</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>Anti-SRP</td>
<td>anti-signal recognition particle</td>
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<tr>
<td>ASAT</td>
<td>Aspartate aminotransferase</td>
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<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>AZA</td>
<td>Azathioprine</td>
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<tr>
<td>CD</td>
<td>Clusters of differentiation</td>
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<tr>
<td>COX</td>
<td>Cytochrome C-oxidase</td>
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<tr>
<td>CyA</td>
<td>Cyclosporine A</td>
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<tr>
<td>CyX</td>
<td>Cylophosphamide</td>
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<tr>
<td>DM</td>
<td>Dermatomyositis</td>
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<td>ECG</td>
<td>Electrocardiography</td>
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<td>FI</td>
<td>Functional index</td>
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<td>GC</td>
<td>Glucocorticoids</td>
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<td>HAQ</td>
<td>Health assessment questionnaire</td>
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<tr>
<td>HLA</td>
<td>Human Leukocyte antigen</td>
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<td>HMGB-1</td>
<td>High mobility group box chromosomal protein 1</td>
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<td>IBM</td>
<td>Inclusion body myositis</td>
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<td>ICAM-1</td>
<td>Intracellular adhesion molecule-1</td>
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<tr>
<td>IIM</td>
<td>Idiopathic Inflammatory Myopathy</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>ILD</td>
<td>Interstitial lung disease</td>
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<td>IMACS</td>
<td>International Myositis Assessment and Clinical Studies Group</td>
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<tr>
<td>IVIg</td>
<td>High-dose intravenous immunoglobulin</td>
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<tr>
<td>LD</td>
<td>Lactate dehydrogenase</td>
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<tr>
<td>MAC</td>
<td>Membrane attack complex</td>
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<td>MITAX</td>
<td>Myositis intensity to treat activity index</td>
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<tr>
<td>MCTD</td>
<td>Mixed connective tissue disease</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MMT</td>
<td>Manual muscle test</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
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<td>MSA</td>
<td>Myositis-specific autoantibodies'</td>
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<td>MTX</td>
<td>Methotrexate</td>
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<tr>
<td>MXA</td>
<td>Myxovirus (influenza virus) resistance 1(Interferon inducible protein)</td>
</tr>
<tr>
<td>MYOACT</td>
<td>Myositis disease activity assessment visual analogue scale</td>
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<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PM</td>
<td>Polymyositis</td>
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<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
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<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
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<tr>
<td>sIBM</td>
<td>Sporadic Inclusion Body Myositis</td>
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<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule-1</td>
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<tr>
<td>VRM</td>
<td>Voluntary repetition maximum</td>
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1 INTRODUCTION

1.1 HISTORY

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of acquired muscle diseases characterized clinically by muscle weakness and histopathologically by inflammatory infiltrates within the skeletal muscle\(^1,2\). The group of these myopathies compromise three major and discrete subsets: polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM)\(^3\). Each subset retains its characteristic clinical, immunopathological, and morphological features regardless of whether it occurs separately or in connection with another systemic disease.

The first description of PM was published 1886 by Wagner\(^4\). Clinical manifestations described were symmetrical edema, stiffness, pain and limited motion of muscles, especially in arm muscles. Lung engagement and skin involvement were also described. At that time the term PM was coined, whereas DM was introduced in 1891 by Unverricht\(^5\). Inclusion body myositis was pathologically described by Chou in 1967\(^6\). The term IBM was coined by Yunis and Samaha in 1971\(^7\).

1.2 CLASSIFICATION AND DIAGNOSIS

The most commonly used criteria for diagnosis and classification of PM and DM were proposed by Bohan and Peter in 1975\(^8,9\):

- Symmetrical proximal muscle weakness of limb-girdle muscles and anterior neck flexors progressing over weeks to months, with or without dysphagia or respiratory muscle involvement.
- Elevation of serum skeletal muscle enzymes, particularly creatine phosphokinase (CK) often aldolase, serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LD).
- Electromyogram (EMG) indicating the classic triad of muscular impairment i.e. polyphasic short small motor neuron potentials, fibrillation, positive sharp waves, increased insertional irritability, and repetitive high frequency discharges.
- Muscle biopsy evidence of Type I and II fiber phagocytosis, regeneration with basophilia, large vesicular sarcolemal nuclei and prominent nucleoli, atrophy in a perifascicular distribution, variation in fiber size and inflammatory exudates, often perivascular.
- Characteristic cutaneous manifestations of dermatomyositis including lilac discoloration of eyelids (heliotrope) with periorbital edema, a scaly, erythematosis dermatitis over dorsum of the hands (especially the metacarpophalangeal and proximal interphalangeal joints (Gottron’s sign). This type of distribution is considered to be virtually pathognomonic of dermatomyositis.

The diagnosis of PM is considered definite when four criteria (without the rash) are met. With three criteria (without the rash) the diagnosis is probable; and with two criteria (without the rash) the diagnosis is possible. The diagnosis of DM is considered definite when three or four criteria (plus rash) are met, with two criteria (plus rash) the diagnosis is probable and with one criterion (plus rash) the diagnosis is possible.

Bohan and Peter classified patients with myositis into five subclasses:
- primary, idiopathic polymyositis
- primary, idiopathic dermatomyositis
- dermatomyositis/polymyositis associated with neoplasia
- childhood dermatomyositis or polymyositis associated with vasculitis
- dermatomyositis or polymyositis with associated collagen-vascular disease

The criteria of Bohan and Peter do not distinguish between IBM and PM. Criteria for IBM were proposed by Calabrese et al in 1987\(^10\) and by Dalakas in 1991\(^3\). According to more recent criteria\(^11\) definite IBM is established with diagnostic muscle biopsy irrespective of other
features. In contrast, if the muscle biopsy specimen fails to demonstrate the characteristic histology (i.e. invasion of mononuclear cells in nonnecrotic fibers, vacuolated muscle fibers and intracellular amyloid deposits or 15-18 nm tubulofilaments) then the patient can still be diagnosed as having a possible IBM by characteristic clinical and laboratory features.

There are two different forms of IBM, a sporadic disease (s-IBM) and a hereditary form (h-IBM). h-IBM encompasses several different myopathies with different clinical manifestations, it has a low grade of inflammation and is not considered as an IIM.

This thesis concerns mainly adult patients with PM and DM. A grown-up case with juvenile dermatomyositis (JDM) was included as were a few cases with IBM, so JDM and IBM will also be briefly discussed.

1.3 CLINICAL MANIFESTATIONS

1.3.1 Skeletal muscle

Most patients with PM or DM present with subacute or slowly progressive proximal muscle weakness that is usually symmetric and represents a major source of disability in patients with myositis. There is often difficulty in raising the head when supine, lifting, carrying, climbing steps, dressing and even walking on flat ground. Many patients need to use a handrail on stairways as a means of pulling themselves up. The patients also typically experience low muscle endurance and their muscles are easily fatigued. As an example they may be able to walk up one set of stairs, and then they can not lift their legs any more and have to rest. The flexor muscle of the neck is more affected than is the extensor. The musculature of the torso can also be affected, which leads to difficulty in arising from supine positions. In contrast, the facial musculature is generally spared. Muscle pain is less common than muscle weakness and fatigue. Typically the main discomfort arises after a work load and is often expressed by the patients as a sensation of “lactic acid in their legs”.

sIBM causes weakness and atrophy of distal and proximal muscles, and involvement of quadriceps and deep finger flexors are often clues to diagnosis. Patients often present with falls because their knees collapse owing to quadriceps muscle weakness, or they have difficulty performing certain tasks such as turning keys, owing to weakness of finger flexors. Neck flexors and extensors are frequently affected. Dysphagia occurs in up to 60% of patients with sIBM, leading to choking episodes. Facial muscle weakness is common and at times prominent, although the extraocular muscles are not affected. Sensory function is usually normal. The tendon reflexes, although preserved early in the disease, can diminish in the late stages as atrophy of major muscle groups becomes evident. Disease progression is slow but steady and resembles that of dystrophy. Most patients with sIBM require an assistive device, such as walker or wheelchair, within several years of onset. IBM patients rarely have muscle pain.

1.3.2 Extramuscular manifestations

Extramuscular manifestations are common features in PM and DM, but less frequent in IBM. The most common extramuscular manifestations are from skin, affecting all with DM and some with PM. In both PM and in DM the lungs, joints, gastro-intestinal channel and heart may be affected, indicating that IIMs are systemic inflammatory diseases. They also frequently co-occur with other defined rheumatologic diseases such as systemic sclerosis, Sjögren’s syndrome and mixed connective tissue disease (MCTD), and less often with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). IBM is usually evident as an isolated muscle disease, although there are reports of an association with Sjögren’s syndrome.
1.3.2.1 Pulmonary disease
Pulmonary complications constitute important clinical manifestations of PM or DM. The lungs may be involved either primarily with inflammation in the lung tissue, interstitial lung disease (ILD) or as a complication of muscle weakness. Pulmonary involvement as a complication of PM or DM was primarily described by Hepper et al. Aspiration pneumonia, ventilatory insufficiency and interstitial pneumonia being the three most important types of associated lung involvement. Interstitial pneumonia or ILD can be a serious complication of PM or DM. The reported prevalence of pulmonary involvement in PM or DM varies between 5 and 46% in cross-sectional studies depending on whether clinical, radiological, functional or pathological criteria have been used. Interstitial pneumonia or ILD is a common (65%) early manifestation in patients with PM or DM and is not always related to clinical symptoms. The presence of ILD in patients with myositis affects the prognosis, with increased morbidity and mortality, and this often also has an influence on the choice of immunosuppressive treatment.

1.3.2.2 Cutaneous manifestations
Dermatological manifestations usually precede the onset of muscle disease and patients may experience significant cutaneous symptoms; however, studies of DM severity have historically focused greater attention on the muscle disease. The characteristic and possibly pathognomonic cutaneous features of DM are the heliotrope rash and Gottron's papules. The heliotrope rash comprises of a violaceous erythematous rash with or without edema in a symmetrical distribution involving the periorbital skin. Sometimes this sign is quite subtle and may involve only a mild discoloration along the eyelid margin. At other times there may be massive edema that develops. A heliotrope rash is rarely observed in patients with lupus erythematosus and scleroderma. Gottron's papules are present over bony prominences, particularly the metacarpophalangeal joints, the proximal interphalangeal joints and/or the distal interphalangeal joints. They may also be found overlying the elbows, knees and/or feet. These lesions consist of slightly elevated, violaceous papules and plaques. There may be a slight scale on some occasions. Within the lesions there is often telangiectasia. These lesions may be clinically confused with lesions of SLE. Routine histopathological evaluation cannot reliably distinguish the cutaneous lesions of DM from those of LE.

Skin calcinosis is a disabling complication, most commonly evident in children or young adults. Hard yellow nodules can arise over bony prominences. The deposits of calcium may erupt onto the skin surface and be the sites of secondary infection.

1.3.2.3 Heart involvement
Clinically manifest heart problems are relatively infrequent in patients with PM or DM. Subclinical cardiac involvement is more common than manifest heart problems and the frequency varies depending on the methods used. ECG changes are most common and ECG abnormalities were observed in 32.5–72% of patients. ECG abnormalities observed in PM and DM patients include atrial and ventricular arrhythmias, bundle branch block, A-V blocks, high-grade heart block, prolongation of PR-intervals, ventricular premature beats, left atrial abnormality, abnormal Q-waves, as well as non-specific ST-T wave changes.

Whether these manifestations are more frequent than in an age and gender matched population is still uncertain in the absence of controlled studies.

1.3.2.4 GI-involvement
The most common GI symptom is dysphagia, caused by the weakness of the tongue, pharynx or esophagus and disordered esophageal motility. Esophageal dysfunction occurs in 15%-50% of patients.

1.3.2.5 Other extramuscular manifestations
Constitutional symptoms, such as fatigue and fever, often precede or accompany flares of disease and usually respond to glucocorticoids (GC). Weight loss may relate to impaired swallowing. Raynaud’s
phenomenon is rather common and could often be managed by calcium blockers. Joint symptoms which are non-erosive and involve both small and large joints can improve by treatment with GC.

1.4 EPIDEMIOLOGY

1.4.1 Polymyositis and dermatomyositis

The IIMs are rare disorders and the reports of incidence and prevalence are limited. The incidence of hospital diagnosed PM and DM according to the Bohan and Peter criteria in a US population in Allegheny County, Pennsylvania, between 1963 and 1982 was determined to be 5.5/million population [21]. An annual incidence of 7.6 cases/million population was estimated in a Swedish county-based study including definite or probable PM and DM and IBM cases [22]. In an Israeli study, a slightly lower incidence of 2.18/million population of PM and DM was reported [23]. The different incidences could depend on varying inclusion criteria as well as varying case retrieval strategies used in these studies, which makes comparisons difficult, but ethnic or geographical differences can not be excluded.

1.4.2 Juvenile dermatomyositis

An estimated incidence rate for juvenile IIM of 1.9 per million children under 16 years of age was reported from a nationwide study from the UK and Ireland [24]. Most of the juvenile cases were classified as JDM and only 3 of 51 cases were classified as PM.

1.4.3 sIBM

There have been few population-based studies in sIBM. The incidence of sIBM varies between different countries and ethnic groups: the incidence is low in Korean, African-American and Mesoamerican Mestizo [25] and in the Middle East compared with northern European, North American white and white Australian populations. An incidence of 2.2/million population was reported from Gothenburg in Sweden [26]. In a published nation-wide study the prevalence of sIBM was reported to 4.9 patients per million inhabitants in the Netherlands. When adjusted for age distribution the prevalence was 16/million inhabitants over 50 years old [27].

1.4.4 Age and gender aspects

There is a bimodal incidence pattern in DM and PM with one peak in childhood before the age of 20 and another adult peak between 55 and 69 years of age [21, 23]. Among children a peak at age 5-9 was reported [21]. Among PM and DM there is a female predominance in most epidemiological studies although this was most pronounced during childbearing age [21, 23]. The female-to-male incidence ratio was 2.2:1 [21], but during childbearing age this ratio increased to over 5:1 [21]. In juvenile DM, girls were also affected more often than were boys, with a female-to-male ratio of 5:1 [24].

1.4.5 The association between polymyositis/dermatomyositis and malignancy

An association between DM and malignancies was substantiated by a few large population-based studies from Scandinavian countries [26-30]. These three studies were all based upon identifying myositis cases using ICD codes from hospital discharge registers which were matched with national cancer registers. In order to estimate the risk to develop cancer the observed number of cancer cases among myositis patients was compared with the expected number, the Standardized incidence ratio (SIR) or relative risk. An increased risk of cancer was reported in DM patients in all three populations (Swedish, Danish and Finnish) that were investigated.
A meta-analysis of these studies demonstrated an increased risk of malignancies in DM patients that persisted after exclusion of the first year following DM diagnosis, SIR=4.4 although three of the individual studies had failed to demonstrate a significantly increased risk. A pooled analysis of the three Scandinavian studies further confirmed the increased risk of cancers in DM patients. This increased risk even persisted after more than 5 years of follow-up (SIR 1.4, 95%CI 1.0-2.0).

For PM patients the data are more uncertain. In the study by Sigurgeirsson the relative risk of cancer in PM was 1.5 in males and 1.4 in females in the Swedish population, and in the Danish study the SIR was 1.7.

The persisting increased risk to develop cancer after more than five years suggests a true association which could indicate that there could be a shared susceptibility, or alternatively that DM or its treatment could induce malignancies.

1.5 ETIOLOGY

By definition, the causes of the PM or DM are unknown. However, there is increasing evidence that genetic factors are involved in their development, although genetically predisposed individuals may only develop their myositis after environmental exposure to specific triggers. The rarity of myositis has precluded concordance studies in twins, but reports of mult case families support a familial predisposition. Candidate gene studies in non-familial IIM have suggested an association of HLA-DRB1*0301 and HLA-DQA1*0501 with IIMs in Caucasians, especially in patients with anti-aminoacyl transfer RNA (tRNA) synthetase antibodies and/or ILD. According to a recent study by Chinoy et al different immunogenetic profiles influence both clinical phenotype and the pattern of circulation myositis-specific autoantibodies.

Concerning sIBM, there is also evidence for genetic susceptibility. A strong association of sIBM with HLA-DR3 and the 8·1 MHC ancestral haplotype (defined by the alleles HLA-A1, B8, DRB3*0101, DRB1*0301, DQB1*0201) was first reported in patients from Western Australia.

The seasonal occurrence of disease onset and relapses was analyzed retrospectively in a group of 53 patients with treated DM or PM. In DM, the incidence of both myositic and cutaneous relapses was highest in the summer whereas in the PM group relapses were more evenly distributed throughout the seasons, although lowest in summer. The present findings suggest that environmental factors such as intermittent infections and light exposure may be involved in reactivating the disease process and causing relapses in DM, although this is less true for PM. That UV-light is a risk factor for onset of DM is supported by the correlation between UV radiation intensity and the relative proportion of DM, as well as the proportion of patients with anti-Mi-2 autoantibodies, where the DM or PM ratio is higher in geographical areas close to the equator than in Northern Europe. The association between UV light exposure and subtype of myositis suggests that UV light is an exogenous factor that could modify the clinical phenotype in two closely related diseases, PM and DM.

1.6 PATHOGENESIS

1.6.1 Immune mechanisms

The causes and pathogenesis of myositis remain unclear. The predominating symptom, muscle weakness, is shared by all subsets of IIMs, suggesting that some underlying pathogenic mechanisms are shared. There are also some distinct discrepancies; thus the muscle symptoms in PM or DM are characterized by weakness and decreased endurance in proximal muscles, often without prominent muscle atrophy, whereas in IBM the muscle weakness also commonly involves some distal muscles with atrophy of thigh and finger flexor muscles being frequently present. This suggests that some pathogenic mechanisms differ between the subsets. JDM resembles adult DM in muscle features, but
the prognosis is more favorable and many juvenile cases recover their muscle function, which is rare in adults.

Firstly, possible pathogenic mechanisms in adult DM or PM will be addressed. There is suggestive evidence that these diseases are secondary to an autoimmune process that could result in muscle injury. Evidence for both humoral and cellular mechanisms of muscle fiber and capillary injury has been proposed. Autoantibodies are frequently present in PM or DM but less often in IBM. The term ‘myositis-specific autoantibodies’ (MSA) has been applied to autoantibodies predominantly occurring in the serum of patients with PM or DM. They have usually included anti-Jo-1 and other autoantibodies to aminoacyl-tRNA synthetases (aaRS), anti-Mi-2, and anti-signal recognition particle (anti-SRP).

The role of autoantibodies in PM or DM in pathogenesis is not clear. CD8+ T cells and macrophages predominate in the endomysial infiltrates in PM and B cells and CD4+ helper T cells predominate in the perivascular regions in DM muscle biopsies, again suggesting some different pathogenic mechanisms in PM or DM. Capillary endothelial cells are an important constituent of the muscle microenvironment in myositis. The recirculation and homing of lymphocytes is accomplished via interaction with endothelial cell structures. A role of microvessels in the pathogenesis of DM was previously suggested by the observed loss of capillaries in muscle tissue. More recently severe morphological abnormalities in small blood vessels, not only in DM but also in PM, has been suggested by expression of adhesion molecules and proinflammatory cytokines such as interleukin (IL)-1α. The capillaries in patients with DM or PM are fewer in number and are larger in size than in normal muscle tissue. The expression of antigen-presenting (major histocompatibility (MHC) and adhesion molecules (intercellular cell adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1)) during inflammation suggests that these cells are capable of interacting with infiltrating T cells. These results suggest that capillary abnormalities are common in all inflammatory myopathies and may affect muscle function in these diseases.

The role of the invading T lymphocytes and macrophages in muscle tissue is also not yet clear. One important function of these cells is to produce effector molecules such as cytokines. These are signaling molecules, potent mediators of a number of cell functions and are essential in coordinating inflammatory responses. They can be produced by a large variety of cells and exhibit proinflammatory as well as anti-inflammatory effects, and they act in networks often in a hierarchical fashion. Their key role in chronic inflammatory diseases has been well documented by the often strikingly good response to therapies targeting proinflammatory cytokines. One of the best examples is tumor necrosis factor (TNF) blockade in patients with rheumatoid arthritis and Crohn's disease.

The role of cytokines in chronic IIM is less certain. IL-1α is one of the most consistently observed cytokines in muscle tissues of patients with IIM. IL-1α is mainly produced by activated macrophages but can also be expressed by other cells, including endothelial cells, and is considered as one of the central mediators of inflammatory reactions. IL-1α acts on target cells both directly and via induction of other cytokines, and its main biological activity is to promote proliferation of T and B cells and to induce upregulation of adhesion molecule expression on endothelial cells. The synthesis of IL-1α can be induced by several other cytokines including TNF, IFN-γ and IL-1α itself.

TNF is a proinflammatory cytokine which is mainly produced by macrophages, but it is also secreted by monocytes, neutrophils, T cells and NK cells. TNF exists in both a transmembrane bound and in a secreted mature form which can induce cytolysis of many cell types, and in immune responses it induces synthesis of IL-1α, stimulates phagocytosis and expression of MHC I and II on
lymphocytes. In addition, TNF can induce MHC class I expression and strongly upregulate ICAM-1 on human skeletal muscle cells. Production of TNF is influenced by various cytokines; thus its production is stimulated by IL-1 and IFN-γ but inhibited by IL-6. TNF gene expression was detected in muscle tissue from most patients with idiopathic inflammatory myopathies, but also occasionally in healthy control subjects. In muscle biopsies from myositis patients TNF was expressed by scattered inflammatory cells, in the cytoplasm of macrophages, in myonuclei in regenerating muscle fibers and was freely dispersed in endomysial and perimysial connective tissue. Several physiological functions in muscles have been associated with TNF, including in vitro reduction of membrane potentials, inhibition of myoblast proliferation, affects on glucose metabolism, and in vivo enhancement of protein breakdown. Furthermore, an excessive production of TNF which is known to act synergistically with IL-1α is believed to mediate muscle wasting by shifting protein metabolism toward net catabolism, suggested to result in a loss of body cell mass of predominantly skeletal muscle. This is apparent in severe infections and rheumatoid arthritis, and is also one of the characteristics for patients with myositis.

High mobility group box 1 (HMGB1) is a proinflammatory cytokine released into the circulation late during the course of endotoxemia, bacteremia and septic or hemorrhagic shock. HMGB1 can be detected in mammalian tissues and in all cell types. It is a DNA-binding protein that stabilizes nucleosomes, and it also stimulates gene transcription. In chronic inflammatory diseases HMGB1 has been suggested to participate by amplifying and extending the inflammatory cascade. In muscle tissues from patients with myositis, cytoplasmic HMGB1 expression could be detected in infiltrating mononuclear cells, vascular endothelial cells and in muscle fibers.

There are several interferons (IFNs) and they constitute an important part of the innate immune response against viral infections. The type I IFNs, IFN-α, IFN-β, have a broad antiviral spectrum but also exhibit anti-proliferative and immunomodulatory properties. They have been implicated as having an important role in specific T cell memory. IFN-α has the ability to induce MHC class I on muscle fibers. IFN-γ is distinct from the type I IFNs and is produced almost exclusively by activated NK cells and certain subpopulations of T lymphocytes. IFN-γ is a major inducer of MHC class I and II expression molecules in professional as well as non-professional antigen-presenting cells.

Despite some differences in the predominating inflammatory cell types in muscle tissues between PM or DM, the cytokine profiles have so far been determined to be similar, with a predominance of proinflammatory cytokines. Although the clinical manifestations differ between IBM and PM some features are shared in the muscle biopsies, namely the predomination of CD8+ T cells and macrophages surrounding muscle fibers which often appear non-necrotic. In addition, IBM is characterized by intranuclear inclusions containing amyloid β and several other Alzheimer-type proteins, and segmental loss of cytochrome c oxidase (COX) activity in muscle fibers. The role of inflammatory cells in IBM has not been clarified. The cytokine profile in muscle tissue of IBM patients resembles that of PM and DM. However, one distinct clinical feature is the limited or in most cases lack of clinical improvement of muscle strength after treatment with immunosuppressive drugs, and the inflammatory infiltrates may persist despite treatment with high doses of GC. These observations question the role of the immune system in the disease mechanisms in IBM and indicate that the inflammatory process may be a secondary phenomenon. As yet the role of cytokines such as TNF in myositis has not been clarified.

1.6.2 Non-immune mediated mechanisms

Treatment with immunosuppressive drugs reduces the amount of inflammatory cell infiltrates and leads to improved muscle performance in most patients with PM or DM. However, despite treatment with high doses of GC and other immunosuppressive drugs over long periods of time most patients experience persisting impaired muscle function, with decreased muscle strength and endurance. The mechanisms that cause the clinical symptoms have still not been clarified and could vary during different phases of the disease. There may even be different mechanisms underlying the clinical
muscle symptoms in PM, DM or sIBM. During the early phase of myositis muscle weakness may be severe without accompanying muscle atrophy, suggesting that other factors than loss of muscle mass are the cause of muscle weakness. During the later phases muscle atrophy and chronic damage of muscle tissue may be pronounced, particularly in sIBM, but muscle atrophy may still not be evident in the late phase. This suggests that other factors also contribute to muscle weakness in the later phases of myositis.

Muscle fiber degeneration or necrosis is a characteristic histopathological feature in patients with myositis and would affect muscle strength. However, there is a discrepancy between the degree of histopathological changes in muscle biopsies (including both muscle fiber degeneration or regeneration) on the one hand, and the degree of muscle weakness on the other hand. One possible explanation for the muscle weakness and the decreased muscle endurance, which are the main complaints of patients with PM, DM or sIBM, could be that molecules that are produced in the inflamed muscle, such as cytokines, could cause metabolic disturbances and affect muscle fiber function.

An alternative explanation to an altered muscle metabolism in patients with PM or DM is the development of a hypoxic condition in the inflamed muscle. Local hypoxia in muscle tissue could be induced both by impairment of the circulation to the muscle and by the inflammatory cell infiltrates, the latter being a well-known cause of tissue hypoxia, for example, in the synovial tissue in patients with rheumatoid arthritis (RA). One interesting observation from this perspective is the decreased number of capillaries in muscle tissues of patients with DM. Capillaries in muscle tissue are important for supporting the muscle cells with adequate nutrients, including oxygen. A reduced capillary density may lead to decreased tissue oxygenation, which might in turn contribute to metabolic abnormalities such as decreased Adenosine triphosphate (ATP) levels. A hypoxic condition in the muscle will change its metabolism from aerobic to anaerobic, making the muscle less fatigue resistant, and patients will experience reduced muscle endurance. That this could be the case in the muscle tissue of patients with DM was further supported by the decreased content of ATP and phosphocreatine observed by P-31 magnetic resonance spectroscopy (MRS). These aberrations were enhanced with exercise but improved with immunosuppressive treatment, the latter being associated with clinical improvement.

These changes in energy use support the notion of a metabolic cause that could at least partly explain the muscle weakness associated with DM. Although these metabolic disturbances have been less well investigated in patients with PM, the clinical muscle symptoms are similar to those in patients with DM and a metabolic disturbance in muscle tissue as increased resting inorganic phosphate/phosphocreatine levels has also been reported in patients with PM. Moreover, it has been observed that the capillaries in muscle tissue are affected in patients with PM. In both PM and DM, the endothelial cells in muscle tissue express molecules that indicate that they are activated, such as ICAM-1 and VCAM-1 and also the proinflammatory cytokine interleukin IL-1α. Furthermore, the endothelial cells of capillaries in both these conditions are thick and clumsy instead of normally being flat. Whether the number of capillaries is reduced in patients with PM was recently suggested but has not yet been confirmed. However, even with a normal number of capillaries it is likely that these thick endothelial cells could slow down the diffusion of nutrients, including oxygen, and affect the mitochondrial function of the muscle fibers, thereby causing muscle weakness and impaired muscle endurance. Another sign indicating mitochondrial dysfunction and metabolic disturbances in patients with inflammatory myopathies is the increased number of cytochrome C oxidase negative fibers (COX), which were frequent in patients with all three subsets of myositis; DM, PM or sIBM.

A main question is how we can induce reversion of hypoxia and metabolic disturbances in these patients. One hypothesis that we wanted to test in this thesis was whether physical training could be useful in this aspect in patients with PM or DM. In this context a report that six week’s endurance training in ten healthy, sedentary individuals, five women and five men, led to enhancement of the oxidative capacity in all muscle fiber types was encouraging. Thus the percentage of type I (Slow twitch) increased significantly (12%) in ten muscles during this training period.
1.7 TREATMENT
1.7.1 General
The primary aim for treatment of myositis is to improve muscle function. During the last twenty years patients with PM, DM or sIBM have been treated with different immunosuppressive drugs such as glucocorticoids (GC), often in combination with methotrexate (MTX), azathioprine (AZA), high-dose intravenous immunoglobulin (IVIg), cyclophosphamide (CyX), cyclosporin A (CyA) or mycophenylate mofetile. The results are not encouraging.

1.7.2 Treatment in polymyositis and dermatomyositis
1.7.2.1 Glucocorticoids (GC)
The general recommended treatment of PM or DM consists of GC in high doses for the initial few months, with or without other immunosuppressive therapies. Placebo-controlled trials of GC treatment have never been performed and the optimal initial dosage of GC, as well as duration of treatment, is therefore uncertain.

1.7.2.2 Other immunosuppressive treatment
Although most patients with PM or DM respond, at least partially, to GC, about 30% of patients do not respond at all. Moreover, in a long-term follow-up study, a substantial number of patients developed increased disability with time due to the side-effects of GC treatment. Several immunosuppressive agents have been reported both as steroid-sparing agents as well as being beneficial in patients who are steroid resistant, but few controlled therapeutic trials have been performed. AZA was reported to have a steroid-sparing effect as well as a favorable effect on clinical function compared to prednisone alone in a placebo-controlled trial. In another placebo-controlled trial high-dose IVIg was beneficial in treatment-resistant DM patients, both regarding muscle function and muscle histopathology. However, the effect was not sustained and the treatment had to be repeated. The molecular mechanisms of high dose IVIg in DM are not known. Although the clinical effects were accompanied by decreased ICAM-1 and MHC class I expression in repeat muscle biopsies, the number of patients that were subject to a repeat biopsy was small. The effect of IVIg in PM patients was less certain. High doses of IVIg are usually well tolerated, but a drawback of high dose IVIg treatment is the high costs for society.

A combination of oral MTX and AZA may benefit treatment-resistant PM or DM cases according to a published controlled trial. However, the study lacked power to compare the two treatment regimens directly. Another controlled trial was conducted by Vencovsky et al. They investigated effectiveness and tolerance of treatment with CyA or MTX added to GC in patients with severe, active PM and DM. Administration of MTX or CyA added to GC was associated with clinical and laboratory improvement. Changes in CK and IL-1Ra levels were not associated with parameters of clinical disease severity measured in this study. No patient recovered completely.

In addition to these controlled trials, open studies and case reports indicate a positive effect of MTX on muscle function in steroid-resistant cases PM or DM. In an open retrospective study, male myositis patients with Jo-1 antibodies with incomplete clinical response to CG were reported to have a more favorable clinical response to additional treatment with MTX compared to AZA. The effect of CyX in PM or DM is more controversial. In one open study some patients were reported to benefit from daily oral CyX.

There are few studies reporting varying effects of treatment with tumor necrosis factor (TNF) blockers in patients with PM or DM. Infliximab, a chimeric monoclonal antibody against TNF was used in two newly diagnosed patients with good initial effects. The long-term follow-up report of those cases was less encouraging. In a retrospective study in which patients with similar refractory PM or DM were treated with etanercept or infliximab, a favourable response was reported.
in 6 of 8 cases. Notably, the improvement was based on reduced serum CK levels as well as improved physician’s global assessment but not on objective clinical outcome measure and analysis of muscle biopsy. However, it cannot be excluded that etanercept, which was used in most of the patients that had improved, may have a more advantageous effect in myositis patients than does infliximab, but this still needs to be tested with validated outcome measures.

1.7.3 Treatment in sIBM
Results of several uncontrolled trials of GC have shown stabilization or temporary improvement in muscle strength in some patients; however, these improvements are not usually maintained. In a prospective trial of high-dose prednisolone for up to 12 months in eight patients with sIBM despite a fall in the serum CK concentrations muscle strength continued to deteriorate, and repeat muscle biopsies exhibited an increase in the numbers of vacuolated and amyloid-containing fibers, despite a reduction in the numbers of T cells.

In one randomized double-blind placebo-controlled study 44 patients with sIBM were treated with MTX. Quantitative muscle strength testing sum scores declined in treatment groups, -0.2% for MTX and -3.4% for placebo. There were no differences in MMT sum scores, activity scale scores and patients’ own assessments after treatment. CK decreased significantly in the MTX group. The conclusion was that oral MTX did not slow down progression of muscle weakness.

A pilot trial of the TNF-blocker etanercept did not yield an improvement in composite muscle strength scores at 6 months, although there was a slight improvement in grip strength after 12 months of treatment.

1.7.4 Non-pharmacological therapy
1.7.4.1 Exercise
It was more than 2000 years ago that Plato observed that ‘a lack of activity destroys the good conditions of every human being, while movement and methodical physical exercise save and preserve it’. About fifty years ago cardiologist Dr. Paul Dudley pointed out that almost any kind of exercise plays a role in maintaining health in patients with heart disease and there is increasing consensus that people of any age or fitness level can benefit from some type of aerobic exercise, whether it is running, walking, ballroom dancing, water aerobics, gardening, or any activity you choose.

Over the past decades considerable knowledge has accumulated concerning the significance of exercise in the treatment of a number of diseases, including diseases that do not primarily manifest as disorders of the locomotive apparatus. Nowadays, exercise is indicated in the treatment of a large number of medical disorders. In the medical world it is traditional to prescribe the evidence-based treatment known to be the most effective and entailing the fewest side-effects or risks. However, the evidence suggests that in selected cases exercise therapy is just as effective as medical treatment – and in special situations more effective – or adds to the medical effect. In this context exercise therapy does not represent a paradigm change – it is rather that the accumulated knowledge is now so extensive that it has to be implemented. Evidence exists today for prescribing exercise therapy in the treatment of metabolic syndrome-related disorders (type 2 diabetes, hypertension, obesity), heart and pulmonary diseases (chronic obstructive pulmonary disease, coronary heart disease, chronic heart failure), muscle, bone and joint diseases (osteoarthritis, rheumatoid arthritis, osteoporosis).

Thus the main question is in the present thesis is if there is any evidence for exercise as therapy in myositis? Despite the pronounced functional deficit in patients with myositis, non-pharmacological interventions have until recently received little attention. An important but controversial treatment of IIM is physical exercise. Because of the fear of causing disease flare-ups, patients with myositis have been recommended to avoid active exercise. Recent studies, however, have demonstrated that active exercise could be safely performed in patients with chronic, stable PM or DM without increased muscle inflammation as assessed by CK muscle biopsies and magnetic resonance imaging (MRI).
and with beneficial effects on muscle function. Beneficial effects on muscle strength after bicycle and step aerobic training for 6 weeks were reported in patients with chronic, inactive PM or DM \(^{99}\). Two studies \(^{100, 101}\) have confirmed the efficacy and safety of strength training and aerobic conditioning in patients with sIBM. The collective results of these studies demonstrate that exercise therapy can improve or stabilize muscle strength and functional ability without leading to an increase in serum CK concentrations or histological markers of disease activity.

The effects of exercise in addition to drug treatment in patients with active, recent-onset disease have only been investigated in a limited number of patients to date, although with beneficial effect on muscle strength and function and without signs of increased disease activity \(^{102}\). These studies indicate a positive effect of physical training on muscle performance in myositis patients, although the biological and the molecular mechanisms underlying this effect were not explored. In addition, there is also evidence that exercise can diminish the risk of osteoporosis fractures in elderly and postmenopausal women through several mechanism such as improved strength \(^{103}\) and balance, leading to decreased fall risk \(^{104}\).

1.7.5 TREATMENT SUMMARY

Based on the limitations of the currently available therapies there is an evident need for improved therapy in patients with IIMs. The fast growing development in molecular biology and immunology research will enhance the possibilities to achieve increased knowledge of key molecular pathways in different diseases, including IIMs. Such molecular knowledge is a prerequisite to develop new and more targeted therapies, such as cytokine blockers. Different approaches can be undertaken to acquire such knowledge of molecular disease mechanisms, these include \textit{in vitro} studies, studies in animal models and thirdly in clinical settings by using longitudinal studies in which careful clinical evaluation is combined with effects on molecular expression in the target organ of disease, such as muscle. In this context the IIMs are particularly interesting as the muscle is tissue that is relatively easy to sample and from which repeated tissue biopsies could be taken in out-patient clinics.

When I started my PhD studies there were only a few treatment studies in which investigations of repeated muscle biopsies had been included as an outcome measure. Indeed, very few studies had actually assessed the effects of a given therapy on muscle inflammation by investigating the histology of repeated muscle biopsies and correlated these findings to muscle function \(^{80, 105-108}\). Thus the molecular effect on muscle inflammation of most immunosuppressive drugs used today is largely unknown.

1.8 PROGNOSIS

The prognosis concerning mortality for patients with PM or DM has improved dramatically since introduction of GC treatment in the 1950s. Long-term survival data from five studies conducted over one or two decades report a five year survival rate of 65-95\% and 10-15 years survival rate of 53-85\% \(^{109-112}\). There seems to be no difference in survival rates between studies conducted during the last 20-30 years.

The remission rate varies between studies and with definitions of remission. Marie \textit{et al} reported remission of DM or PM in 40-77\% patients in France \(^{113}\). Those who achieved remission were younger and had a tendency toward a shorter period of time between onset of symptoms and diagnosis compared to patients who experienced relapse of disease. At an age of 50 years, myositis associated with cancer or lung involvement was predictive for a poorer prognosis \(^{110}\). Another recent study reported full remission in only 15\% of patients \(^{112}\). Despite the improved survival with the introduction of GC treatment, a majority of patients have persisting low muscle endurance and muscle weakness. There are still a number of patients that do not improve at all or only very slightly with
GCs and conventional immunosuppressive treatment, but the reason for this is unknown. Even more common is that patients who respond with partial improvement of muscle performance yet are left with persisting reduced muscle strength and endurance despite the absence of classical signs of inflammation. Why these patients are still weak is even more confusing.

Another clinically relevant problem is that currently used treatment is accompanied by frequent side-effects. One of the most common side-effects of long-term oral GC treatment is osteoporosis, which can lead to severe disability. As DM or PM mainly affect women in their middle age, who already have an increased risk of developing osteoporosis, much effort should be taken to minimize the use of GC treatment. Additionally GC has negative effects on muscles including development of muscle atrophy.
2 AIMS OF THE THESIS

The aim of this thesis has been to achieve an increased knowledge of disease mechanisms that are important in PM or DM. The overall approach to address the question of pathogenic mechanisms has been to use pharmalogical and non-pharmalogical therapies to study the effects on clinical outcome measures and to correlate these effects to effects on molecular expression in the target organ of inflammation in myositis, the skeletal muscle, by using repeated muscle biopsies. For this I have used traditional pharmacological therapy (high-dose IVIg) and a new biological treatment (targeting TNF) as well as non-pharmacological intervention, physical exercise. More specifically the aims were.

- To study effects of high-dose intravenous immunoglobulin (IVIg) treatment on molecular expression in muscle tissue of patients with chronic, active PM, DM or IBM
- To study effects of a TNF-blocking agent, infliximab, in patients with chronic, active PM, DM or IBM
- To study effects of endurance training on muscle fiber composition and muscle fiber cross-sectional area in patients with chronic PM or DM
- To study the effects of resistance exercise on gene expression in muscle tissue from patients with chronic PM or DM and how resistance training can influence genes signaling for inflammation and fibrosis
3 MATERIALS AND METHODS

The following section describes the main features of the methods used in the studies. Detailed protocols are described in the respective papers. All studies were approved by the ethical committee at the Karolinska Institutet/Karolinska University Hospital, Stockholm, Sweden. Study in paper IV was also approved by Children’s National Medical Center (CNMC), Washington, D.C. Institutional Review Board. All patients gave their informed consent to participate.

3.1 STUDY POPULATION (I-IV)

Most patients included in this thesis were recruited from the Rheumatology Unit at Karolinska University hospital, Stockholm. This is a nonselective referral center for the Stockholm area and nearby counties. Patients were diagnosed with definite or probable PM or DM according to the criteria of Bohan and Peter 8, 9. A few patients in cohort two in paper 2 were recruited from the Department of Neurology, Karolinska University hospital, Stockholm, from the Department of Medicine Västerås hospital, the Department of Medicine Karlstad hospital, or from the Department of Orthopedics, Danderyd’s hospital.

3.2 CLINICAL OUTCOME MEASURE

3.2.1 Myositis disease core set (II, IV)

An international collaboration, the international Myositis assessment and Clinical Studies (IMACS), 115 has reached a consensus on a three component outcome measure: myositis disease activity, myositis damage index and health-related quality of life.

- Disease activity core set
- Damage index
- SF-36

This outcome measure was developed for adult and pediatric patients with PM or DM. The core set consists of 6 domains: the patient’s and physician’s global assessment, the manual muscle test (MMT), the health assessment questionnaire (HAQ); assessment of serum levels of muscle enzymes (e.g. creatine kinase (CK), lactate dehydrogenase (LD)); and an extramuscular disease activity score. The extramuscular disease score includes disease activity in constitutional, cutaneous, articular, gastrointestinal, pulmonary, cardiac and muscular systems. It has two components: the myositis disease activity assessment visual analogue scale (MYOACT) and the myositis intension to treat index (MITAX). Disease activity core has partly been validated115. The myositis damage index is used to register persistent, irreversible changes in anatomy, pathology and function from patient’s history and by clinical examination that have been present for at least 6 months. For the third aspect, health-related questionnaire the generic questionnaire SF-36 was recommended116.

3.2.2 Assessing muscle impairment

3.2.2.1 Manual muscle test (MMT) (II, IV)

MMT for muscle impairment is based on the medical research Council scale and is the most frequently used measure of muscle strength in clinical trials 88, 92, 106, 117-119. The MMT has been used in trials to assess isometric muscle strength in myositis. However, the MMT could have limited measuring reliability in patients with mild impairment 120. There are different versions of the manual muscle test, and the 8-muscle group version is included in the disease activity core set proposed by IMACS 121. It has been partly validated and inter-rater reliability was good in six out of eight tested muscles in adult myositis patients 121.
3.2.2.2 Functional Index in myositis (FI) (I-IV)

The Functional Index in myositis (FI) was the first functional impairment outcome measure developed specifically for patients with PM and DM. The FI is based on repetitive movements involving selected muscle groups to capture decreased muscle endurance and was reliable and validated regarding its ability to discriminate patients from healthy individuals. The FI is useful in assessing patients with moderate to severe impairment. However, it is time-consuming, some tasks might be inadequate, and ceiling effects have been observed in patients with mild impairment. The revised FI, the FI-2 is validated and is a reliable disease-specific measure of impairment in patients with PM and DM. The FI-2 contains no redundant tasks, has no ceiling or floor effects, and possesses satisfactory construct validity and inter-rater and intra-rater reliability. It is not time-consuming to administer, is well-tolerated by patients with all stages of disease, and does not require any expensive equipment or formal training.

The content of the FI-2 represents measurement of muscle impairment of the upper and lower limbs and neck. This content was confirmed by extensive analysis of many previously-performed FI, as well as input by patients and health professionals with a variety of training and experience. The inclusion of shoulder flexion, shoulder abduction, hip flexion, step test, and head lift tasks in the FI-2 is in accordance with the disease phenotype of myositis. It has also been suggested that distal muscle groups may be involved in later stages of the disease, which supports the inclusion of heel and toe lift tasks. This also indicates that grip strength, although not included in the FI-2, is important to measure and our results support the reliability of the Grippit as a grip strength measure for patients with myositis.

3.2.2.3 Voluntary repetition maximum (VRM) (IV)

For evaluation of impairment 10 VRM, e.g. the weight permitting the performance of 10 correct repetitions, but not more, was carried out for five muscle groups; mm deltoideus on right and left sides, mm quadriceps on right and left sides, mm biceps/latissimus dorsi, the gastrocnemius muscles bilaterally, and the trunk muscles. The load of 10 VRM was approximately 70% of 1 VRM.

3.2.3 SF-36 (III)

A 36-item short-form (SF-36) is constructed to survey health status in the Medical Outcomes Study. The SF-36 is designed for use in clinical practice and research, health policy evaluations, and general population surveys. The SF-36 includes one multi-item scale that assesses eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. The survey is constructed for self-administration by persons 14 years of age and older, and for administration by a trained interviewer in person or by telephone.

3.2.4 PERFORMANCE tests (IV)

Standard indicators of physical performance were measured by maximal oxygen uptake per kilogram at peak exercise (VO\textsubscript{2, max}). Ventilation was measured by adding a mouthpiece to a SensorMedics Vmax 229. A standard incremental cycling was performed starting at a rate of 40W with a 10W increase work rate every 60 second until respiratory quote $>$1.1.
3.3 LABORATORY ASSESSMENT

3.3.1 Muscle BIOPSY (I-IV)

Of all the laboratory investigations methods muscle biopsy forms the golden standard for diagnosis. Obtaining a muscle specimen for histopathological study is an important component of the diagnostic evaluation for most suspected myopathies. Histopathological evaluation of muscle biopsies is also important to exclude other myopathies and to identify subsets of IIM. A muscle biopsy technique that allows for repeated biopsies is important to assess the effect of treatment at the tissue level. One such muscle biopsy technique is the percutaneous conchotome biopsy or the semi-open muscle biopsy. This method has become widely used in Sweden and other Scandinavian countries, including our rheumatology clinic, during the last 20 years for both diagnostic and research purposes, but has received little attention outside of Scandinavia. The percutaneous conchotome muscle biopsy technique gives a good size sample that allows for diagnostic evaluation and has a high yield in patients with myositis. It is a simple procedure, easy to learn and to perform, with a low complication rate and minimum discomfort for the patient. The method can preferably be used as a diagnostic tool and to perform repeated biopsies to assess the effect of a given therapy for both clinical and research purposes. The percutaneous conchotome muscle biopsy technique is a sensitive method to simply and safely make diagnostic evaluations and to assess the effect of a given therapy on muscle tissue in patients with idiopathic inflammatory myopathies. This semi-open biopsy technique can easily be performed by the rheumatologist in an out-patient clinic with a very low complication rate, and could be included as a tool for assessment of disease activity in clinical trials. The problem with ‘skip lesions’ could be reduced by taking several biopsies from the same incision. Another way to overcome this problem is to use MRI to select an appropriate site for muscle to be biopsied. Further knowledge of disease pathogenesis and the effect of treatment at a molecular level could be obtained by performing repeated muscle biopsies and by correlating histological findings to clinical outcome.

Various methods for sampling muscle tissue are available, and each has advantages and disadvantages. An open biopsy generally harvests the largest amount of tissue and can be performed on numerous muscles, but this technique is invasive and gives a large scar. Conversely, needle biopsy has become or is becoming the standard method of muscle biopsy at some institutions in North America, Europe and elsewhere in the world. Needle biopsy can be performed rapidly and is less invasive and less expensive, but the disadvantage is the smaller amounts of tissue per sample.

3.4 ANALYSES

3.4.1 Immunohistochemistry (I-IV)

Muscle biopsies were analyzed for routine purpose and research. In papers I, II and IV: T cells, macrophages, endothelial cells, adhesion molecules and MHC class I and II were analyzed using an immunohistochemistry (IHC) technique. Cytokine staining of the cross-sections was performed using anti-IL-1α, anti-IL-1β, anti-IL-1ra, anti-IL-RI, IL-1RII, anti-INF-γ, anti-TNF-α, anti-MXA (interferon inducible protein), anti-FOXp3 according to a modified IHC protocol. The CD31 (EN4) was used as an endothelial cell marker. In paper III the muscle biopsy cross sections were stained for different isoforms of myosin using monoclonal antibodies against fast, slow and neonatal myosin heavy chain (MHC) (MHC-f, MHCs, MHCn). The muscle biopsy cross sections were also stained with antibodies against intermediate filaments vimentin (VIM-V9) and anti CD56 (NCAM), a marker for muscle fiber regeneration. IHC assessment was performed by conventional microscope evaluation and computed image analysis.

3.4.2 Fiber type composition and fiber cross sectional area (I, III)

In studies I and III, the serial cross-sections of muscle tissue were analyzed enzymatically for fiber types I, IIA, IIB, and IIC with myosin ATPase (mATPase) staining. Three slides were used for each
patient and the sections were separately preincubated at pH 4.3, 4.6, and 10.4\textsuperscript{135-137}. The classification of muscle fiber types was based on their mATPase staining characteristics. Thus fibers with high content of acid-stable mATPase and low content of alkali-stable mATPase were termed type I (slow twitch) while fibers with opposite staining pattern were termed type II (fast twitch). Subtypes of type II were also observed by using different pH levels (4.3 and 4.6) for acid preincubation low content of alkali-stable ATPase\textsuperscript{135, 136}. The relative number of the different fiber types was determined after classification of the fiber types in approximately 500 fibers in each muscle tissue section. The results are presented as fiber type percentage of total amount of fibers counted in the biopsy section.

Cross-sectional muscle fiber areas of type I and II were established by calculating the mean value out of the individual area of 20 fibers of each fiber type per biopsy. This was achieved by applying computerized image analysis (Leica system) from a NADH-staining (nicotinomide adenine dinucleotide-tetrazolium reductase)\textsuperscript{138}.

3.4.3 Microarray (IV)

Microarrays represent a major advance in surveying tissues for qualitative and quantitative alterations in gene expression. Thousands of gene transcripts can be evaluated simultaneously and such gene profiles can be utilized to improve diagnostic procedures and to identify potentially important pathophysiological pathways. Investigations of paired samples taken from the same individual before and after specific interventions reduce some of the problems associated with biological variations between individuals. However, microarray analysis of mRNA data needs to be combined with protein expression analysis, to ensure that results from the microarray are biologically relevant.

Expression profiling was performed for each individual sample using Affymetrix microarrays following standard operating procedures and quality controls as reported (The Tumor Analysis Best Practices Working Group 2004). All processing and hybridization steps were carried out following the instructions provided by Affymetrix (www.Affymetrix.com). Briefly, total RNA was extracted from frozen muscle biopsies using TRIzol (Invitrogen, Carlsbad, CA), cleaned and concentrated using the RNeasy Minielute Kit (Qiagen, Santa Clara, CA), and quantified spectrophotometrically at 260nm. Following quantitation, RNA integrity was verified by agarose gel electrophoresis and 2 ug was used for cDNA synthesis. Double stranded cDNA was then subjected to a 16 hr \textit{in vitro} transcription at 37°C in the presence of biotin labeled nucleotides using a one round amplification strategy. The resulting biotin labeled cRNA was cleaned, fragmented and hybridized to whole genome Affymetrix U133 plus 2.0 arrays. Following overnight hybridizations at 45°C, microarrays were washed and stained on an Affymetrix Fluidics Station 450 and then scanned using Affymetrix GeneChip Scanner 3000, Figure 1.
Figure 1. Flash-frozen tissue (≈50 mg) is homogenized to isolate total RNA. Single-stranded (ss) cDNA and then double-stranded (ds) cDNA is made from ≈5 μg of total RNA. Double-stranded cDNA contains a T7 RNA-polymerase promoter adjacent to the 3’ polyA tail of each transcript. It is transcribed in vitro to generate more than 400 biotinylated cRNA molecules for each ds cDNA molecule. The biotinylated cRNA is fragmented and hybridized to the microarrays. Each transcript is queried by one or more probe sets of 11 perfect-match and 11 paired-mismatch oligonucleotides (the latter contain a centrally located point mutation as a form of hybridization specificity control). Currently available Affymetrix microarrays have ≈54,000 probe sets on each 1.28 cm² glass microarray (≈1.2 million 25-mer oligonucleotides on the HG-U133Plus 2.0 array). The biotinylated cRNA fragments hybridize to the appropriate oligonucleotide features. A laser scanner determines the amount of bound biotinylated cRNA indirectly through the streptavidin-conjugated phycoerythrin fluorescence at each feature within a probe set. The component probe pairs are interpreted and averaged to arrive at a single signal that reflects the relative abundance of the original mRNA. Probe sets are interpreted by any one of a number of probe-set algorithms, each providing a signal that reflects the relative hybridization intensity across the probe set. RT, reverse transcriptase.

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3.4.4 **RT-PCR (IV)**

Real-time quantitative RT-PCR (qRT-PCR) was performed to validate the expression of selected genes obtained with the microarray platform. Briefly, cDNA was synthesized from total RNA (100ng) from the same patient population using the cDNA archive kit (Applied Biosystems, Foster City, CA). Labeled primers (FAM) were added to the samples together with 12.5 ul of TaqMan Universal PCR Master Mix. Human GAPDH (GAPDH) (VIC labeled) was used as endogenous control. Reactions were performed on ABI PRISM 7900HT Sequence Detection System as recommended by manufacturers. All PCR amplifications were performed in triplicate multiplexed with the endogenous control primers. PCR quantitations were calculated using the $\Delta\Delta^Ct$ method as described previously by the manufacturer. This method is based on the difference in threshold cycles ($\Delta^Ct$) between the target gene and the endogenous control.

3.4.5 **Magnetic resonance image (MRI) (II)**

Noninvasive muscle MRI using short tau inversion recovery (STIR) as a method to suppress fat signals to clarify the presence of inflammation, or combined T1-weighted and T2 weighted images to distinguish between inflammation and damage with replacement of muscle with fibrosis or adipose tissue, is a useful diagnostic tool in clinical practice. Another advantage of MRI is that the examination covers an area containing several muscles. The sensitivity of this method compared with muscle biopsy is uncertain as no comparative studies have been carried out. Changes in MRI signaling in some longitudinal studies using immunosuppressive treatment have correlated with clinical improvement suggesting that it as a value in longitudinal studies. High cost of MRI investigation limits its usefulness.

3.5 **EXERCISE PROTOCOLS**

3.5.1 (III)

When developing the home exercise program used in exercise study in paper III, no previous well-described exercise program had been developed for patients with PM and DM. The program was designed to target affected muscle groups and also to be adjustable to different levels of muscle impairment.

Each patient was individually instructed to perform a standardized 15 minute home exercise program five days a week for 12 weeks. The program contained a warm-up by climbing up and down a stool, exercise for shoulder mobility and grip strength using a pulley apparatus, strength exercises for quadriceps and hip muscles, sit-ups without support of the neck and careful stretching. For those with small to moderate reduction of muscle functions determined by their functional index in myositis (FI) scores >38 per right and left side, the program included also exercises with weight (0.25-2 kg) and upper limb strengthening exercises. Furthermore, patients were instructed to supplement the exercise program with a 15 min walk at a self-selected speed.

Along with the exercise program the patients were given taped-recorded instructions with music and they were also instructed to write an exercise diary. The same physiotherapist instructed all patients, and also contacted the patients by telephone once a week during the 12-week exercise period.

3.5.2 (IV)

The exercise in study IV included resistance exercise with a well-defined load. The one-hour program started with a 10 minute warm-up either on a treadmill or an ergometer cycle at approximately 50% of the individual estimated maximal heart rate. Then exercises of shoulder flexor, the quadriceps, the latissimus dorsi/biceps, the gastrenemious and the abdominal muscles were performed with a load of
10 voluntary repetition maximum (VRM). The program ended by stretching of trapezius, quadriceps, and the pectoralis muscles. The patients exercised three days a week for seven weeks at the physical therapy department at Karolinska Hospital, figure 2.

7-week exercise program

![Images of exercise equipment and muscle groups]

Figure 2

3.6 STATISTICAL METHODS

3.6.1 Study (I)
Data were analysed using GraphPad Prism 4.0 statistical software (GraphPad Software Inc., San Diego CA). Friedmans test (multiple ANOVA) with Dunn’s Multiple Comparison Post Test was used to compare expression of the different molecules at the first biopsy and the two repeat biopsies. Non-parametric Wilcoxon’s signed rank test was used for pairwise comparisons. Spearman’s rank correlation was used to test for correlations. P values ≤0.05 were considered statistically significant.

3.6.2 Study (II)
Wilcoxon’s signed rank test was used to compare variables before and after infliximab treatment. Adjustment for multiple comparisons was made by the use of the Bonferroni correction method; p values ≤0.05 were considered statistically significant.

3.6.3 Study (III)
Values are stated as means ±SD. The p-values were accepted as statistically significant at p<0.05. Student group t-test and Mann Whitney was applied to test the difference between groups for the
muscle characteristic variables before training (basal). Wilcoxon Signed Rank test for paired observations was applied to test training response for the clinical variables FI and Physical Functioning in the patient group. Student t-test for paired observations was applied to test training response for the different fiber types, CD56 % and vimentin %. Regarding cross-sectional muscle fiber area a 2-factor ANOVA (fiber type and time), was applied to test the difference between fiber type in response to time. Single regression analyses were applied to determine the relationship between exercises induced changes in Physical Functioning versus exercise induced changes in proportion of type I fibers and type II cross-sectional muscle fiber area.

3.6.4 Study (IV)
The Wilcoxon signed rank procedure tested for within-group changes. The same non-parametric methods were also applied for investigating serum analysis as well as clinical outcome, MITAX, MMT, VRM 10-15. Concerning microarray statistical analysis was done using GeneSpring 7.2 (Silicon Genetics, Redwood City, CA). In order to reduce background noise, additional stringent quality control measures were done by filtering out probe set intensities close to 0 and using only the probe sets flagged as “present” in half of the samples (50% P calls) as described in the GeneSpring user manual. Sample normalizations included a “per chip” normalization (50\textsuperscript{th} percentile) and a paired sample normalization, in which each subject’s pre-training samples served as its own control. Statistical significance was determined using a paired T-test with at alpha level of 0.05 and a fold difference between pre-and post of 1.5 fold.
4 RESULTS AND DISCUSSION

4.1 LIMITED EFFECTS OF HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN (IVIg) TREATMENT ON MOLECULAR EXPRESSION IN MUSCLE TISSUE OF PATIENTS WITH INFLAMMATORY MYOPATHIES

High dose IVIg had successfully been used in patients with DM in a previous placebo-controlled study, and a few open studies suggested some beneficial effects in PM and sIBM. However, the mechanisms of actions of IVIg in inflammatory myopathies had not been clarified. In vitro studies suggested that IVIg could act by increasing IL-1Ra. IL-1Ra is an antagonist of the pro-inflammatory cytokine IL-1, which is one of the molecules that had been consistently detected in muscle tissue of all subsets of inflammatory myopathies. In addition, studies in vasculitis disorders where IVIg has proven to be beneficial have suggested that IVIg has an effect on blood vessels by reducing activation markers on endothelial cells. This could be another possible mechanism of action in patients with IIM. In order to investigate the mechanism of action of high dose IVIg in inflammatory myopathies we included thirteen treatment resistant patients, 6 with PM, 4 with DM, 2 with IBM, and 1 adult person with juvenile onset DM. They all had signs of persisting inflammatory active disease.

They were all treated with 2g/kg of IVIg 3 times with a monthly interval. Clinical evaluation as well as FI, CK-levels and muscle biopsies were performed before treatment and after the third IVIg infusion. Molecules were also studied in biopsies taken 24-48 hrs after the first infusion.

Improved muscle function as measured by FI was recorded in three patients (1PM, 1DM, 1 IBM) and CK-levels decreased by >50% in five out of nine individuals with elevated levels, but median CK levels were not changed. Skin rash improved in three of four DM patients. T cells, macrophages, MHC class I antigen on muscle fibers, IL-1, ICAM-1 and VCAM-1 expression and MAC-deposits on capillaries were present to an equal degree in biopsies before and after IVIg treatment. The relative frequency of muscle fiber type I and type II fibers was unchanged. No correlation was determined between the clinical response and molecular changes of inflammatory molecules or fiber type characteristics.

The clinical effects of high-dose IVIg on muscle function in patients with refractory inflammatory active myositis did not correspond with effects on any of the investigated molecules in our study. T cells, macrophages, phenotypical changes in muscle fibers and endothelial cell activation, including IL-1 α expression, were still present after treatment. Thus we could not explain the positive effects of IVIg treatment on the clinical parameters that we recorded in a few patients through changes in immunological molecules in muscle or muscle fiber characteristics. In contrast, in a previous study treatment with similar IVIg doses in adult DM cases muscle function improved and there was an increased number of capillaries, resolution of complement deposits on capillaries and a reduction in the expression of ICAM and MHC class I in muscle fibers in those with good clinical responses. The explanations for the discrepancy in outcome between this study and ours could probably be several but have not been clarified. One possibility could be the difference in patient selection, concurrent treatment or that in the previous study only a few cases were selected for repeated biopsy.

Our findings suggest that the effects of IVIg treatment in IIM are limited. Although the effect on muscle performance and on muscle tissue inflammation was limited, we still noticed positive effects of IVIg on skin rash, as has previously been reported. Thus severe skin rash could be an indication for treatment with IVIg in patients with DM where other types of medication have failed or were not tolerated. In our study we could not clarify the mechanisms of actions of high-dose IVIg in patients with IIM, and the clinical relevance of using IVIg to treat muscle symptoms and muscle inflammation was not revealed by our study.
4.2 A HIGH INCIDENCE OF DISEASE FLARES IN AN OPEN PILOT STUDY OF INFliximab IN PATIENTS WITH REFRACTORY INFLAMMATORY MYOPATHIES

Proinflammatory cytokines predominate in muscle tissues from patients with inflammatory myopathies, indicating their role in disease mechanisms in these disorders, the most consistently detected cytokines being IL-1α and IL-1β. At the time when we conducted our clinical trial, IL-1 blockade was not available for use in clinical practice. However, a TNF-blockade using monoclonal antibody, infliximab, was available and was known to induce IL-1 production. Infliximab treatment had also been successfully used in patients with RA and Crohn’s disease. In addition, there was one report of two new onset patients with DM who had been successfully treated with infliximab. This encouraged us to investigate the effects of infliximab in IIM patients who had not responded satisfactorily to conventional immunosuppressive treatment and still had signs of persisting inflammatory active disease.

Thirteen patients with PM, DM as defined by Bohan and Peter or IBM and with treatment-resistant disease were treated with TNF-blockade using four infliximab infusions (5mg/kg body weight) during a period of 14 weeks. Outcome measures included myositis disease activity score with improvement defined according to IMACS and MRI. Repeated muscles biopsies were investigated by immunohistochemistry for cellular infiltrates, MHC class I and II, TNF, IL-1α, IL-6, HMGB-1, IFN-γ, MxA, and MAC expression and were assessed by conventional microscopic evaluation and by digitalized computed tomography.

Nine patients completed the study. Three patients discontinued due to adverse events and one due to a discovered malignancy. Three of the completers improved by ≥ 20% in three or more variables of the disease activity core set, four were unchanged and two worsened ≥ 30%. No patient improved in muscle strength as assessed by the manual muscle test (MMT). At baseline two completers had signs of muscle inflammation by MRI, and five had at follow-up. T-lymphocytes, macrophages, cytokine expression, and MAC deposition in muscle biopsies were still present in the muscle tissues after treatment.

Infliximab was not an effective therapy in refractory inflammatory myopathies. Instead some patients flared during treatment. This made us stop the trial prematurely after having included thirteen instead of the planned fifteen cases. The beneficial effects were mainly apparent in arthritis score. There was no improvement in the muscle performance test. The explanations for lack of efficacy, in contrast to previous reports, could not be answered by our study. One difference was that our patients had established disease and had been resistant to treatment with conventional immunosuppressive therapies. In a retrospective study in which patients with similar refractory PM and DM were treated with TNF-blockade a favourable response was reported in 6 of 8 cases. Notably, the improvement was based on reduced serum CK levels as well as improved physician’s global assessment. No outcome measure was used that reflects muscle performance or patient perception. Another difference was that in this retrospective case series etanercept or infliximab was used, with most of the patients receiving etanercept. It cannot be excluded that etanercept may have a more advantageous effect in myositis patients than does infliximab, but this still needs to be tested with validated outcome measures. A limitation of our trial was the open uncontrolled design. Nonetheless, we believe that by using objective measures of muscle inflammation such as MRI and repeated muscle biopsies in addition to clinical outcome measures our results are reliable.

Not only did we fail to demonstrate any improved muscle performance, we even recorded flares with increased muscle inflammation recorded by MRI. Although we cannot exclude that worsening occurred by chance, we find it intriguing that the results are reminiscent of what was reported in some patients with multiple sclerosis who were treated with infliximab where an increased signal in MRI of the brain indicated increased tissue inflammation. From this perspective the case report of a RA patient who developed clinical myositis, anti-Jo-1 auto-antibodies, interstitial lung disease and muscle biopsy changes typical of myositis during treatment with infliximab is interesting. One suggested explanation for the increased inflammation seen in some conditions treated with TNF blockade is that blocking TNF may cause activation of T lymphocytes. However, despite careful assessment of
molecular expression that could indicate T lymphocyte activation (number of T cells, T cell proliferation marker Ki67, or IFN-γ production) in the muscle tissues of our patients, we could not confirm such a mechanism. There are also reports that suggest blocking TNF may activate the type I interferon system, but we could not determine an increased IFN-α activity, using MxA protein as a marker for IFN-α production in muscle tissue. Activation of T-lymphocytes could lead to autoantibody formation and, notably, three of our patients, two of whom had clinical worsening, developed ANA and/or cardiolipin IgM during the trial. Although the number of cases included was low, this could be support for immune activation through TNF blockade in these cases.

In summary, the absence of clinical improvement and the persisting inflammatory infiltrates in muscle tissue using anti-TNF treatment makes it unlikely that TNF is a key molecule in the disease mechanism in treatment-resistant myositis cases. In a clinical perspective, the radiological and clinical worsening evident in a several cases suggests that caution should be exercised with the use of infliximab in myositis patients.

4.3 THE EFFECT OF PHYSICAL TRAINING ON THE PROPORTION OF SLOW-TWITCH TYPE I MUSCLE FIBERS, A NOVEL NON-IMMUNE-MEDIATED MECHANISM FOR MUSCLE IMPAIRMENT IN POLYMYOSITIS OR DERMATOMYOSITIS

Patients with PM or DM are clinically characterized by muscle weakness and in particular low muscle endurance or muscle fatigue, mainly in proximal muscles. Most patients with PM or DM experience at least a partial improvement of muscle function through long-term treatment with GC and other immunosuppressive agents. The pathophysiological background to the sustained low muscle endurance and muscle weakness in patients with chronic PM or DM is not known. Based on the persisting clinical symptoms (low muscle endurance together with the persisting molecular changes in microvessels, increased IL-1 in endothelial cells and MHC class I expression in muscle fibers despite absence of inflammatory cell infiltrates), we hypothesized that these changes could be a consequence of tissue hypoxia. A support for the hypoxia hypothesis was the improved muscle performance that had been recorded after training. Muscle performance in healthy individuals is dependent on muscle fiber characteristics. Thus there is a strong relationship between skeletal muscle function and muscle fiber type composition and cross-sectional muscle fiber area. Importantly, these characteristics are affected by gender and age. Whether the persisting muscle impairment could be influenced by distorted muscle fiber characteristics in patients with chronic PM or DM was not known. We found no published data on muscle fiber characteristics in myositis patients where appropriate gender- and age-matched controls had been included.

Muscle fiber type composition and muscle fiber area were investigated by biochemical and IHC techniques in repeated muscle biopsies before and after a 12 week exercise program in nine patients with PM/DM who had increased their muscle performance by clinical outcome measure (FI). We also investigated the same variables in age- and sex-matched healthy controls. Muscle function was evaluated by FI in myositis and quality of life instrument by SF-36.

Before exercise the proportion of type I fibers was significantly lower (32±10%) and the proportion of type IIC fibers was significantly higher (3%±3) in the patients compared to in healthy controls. After exercise the percentage of fiber type I had increased significantly to 42±13% (p<0.05), and type IIC had decreased to 1%±1%. Fiber types IIA and B did not change. An exercise-induced increase of the mean fiber area by 20% was also observed in type II fibers. The functional capacity measured by the FI of myositis and the subscale of Physical Functioning of the SF-36 increased significantly. A positive correlation was determined between improved Physical Functioning and the proportion of type I fibers as well as with type II muscle fiber area (r=0.88; p<0.01, r=0.70; p<0.05).
The first striking and novel result was the low proportion of oxidative, slow twitch, type I fibers in chronic PM or DM patients compared to age- and gender-matched healthy individuals. A low proportion of oxidative, slow twitch, type I fibers has been demonstrated in sedentary individuals compared with physically active individuals. However, a sedentary lifestyle seems a less likely explanation, as low physical activity is usually accompanied by type II fiber atrophy which was not evident in our patients. Moreover, deconditioning by itself does not seem to be an explanation as fiber type composition in men with chronic heart failure was not affected in comparison to sedentary males when matched for aerobic capacity. GC are known to affect both fiber type composition and fiber area, and especially to cause type IIB fiber atrophy. All patients in our cohort had been treated with GC for several years, and some were still receiving GC treatment, although in low doses, at the time of study. A possible explanation for the low percentage of the oxidative type I fibers could be adaptation to muscle tissue hypoxia. This hypothesis is based on previous observations showing a reduced number of capillaries. Interestingly, a similar low relative proportion of type I fibers was reported in patients with chronic obstructive lung disease, another condition that gives rise to local tissue hypoxia. Further support for local hypoxia in muscle tissue is the demonstration of low levels of ATP and PCr in muscle observed by MRS in patients with DM or PM, indicating a metabolic dysfunction which could be a consequence of hypoxia.

The second novel observation was that the clinical improvement of a 12 week training of moderate intensity was reflected by a change in fiber type composition towards a more ‘normal’ pattern and increased fiber size area. This is first study in which improved muscle function in patients with PM or DM correlates to changes in muscle fiber characteristics. Similar significant changes have rarely been recorded after training in healthy individuals. Although the group of patients was small the data were remarkably consistent. One possible explanation for the improved muscle performance and changed fiber type composition could have been regeneration of type 1 fibers. However, this seems to be a less likely explanation according to our study as we did not find convincing evidence of muscle fiber regeneration. Another possible explanation for the improved function and the adaptation of muscle characteristics could be that exercise improved micro-circulation in the muscle, lowered total peripheral resistance and reduced skeletal muscle ischemia, such as has been reported as a result of exercise in patients with chronic heart failure. This could not be proven by our study and still needs to be investigated.

4.4 RESISTANCE EXERCISE REDUCES THE EXPRESSION OF INFLAMMATION AND FIBROSIS ASSOCIATED GENES IN AUTOIMMUNE MYOSITIS PATIENTS

The background for this study was the observed clinical improvement with increased muscle strength in a resistant exercise study in patients with chronic, stable PM or DM with low inflammatory disease activity. We wanted to determine effects of resistance training on gene expression in muscle tissue from myositis patients. The first question we wanted to address was if there were molecular correlates in muscle tissue that associated with the clinically improved muscle performance. Particularly, we were interested in genes that are regulated by hypoxia and are involved in metabolic processes.

Eight patients with PM or DM with stable condition underwent resistance training using a load of 10 VRM three times a week for seven weeks. Clinical outcome measures were used to define disease activity. Muscles biopsies were obtained from the vastus lateralis muscle before and after the last training session. RNA was hybridized to Affymetrix U133 gene chip arrays. Absolute expression values were calculated using MAS.5. Real-time quantitative RT-PCR was performed to validate the expression of selected genes. Changes of transcript on protein level were investigated by IHC.

The major finding in this study was that seven weeks of intensive resistance exercise induced down-regulation of genes that were associated with inflammation, both T cell and macrophage activation, and genes that down-regulate fibrosis in muscle tissue from patients with PM and DM. In
addition, genes involved in metabolism were also modulated by exercise, indicating a restoration of metabolic homeostasis. Overall, 265 gene transcripts were modified, of which 41 (15.5%) were associated with inflammation, 25 (9.4%) with fibrosis and 7 (2.6%) with metabolic regulation. The changes in gene expression detected following exercise were associated with decreased overall disease activity and clinically improved muscle performance. Additionally \( V_{\text{O2max}} \) improved significantly after training.

The pattern of reduced gene expression of genes involved in inflammation was consistent with almost all genes involved in inflammation being down-regulated (33 of 41). Consistent with this observation was the up-regulation of FOXP3 which is expressed in regulatory T-cells and is known to have a down-regulating effect on autoimmune reactions. Moreover, in agreement with this we observed a consistent pattern of down-regulation of genes involved in the fibrosis process (with a decreased expression of 22 out of 25). As increased fibrosis is often a late and natural consequence of chronic inflammation in many tissues, including muscle, reduction of genes involved in fibrosis may be a physiological consequence of reduced inflammation.

As mentioned above there has been a longstanding fear that exercise and training might worsen muscle inflammation. Although there is no hard data to support this, patients with IIMs have been advised to avoid training. Our study implies that exercise is not only safe and does not lead to increased inflammation but, on the contrary, could even reduce muscle inflammation. Such an effect of training is supported by a significant reduction in the local expression of inflammatory molecules including TNF in skeletal muscle after training in patients with chronic heart failure and in elderly men and women \(^{152}\). Additionally, exercise training in other chronic disease such as chronic obstructive pulmonary disease (COPD) and type 2 diabetes has been established. Many of these studies reveal reduced inflammation and improved endothelial function after intervention \(^{153, 154}\). There are few training studies that investigated inflammatory markers in the target organ, muscle, by microarray except one \(^{155}\). Radom-Aizik et al investigated the effect of 12 weeks' aerobic training in six patients with chronic obstructive pulmonary disease. Patients improved their aerobic capacity. Investigation of muscle (vastus lateralis) of six patients after training revealed up-regulation of several gene signalling related to energy pathways, oxidative stress and COX gene pathways.

This is the first exercise study in patients with PM or DM in which gene expression analysis was performed in the target organ. A weakness of our study is the limited number of patients and the absence of a control group. However, strength is the longitudinal approach, as each individual served as its own control, thereby reducing the effects of genetic heterogeneity. A few selected genes could be confirmed by analysing by qRT-PCR. We could also confirm signs of inflammation in muscle tissue by IHC, but could not reveal any significant changes at the protein level.

In conclusion, resistance exercise can restore muscle function in autoimmune myositis patients by reducing inflammation and fibrosis, the expression of pro-inflammatory and pro-fibrotic genes, and by restoring metabolic homeostasis.

**Overall discussion**

The IIMs are a somewhat heterogenous group of disorders, which is also reflected in their different responses to immunosuppressive therapies. I have used the Bohan and Peter criteria and the Griggs criteria for IBM to subgroup the patients included in this thesis. Although these are the most often used diagnostic criteria, there are some limitations as even the subgroups PM or DM are not always homogenous, as reflected by the difference in response to immunosuppressive treatment. Thus there is a need to revise classification criteria to enhance future studies of disease mechanisms. Of the interventions that we have used in the studies included in my thesis, training was clearly the most successful of them.
To function properly skeletal muscle tissue requires large quantities of energy, which is provided by hydrolysis of ATP. Most cellular energy is produced in mitochondria, and muscle fibers are rich in mitochondria. Oxygen supply is a major factor that limits the aerobic metabolism, which is the predominating metabolism for endurance work and fatigue resistance in muscle. Tissue hypoxia leads to anaerobic metabolism and accumulation of lactate in muscle tissue and muscle fatigue, a symptom that is common in patients with PM or DM. Available data support the hypothesis of muscle tissue hypoxia, which could lead to an acquired metabolic myopathy. Muscle tissue hypoxia in patients with myositis could be caused both by the local inflammation and by a reduced tissue perfusion. Based on this hypothesis, a therapeutic approach that includes both anti-inflammatory treatment and a stimulation of angiogenesis would be ideal. The anti-inflammatory therapy using immunosuppressive agents is already a standard treatment in these patients, but an even better improvement of muscle function might be achieved if the immunosuppressive treatment would be combined with some stimulator of angiogenesis. In healthy individuals exercise is a potent tool to improve muscle strength and function; it increases VEGF production in muscle tissue and induces increased capillarity.

Moderate exercise was recently demonstrated to be safe and beneficial in patients with PM and DM. Based on these studies and on the molecular expression in muscle tissue of patients with myositis, suggesting a local tissue hypoxia, it now seems appropriate to recommend combination therapy of immunosuppressives and moderate exercise. In our studies we have investigated the effects of training in patients during a stable, chronic phase of disease. Whether active physical exercise could be introduced during an early stage of treatment to diminish the risk of persisting, chronic muscle weakness is still unclear. In one open study training was introduced within the first 3 months after the immunosuppressive treatment had been started, without negative effects, indicating that training could safely be introduced early in combination with immunosuppressive pharmacological treatment. Whether early introduced training has positive effects on long-term outcome can only be addressed by a controlled trial. The optimal exercise program for patients with myositis still needs to be determined. My studies indicate positive effects of both endurance training which we implicated in study III, and of resistance training (study IV). Future recommendation may combine these two types of training. There are also pro-angiogenic drugs currently being tested in clinical trials which might be beneficial in patients with myositis. An improved understanding of the metabolic changes in muscle tissue in patients with PM and DM is likely to lead to new therapeutic possibilities and improved function in these patients.
5 CONCLUSIONS AND FUTURE PERSPECTIVE

Investigations of muscle biopsies in longitudinal studies proved to be useful to achieve information regarding the effects of different interventions on molecular expression in muscle tissue. In combination with careful clinical investigations I can make the following conclusions:

- High-dose intravenous immunoglobulin had limited effect on muscle performance and on molecular expression in muscle tissue of patients with inflammatory myopathies with active inflammation. The limited positive effect on muscle function did not correlate with immunological molecules in muscle.

- Treatment with infliximab was not an effective therapy in refractory inflammatory myopathies. We even observed clinical flares in some patients. Treatment with infliximab had no effect on immunological molecules in muscle tissue in these patients, suggesting that TNF is not a key molecule in the disease mechanisms of IIMs.

- Twelve weeks endurance training led to improved muscle function in patients with chronic PM or DM with low degree of inflammation and we could observe positive changes in muscle fiber characteristics which correlated to muscle function.

- Seven weeks resistant training had good effect on muscle function in patients with chronic, stable PM or DM. Improved muscle function was accompanied by reduced expression of genes signaling for inflammation and fibrosis and genes involved in metabolism were also modulated.

There is a subgroup of patients with PM or DM who has persisting inflammation that is resistant to conventional immunosuppressive treatment. High dose IVIg or TNF-blockade were not convincingly helpful in these cases and infliximab may even worsen disease. The key molecular pathways still need to be determined in these patients to facilitate development of new therapies. This is also true for patients with IBM, of which a few were included in my studies, also without significant improvement with the tested drugs.

In the larger group of PM or DM who respond to treatment with GC and other immunosuppressive drugs, the majority still had reduced muscle function after many years of treatment and also here additional information is need regarding disease mechanisms to improve their muscle function and health-related quality of life. In this context my two studies employing training regimes, study III and IV, have revealed important points: training was safe, and training could safely be used in combination with pharmacological immunosuppressive treatment. Furthermore, training induced structural changes in muscle which correlated to improved muscle function. These observations substantiate the hypothesis that chronic muscle inflammation may lead to an acquired metabolic myopathy and that this is not reverted by immunosuppressive treatment on its own, but that this could be positively affected by training. The most recent study even indicates that training could lead to diminished inflammation. This will need to be tested in a larger cohort in a controlled study design in which systemic effects of training could also be measured. These results also open up the challenging question of whether training could be effective on inflammation in the subgroup of patients with refractory disease. This could be a topic for a future study, preferably with both clinical outcome measures and analysis of molecular expression in muscle tissue. As myositis is a rare disorder this would have to be performed in a multi-center study design.

Our overall conclusion is that regular training in supervised form should be prescribed in combination with GC and other immunosuppressive drugs to patients with PM or DM who are in a stable phase of their disease. This is particularly interesting in the context that patients with PM or DM until recently were recommended to avoid active physical exercise due to fear of exacerbation of muscle inflammation.
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