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# INTERSTITIAL LUNG DISEASE IN POLYMYOSITIS AND DERMATOMYOSITIS

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**To my family**

# ABSTRACT

Polymyositis and dermatomyositis are rare disease entities affecting skeletal muscle and other organs such as the lungs. Interstitial lung disease (ILD) is increasingly recognized as a serious complication of poly-/dermatomyositis. The reported prevalence of pulmonary involvement varies widely due to use of different clinical, radiological, functional and pathological criteria. The etiology and pathogenesis of myositis as well as myositis-associated ILD are still unclear and it is not known when during the course of disease ILD develops. Infiltration of T cells and macrophages in the muscle tissue suggests an important role of T cell-mediated immunity in the pathogenesis of the diseases. It is still unknown which antigen these cells recognize and which cytokines are important in the inflammatory process. The role of autoantibodies in disease mechanisms of myositis is also not clear.

In order to establish the prevalence, characteristics and the course of myositis associated ILD, as well as putatively relevant pathogenetic factors we investigated an unselected group of patients with newly diagnosed poly-/dermatomyositis using chest radiography/ high resolution tomography and pulmonary function tests. Furthermore, we investigated T cell receptor (TCR) V gene usage in bronchoalveolar lavage (BAL), muscle biopsy and peripheral blood T cells by using T cell specific monoclonal antibodies. Moreover, we analyzed the relationship between presence of ILD-related autoantibodies, genotype and balance between serum levels of cytokines suggested to be involved in the disease (tumor necrosis factor (TNF), interleukin (IL)-10).

ILD, defined by radiographic changes and/or restrictive ventilatory impairment, was recorded in up to 79% of the patients. The number of patients with ILD had been even higher with the use of bronchoscopy and BAL, as alveolitis was also evident in patients without evidence of ILD through radiographic examinations or lung function tests. Arthritis and positive anti-Jo-1 antibodies were more common in ILD-patients than in patients without ILD. The course of myositis-associated ILD varied. In most cases pulmonary function tests stabilized, improved or even normalized after initiation of immunosuppressive therapy. A common targeted antigen in muscle and lung tissue was suggested by a restricted TCR BV gene usage in the lungs and muscle. The presence of anti-Jo-1 antibodies and anti-Ro52 antibodies was associated with higher TNF/IL-10 ratios in myositis patients and this ratio seemed to have a genetic basis, thus suggesting a role of genes as a predisposing factor for ILD.

In conclusion, ILD is a common manifestation of myositis. We propose that all newly diagnosed patients, regardless of pulmonary symptoms, should be screened for ILD by physical examination, chest radiographic examination, lung function tests and screening for anti-Jo-1 antibodies in order to identify patients with ILD early in the course of disease, when it is likely that the clinical course may improve by immunosuppressive treatment. Restricted TCR BV usage in the lungs and muscle make it important to include the lungs in the search for etiology of myositis. Patients with anti-Jo-1 antibodies have a high risk for developing ILD. This could be genetically determined, mediated through altered cytokine production. An increased knowledge

concerning the pathogenesis will hopefully make it possible to develop more selective therapies for myositis patients.

# LIST OF PUBLICATIONS

I. Fathi M, Dastmalchi M, Rasmussen E, Lundberg I.E, Tornling G
Interstitial lung disease, a common manifestation of newly diagnosed polymyositis and dermatomyositis
*Annals of Rheumatic Diseases 2004; 63:297- 301*

II. Fathi M, Vikgren J, Boijsen M, Tylen U, Jorfeldt L, Tornling G, Lundberg I.E
Interstitial lung disease in polymyositis and dermatomyositis; longitudinal evaluation by pulmonary function and radiology
*Submitted*

III. Englund P/Wahlström J, Fathi M, Rasmussen E, Grunewald J, Tornling G, Lundberg I.E
Restricted TCR BV gene usage in lungs and muscle tissue of patients with idiopathic inflammatory myopathies
*Arthritis & Rheumatism, In press*

IV. Hassan A.B, Fathi M, Dastmalchi M, Lundberg I.E, Padyukov L
Genetically determined imbalance between serum level of tumor necrosis factor (TNF) and interleukin (IL)-10 is associated with anti-Jo-1 and anti-Ro52 autoantibodies in patients with poly- and dermatomyositis
*Journal of Autoimmunity 2006; 27: 62-68*

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AV | Alpha chain variable |
| BAL | Bronchoalveolar lavage |
| BOOP | Bronchiolitis obliterans with organizing pneumonia |
| BV | Beta chain variable |
| CD | Cluster of differentiation |
| CK-19 | Cytokeratin 19 fragment |
| DAD | Diffuse alveolar damage |
| ELISA | Enzyme linked immunosorbent assay |
| FACS | Fluorescence-activated cell sorter |
| FEV1 | Forced expiratory volume in one second |
| FITC | Fluorescein isothiocyanate |
| HLA | Human leukocyte antigen |
| HRCT | High resolution computerized tomography |
| IBM | Inclusion body myositis |
| IIM | Idiopathic inflammatory myopathies |
| IL | Interleukin |
| ILD | Interstitial lung disease |
| KL-6 | Krebs von den Lungen-6 |
| MHC | Major histocompatibility complex |
| MICA | MHC class I-related chain genes |
| MSA | Myositis-specific antibodies |
| NSIP | Non-specific interstitial pneumonia |
| PBS | Phosphate buffer solution |
| PCR | Polymerase chain reaction |
| PMNC | Peripheral blood mononuclear cells |
| RPMI | Roswell Park Memorial Institute |
| TCR | T cell receptor |
| TGFß | Transforming growth factor-beta |
| TLC | Total lung capacity |
| TNF | Tumor necrosis factor |
| UIP | Usual interstitial pneumonia |
| VC | Vital capacity |

# 1 INTRODUCTION

## 1.1 HISTORY

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of muscle disorders with unknown aetiology. They are characterized by muscle weakness and by presence of inflammatory cell infiltrates in muscle tissue (Dalakas MC 1995, Plotz PH 1995). The main disorders defined within the group of IIM are polymyositis, dermatomyositis and inclusion body myositis (IBM) (Dalakas MC 1991). Wagner was the first who described a case as polymyositis in 1863 (Wagner E 1886), and Unverricht described the first patient with dermatomyositis in 1887 and used the term for first time in 1891 (Unverricht H 1891). IBM was described by Chou in 1967, and the term was introduced by Yunis and Samaha in 1971 (Chou S 1967, Yunis E 1971)

## 1.2 CLASSIFICATION AND DIAGNOSIS

The most commonly used criteria for diagnosis and classification of polymyositis and dermatomyositis are those proposed by Bohan and Peter (Bohan A 1975):

- Symmetric proximal muscle weakness
- Elevation of serum skeletal muscle enzymes, particularly creatine phosphokinase and often aldolase, serum glutamate oxalacetate, pyruvate transaminase and lactate dehydrogenase
- Electromyogram indicating the classic triad of muscular impairment, i.e. polyphasic short small motor unit potentials, fibrillation, positive sharp waves, increased insertional irritability, and repetitive high frequency discharges
- Muscle biopsy with typical histopathological findings i.e. degeneration, necrosis and/or regeneration with interstitial mononuclear infiltrates
- Characteristic cutaneous manifestation of dermatomysositis including heliotrope rash or Gottron's sign

The diagnosis of polymyositis 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 dermatomyositis is considered *definite* when three or four of these criteria (plus the rash) are met. With two criteria (plus the rash) the diagnosis is *probable*; and with one criterion (plus the rash) the diagnosis is *possible*. Bohan and Peter classified patients with myositis into five subclasses:

- Primary, idiopathic polymyositis
- Primary, idiopathic dermatomyositis
- Polymyositis/dermatomyositis associated with malignancy
- Childhood polymyositis/dermatomyositis
- Polymyositis/dermatomyositis associated with other defined connective tissue disease

Criteria for inclusion body myositis were proposed by Calabrese *et al* in 1987 (Calabrese LH 1987) and by Dalakas 1991 (Dalakas MC 1991). According to more recent criteria (Griggs RC 1995) *definite* IBM is established with diagnostic muscle

biopsy irrespective of other features. In contrast, if the muscle biopsy specimen fails to demonstrate the characteristics histology (i.e. invasion of mononuclear cells in non-necrotic fibres, vacuolated muscle fibres and intracellular amyloid deposit or 15-18 nm tubulofilaments) then the patient can still be diagnosed as having a *possible* inclusion body myositis by characteristic clinical and laboratory features.

The criteria of Bohan and Peter do not include a quantitative definition of elevated muscle enzymes in serum or a definition of the characteristic skin changes. Furthermore, they do not comprise a classification of IBM. However, these criteria are still generally accepted and used in both clinical practice and research. Since 1975 a few new proposals for the classification of myositis have been raised based on distinct clinical, histopathological and immunohistopathological criteria and the presence of so-called myositis-specific autoantibodies (MSA) (Love LA 1991, Targoff IN 1997), but these new classifications are still not generally used.

## 1.3 AUTOANTIBODIES

Autoantibodies are present in 66% of myositis patients (Love LA 1991). They are divided into two groups, myositis-specific autoantibodies (MSA) and myositis-associated autoantibodies (MAA).

MSA occur in 20-30% of myositis patients (Love LA 1991). These autoantibodies target ribonucleic acids (RNAs) involved in protein synthesis and are associated with certain clinical features, as well as with the course and prognosis of the disease, and can therefore be useful in diagnosis and classification of patients with myositis. The MSAs arise months before the onset of myositis and may disappear after complete remission of the disease (Love LA 1991). The MSAs may be divided into three groups.

The first group, the most common, comprises autoantibodies against one of the five aminoacyl-transfer-RNA synthetases which catalyse the binding of each amino acid to its tRNA (Love LA 1991). The presence of these antibodies is strongly associated with an acute disease onset and the so-called anti-synthetase syndrome comprising in myositis of, arthritis, mechanic hands, Raynaud's phenomenon and interstitial lung disease (Love LA 1991, Hausmanowa-Petrusewicz I 1997). Patients with anti-aminoacy-tRNA syntheatasis have a moderate response to therapy. Among the antisynthetase antibodies, anti-histidyl tRNA synthetase antibodies (anti-Jo-1 antibody) are the most common and have been detected in 20-30% of patients with myositis (Yoshida S 1983, Love LA 1991).

The second group of MSAs is called anti-signal recognition particles (anti-SRP). These autoantibodies are directed against a complex of six proteins and an RNA molecule that escorts newly synthetized protein to the endoplasmatic reticulum (Targoff IN 1990). The clinical features associated with these autoantibodies vary. In one study they were associated with a severe disease with very acute onset, high frequency of cardiac involvement, relative resistance to treatment and high mortality (Love LA 1991). In a more recent study they were associated with a subtype of myopathy often called immune-mediated necrotizing myopathy (Hengstman GJ 2006).

The third group of MSAs is known as anti-Mi-2 autoantibodies. These autoantibodies are directed against a nuclear protein of unknown function. Occurrence of this autoantibody type is associated with an acute onset of dermatomyositis with prominent skin changes which responds well to therapy and has a good prognosis (Love LA 1991, Hengstman GJ 2006).

In contrast to MSA, mysositis-asssosiated autoantibodies are frequently reported in patients without myositis. Among the MAA, antinuclear antibodies (ANA) are the most commonly detected antibodies. ANA is not specific but its occurrence may suggest an autoimmune disease. The frequency of ANA is highest in those with myositis overlap syndromes with other connective tissue diseases (Love LA 1991). Besides ANA and MSA, anti-Ro autoantibodies are the most common antibodies in myositis (Love LA 1991). Anti-Ro has been detected in up to 17% of myositis patients (Arnett FC 1996). Recent data reveal a high frequency of anti-Ro52 (25%), often without anti-Ro60, in patients with myositis (Frank MB 1999, Rutjes SA 1997). Anti-Ro52 has been found in 58% of patients with anti-Jo-1 antibodies (Rutjes SA 1997). For this reason anti-Ro52 can be valuable in diagnosis of myositis.
Other MAA such as anti-PM-Scl autoantibodies and anti-$U_1$RNP occur in a small percentage of myositis patients with characteristic overlap syndrome with features of systemic sclerosis and mixed connective tissue disease, respectively (Love LA 1991).

Why these autoantibodies (MSA, MAA) are produced is still unknown; their role in muscle tissue injury still remains to be elucidated.

## 1.4 ETIOLOGY

Little is known about the causes of myositis. However, for many years it has been suggested that hormonal, environmental exposures and genetic factors contribute to the onset of the IIM. The fact that women have a higher risk than men of developing myositis is a clear indication that sex hormones are involved. Environmental factors, such as different viral infections could possibly trigger the disease, although attempts to isolate viruses from muscle tissue have failed to date (Fox SA 1996, Leff RL 1992).

There is increasing evidence that genetic factors are involved in the development of poly-/dermatomyositis. Genetically predisposed persons may develop myositis after exposure to specific environmental triggers such as viruses and sunlight. Of the genetic factors studied in human diseases, human leukocyte antigens (HLA) are the best detailed. The HLA-types HLA DRB1 0301 and HLA DQA1 0501 alleles have been implicated to be susceptibility factors by association studies in Caucasian patients with polymyositis and dematomyositis (Love LA 1991, Friedman JM 1983, Hirsch TJ 1981). According to a recent study by Chinoy *et al* different immunogenetic profiles influence both clinical features and the pattern of circulating myositis-specific autoantibodies (Chinoy H 2006).

Although all these factors can be involved in development of the disease, the primary cause of myositis remains obscure.

## 1.5 PATHOGENESIS

The pathogenesis of myositis is still unclear. Clinical signs, histopathological features and autoantibody profiles suggest that different mechanisms may be involved in the development of different subsets of myositis. However, there is evidence that these mechanisms are autoimmune processes resulting in muscle injury. One of the hallmarks of myositis is inflammatory infiltrates in muscles with signs of muscle fiber damage, atrophy, degeneration and regeneration. Mixed exudates of T cells, B cells and macrophages identified by immunohistochemical analyses of inflammatory cells in myositis suggest that activated lymphocytes play a pathogenetic role (Arahata K 1984, Engel AG 1984). The different inflammatory myopathies have various proportions of lymphocytes at different locations. In dermatomyositis inflammatory infiltrates with a high proportion of $CD4^+$ T cells and macrophages are mainly localized to perivascular areas surrounding blood vessels and to the perimysium (Figure 1). By contrast, in polymyositis many non-necrotic myofibers are surrounded and invaded by $CD8^+$ lymphocytes and macrophages (Figure 2). Based on these findings it has been suggested that dermatomyositis and polymyositis have different immunopathological etiologies. The muscle fibers of patients with poly-/dermatomyositis have high levels of MHC class I (Nyberg P 2000). This has been observed both in muscle biopsies from patients with active inflammation and in muscle of patients without inflammatory activity. These data together with the presence of T cells in muscle tissue may suggest that T cells react against an antigen presented on the surface of muscle fibers. Despite extensive studies regarding the phenotype and localization of the inflammatory cells in polymyositis and dermatomyositis, it is still unknown which antigen these T cells recognize.

The role of myositis-specific autoantibodies in muscle injury is not clear. The occurrence of myositis-specific autoantibodies is associated with specific clinical characteristics. These antibodies can appear months before the onset of myositis and their levels reflect activity of the disease. Presence of anti-Jo-1 antibodies is strongly associated with the anti-synthetase syndrome. The fact that the anti-Jo-1 antibody specifically detected in polymyositis patients and that the level of circulating anti-Jo-1 antibodies changes with disease activity and therapy suggests that this antibody plays a significant role in the pathogenesis of polymyositis.

Recent data suggest that pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-$\alpha$) and interleukin (IL)-1 play important roles in the inflammatory processes of many autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (Farahat MN1993, Mitamura K1991). The role of different cytokines in poly-/dermatomyositis is not clear. There is no difference between poly- and dermatomyositis as regards the pattern of cytokines in muscle tissues. The most frequently reported in patients with myositis are the pro-inflammatory cytokines IL-1 and TNF-$\alpha$. Immunohistochemical studies of muscle biopsies have demonstrated IL-1 expression in mononuclear cells and endothelial cells and TNF-$\alpha$ positive macrophages and lymphocytes expressed in the endomysium and in the perivascular tissue in muscles from myositis patients (Lundberg IE1997, Tateyama M 1997). Increased expression of another cytokine, TGFß which has an anti-inflammatory effect and can stimulate fibrosis in muscle biopsies of patients with myositis has also been reported

(Lundberg IE 1997). These results suggest that several different cytokines are involved in the pathogenesis of mysositis.

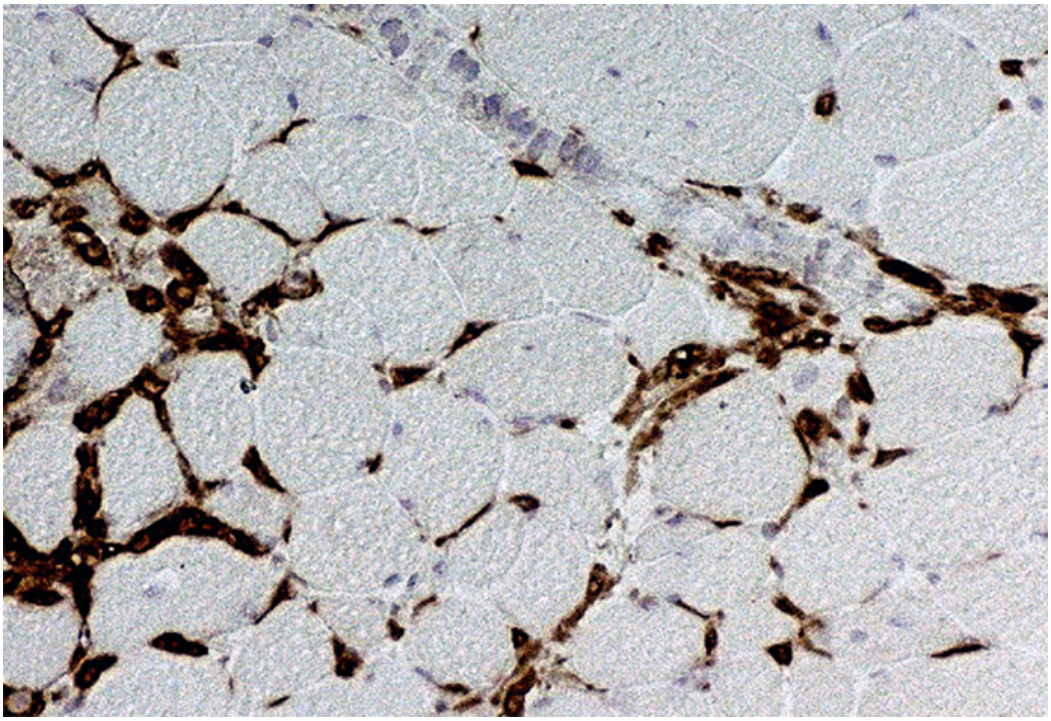

**Figure 1**. Immunohistochemical localization of $CD4^+$ T cells, visualized as brown staining in muscle tissue from a 66 year old man with dermatomyositis

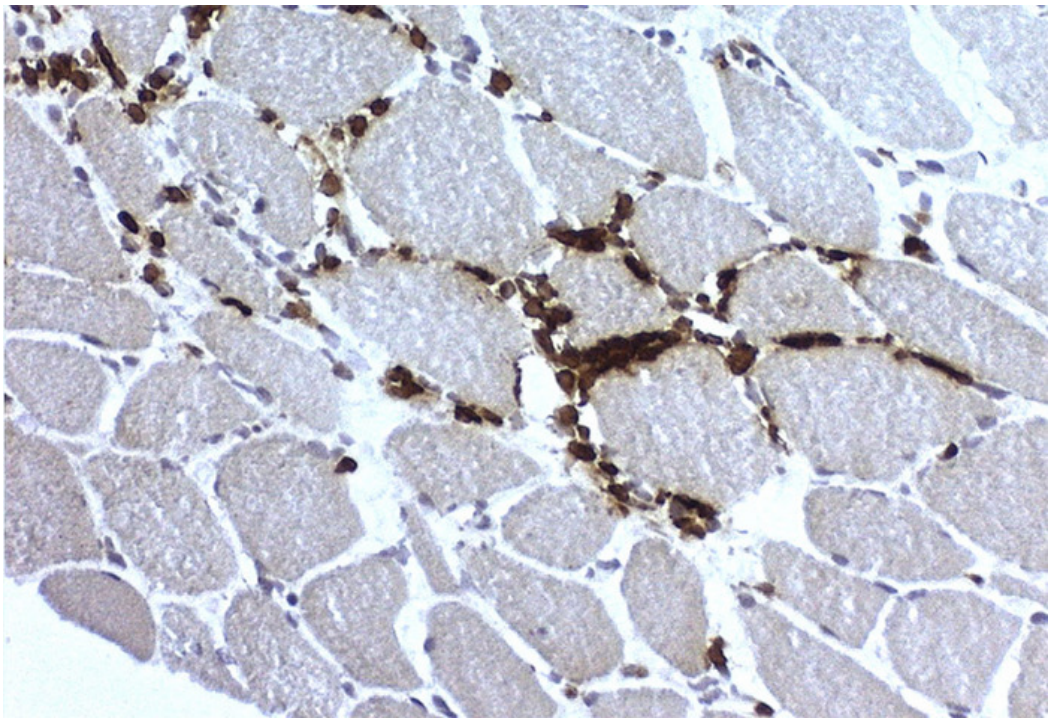

**Figure 2**. Immunohistochemical localization of $CD8^+$ T cells, visualized as brown staining in muscle tissue from a 56 year old woman with polymyositis

## 1.6 EPIDEMOLOGY

Polymyositis and dermatomyositis are rare disorders and the number of studies reporting incidence and prevalence is limited. The reported incidence of poly-/dermatomyositis varies between 4 and 10 cases per million population and year. The variation depends partly on which inclusion criteria have been used in the studies, but it also reflects ethnical and geographic differences (Oddis CV 1990, Medsger TA 1971, Hoschberg MC 1988, Hengstman GJ 2000, Weitoft T 1997, Kapipianinen-seppänen O 1996). Nevertheless, there are data suggesting that the incidence of myositis is increasing, which could be due to several factors, such as a higher referral rates to hospitals, an increased awareness and knowledge about the diseases and the increasing availability of laboratory analyses (Oddis CV 1990, Medsger TA 1971, Benbassat J 1980, Hoschberg MC 1988).

The incidence pattern of poly-/dermatomyositis is bimodal with one peak during childhood, and another peak between 55 and 69 years of age (Oddis CV 1990, Medsger TA 1970, Hoschberg MC 1988, Hengstman GJ 2000, Weitoft T 1997, Kapipianinen-seppänen O 1996). Poly-/dermatomyositis occur with a higher prevalence in women than in men with an approximate ratio of 2:1 (Oddis CV 1990, Medsger TA 1971, Benbassat J 1980, Hoschberg MC 1988). IBM is a more rare disease with incidence rates ranging from 2.2 to 4.9 cases per million population and year, with a male predominance (Lindberg C 1994, Badrising UA 2000).

## 1.7 CLINICAL MANIFESTATIONS

### 1.7.1 Skeletal muscle

The typical clinical feature of skeletal muscle involvement in poly-/dermatomyositis is proximal, often symmetric, muscle weakness that develops relatively slowly during weeks to months, but rarely acutely. Muscle tenderness and myalgia are frequent symptoms, but severe pain is uncommon. Patients usually report increasing difficulty with everyday activities which require the use of proximal muscles such as getting up from a chair, lifting objects and combing their hair. Fine motor skills, such as writing or buttoning a shirt are affected only late in the course of poly-/dermatomyositis. However, they may be impaired early in the course of inclusion body myositis. In advanced cases respiratory muscles may become affected. Dysphagia, regurgitation and even aspiration caused by involvement of the pharyngeal and oesophageal skeletal muscles may complicate the disease. Progressive and recurrent disease can result in atrophy and fibrosis of the affected muscles.

### 1.7.2 Systemic and cardiac manifestations

Extramuscular features are common in patients with poly- and dermatomyositis. Systemic features are fever, weight loss, Raynaud's phenomenon, non-erosive polyarthritis and Sjögren's syndrome. In addition, inflammatory processes in the cardiac muscles may result in dilated cardiomyopathy, congestive heart failure and arrhythmia.

### 1.7.3 Cutaneous manifestations

Patients with dermatomyositis are distinguished from those with polymyositis by the occurrence of skin rash. The most characteristic is purple discoloration of the eyelids (heliotrope rash) with periorbital oedema and Gottron's papules, which is a raised, erythematous dermatitis over the dorsum of the hands and especially the metacarpophalangeal and proximal interphalangeal joints. Erythema on the extensor surface of the knees, elbows as well as face, neck and upper torso is also common.

### 1.7.4 Pulmonary complications in myositis

Pulmonary complications are now recognized as important determinants of the clinical course of myositis. The lungs may be involved either primarily or as a complication of muscle weakness. In 1964, Hepper *et al* were among the first investigators to describe pulmonary complications of poly-/dermatomyositis. They considered aspiration pneumonia, ventilatory insufficiency and interstitial pneumonitis as the three distinct types of lung involvement associated with poly-/dermatomyositis (Hepper NG1964). The reported prevalence of pulmonary complication in poly-/dermatomyositis varies between 5 and 46% in cross-sectional studies depending on whether clinical, radiological, functional or pathological criteria are used (Dickey BF 1984, Benbassat J 1985, Frazier AR 1974, Salmeron G 1981, Tazelaar HD 1990, Marie I 1998). The actual frequency of pulmonary complication in unselected patients with myositis is unknown. Pulmonary complications are a major cause of morbidity and mortality in poly-/dermatomyositis (Winkelmann RK 1968, Dickey BF 1984, Benbassat J 1985, Marie I 2002). Thus the prevalence and signs are clinically relevant and need to be determined by means of sensitive techniques.

#### *1.7.4.1 Aspiration pneumonia*

Recurrent aspiration pneumonia has been considered to be the most frequent pulmonary complication of poly-/dermatomyositis, being reported in 5-20% of patients (Dickey BF 1984, Marie I 2005). As in other diseases causing diffuse muscle weakness, patients with myositis are prone to develop aspiration pneumonia due to pharyngeal dysfunction, but also due to impairment of the cough clearance mechanism (Donoghue FE 1960, O`Hara JM 1967). However, less than 50% of patients with aspiration pneumonia report some degree of dysphagia (Dickey BF 1984, Marie I 2005). Patients with dysphagia and aspiration pneumonia are considered to have a poor prognosis with high mortality because of the more extensive muscle and skin disease and require special attention (Medsger TA 1971).

#### *1.7.4.2 Hypoventilation*

Respiratory failure caused by hypoventilation has been reported in 5% of patients with polymyositis (Braun NM 1983). Hypoventilation, which is a result of inflammation of inspiratory and expiratory respiratory muscles, occurs in patients with severe generalized muscle weakness.

The cough reflex is diminished in patients with respiratory muscle weakness, and atelectasis and pneumonia may develop due to the inability to maximally inspire. Patients with respiratory muscle weakness have a restrictive pattern in pulmonary

function tests but lack evidence of ILD. The lung function tests demonstrate reduced lung volumes and maximal inspiratory and expiratory pressures, increased residual volume, but without decrease in the $FEV_1/VC$ ratio (Braun NM 1983). The chest radiograph reveals small lung volumes and basilar atelectasis with elevation of the diaphragm.

Although hypoventilation represents an unusual presenting manifestation of myositis, a few patients may present with progressive dyspnoea and acute respiratory failure, which may require intubation and mechanical ventilation.

#### *1.7.4.3 Interstitial lung disease*

In 1956, Mills and Matthews reported the first cases of ILD in association with dermatomyositis (Mills ES 1956). Since that original description, the association between ILD and poly-/dermatomyositis has become well recognized, and ILD is considered to be associated with high morbidity and mortality in patients with myositis (Marie I 2002, Dickey BF 1984, Benbassat J 1985). There has been no prospective cohort analysis of patients with poly-/dermatomyositis to determine the incidence of ILD. It is still unclear when during the course of myositis ILD develops. According to earlier cross-sectional studies the incidence of ILD in polymyositis and dermatomyositis is between 5 and 50%. The variability in the incidence may be due to lack of uniform diagnostic criteria, different stages of disease in which patients were studied, source of patient referral and sensitivity of the used diagnostic methods (Dickey BF 1984, Benbassat J 1985, Frazier AR 1974, Salmeron G 1981, Tazelaar HD 1990, Marie I 1998). It is likely that the reported incidence of ILD will increase with more frequent use of diagnostic methods such as high resolution computerized tomography (HRCT) and broncheoalveolar lavage (BAL), as has been reported in patients with other connective tissue diseases (Wallaert B 1986, Remy-Jardin M 1993).

The clinical manifestation of ILD in patients with polymyositis or dermatomyositis may vary from asymptomatic to severe, rapidly progressive dyspnoea with respiratory insufficiency and fatal outcome. Three different patterns of the ILD have been described based upon the clinical symptoms: 1) acute onset of symptoms; 2) chronic, slowly progressive symptoms; and 3) no pulmonary symptoms but abnormal chest radiographs or abnormal results of pulmonary function tests (Frazier AR 1974). As for other ILD the most common presentation is slowly progressive dyspnoea with or without cough, but ILD may also occur in patients lacking overt clinical symptoms (Marie I 2002). In a recent study, Fujisawa and co-workers found differences between polymyositis and dermatomyositis as regards the prognosis of ILD. Dermatomyositis-ILD had worse prognosis and was more often refractory to corticosteroid therapy than polymyositis-ILD (Fujisawa T 2005). An aggressive course of ILD, often with fatal outcome, is also reported in amyopathic dermatomyositis, a variant of dermatomyositis that is characterised by the typical skin rash but without any signs of myositis (Sakamoto N 2004, High WA 2003, Lee CS 2002).
The development of ILD does not seem to correlate with extent and severity of the myositis. In several studies arthritis has been identified as a risk factor for development of ILD in patients with myositis (Dickey BF 1984, Schumacher HR 1979, Marie I 1998). ILD may appear concomitantly with, before, or after the onset of the skin or

muscle manifestations of myositis (Marie I 2002, Schwarz MI 1998, Schnabel A 2003). There are even case reports of ILD and polymyositis or dermatomyositis "sine myositis" at presentation, in some cases with an acute onset, rapidly progressive ILD (Sakamoto N 2004, High WA 2003, Lee CS 2002).

Crackles are heard at physical examination, but digital clubbing is rarely evident in myositis patients.

Pulmonary function tests in patients with ILD typically reveal restrictive ventilatory defects with reduced lung volumes, diminished residual volume, forced expiratory volume in one second ($FEV_1$) with normal or elevated $FEV_1$/VC and a decreased diffusing capacity for carbon monoxide. However, occasional patients with ILD have normal results of pulmonary function tests. As in other diffuse ILD chest radiography, in particular serial examinations are useful both for screening and for detection of complications of ILD such as infections and pneumothorax. However, chest radiography is rarely sensitive enough to detect early changes. Normal chest radiographs has been confirmed in approximately 10% of the patients with biopsy-proven diffuse ILD (Epler GR 1978). HRCT is a more sensitive method that is widely used not only for detection of ILD but also for identifying the extent and severity of the disease as well as to discriminate between fibrotic disease and active inflammation of the lungs. The most common HRCT findings in myositis patients with lung involvement are irregular linear opacities with areas of consolidation and ground glass attenuation. Honeycombing is not a common finding in myositis patients (Bonnefoy O 2004, Mino M 1997, Arakawa H 2003, Douglas WW 2001) (Figure 3).

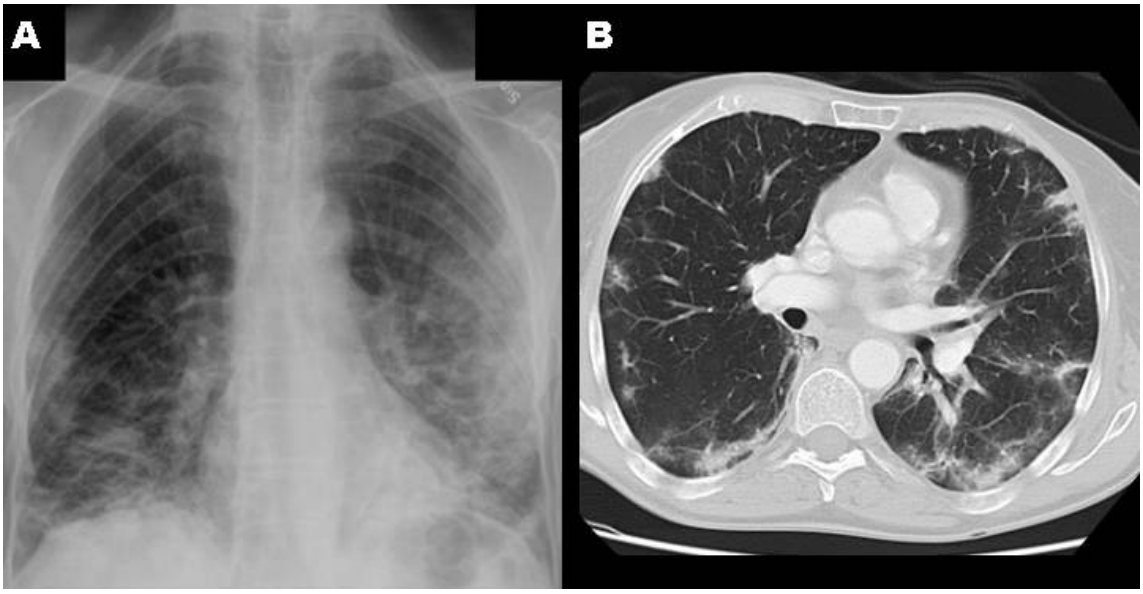


**Figure 3**. A. Chest radiograph of a 73 year old woman with polymyositis shows patchy bilateral areas of consolidation involving mainly the lower lobes. B. HRCT demonstrates that the consolidation involves predominately the subpleural lung regions.

Bronchoalveolar lavage (BAL) is a safe, non-invasive and generally well tolerated procedure. It is useful in identifying other causes of ILD such as infections, drug-induced pneumonitis and sarcoidosis. It may have a supportive role in the assessment of

disease activity and prognosis in myositis-patients with ILD. In idiopathic pulmonary fibrosis a high number of lymphocytes in BAL fluid represent a favourable outcome, while neutrophils and/or eosinophils in BAL fluid are associated with a poor prognosis (Haslam PL 1980). Resembling the idiopathic pulmonary fibrosis, poly-/dermatomyositis-associated ILD has a poor prognosis in patients with neutrophil-dominated alveolitis (Schnabel A 2003, Marie I 2002). In one study, patients with progressive ILD had neutrophils in BAL fluid and also tended to have a higher eosinophil count than patients with a non-progressive disease (Schnable A 2003).

Open lung biopsies are not routinely performed in myositis patients with evidence of ILD due to the potential morbidity associated with surgical lung biopsy (Tazelaar HD 1990) and the fact that knowledge of the specific histopathological lesion rarely changes the choice of therapy. The histopathological and radiological findings of ILD associated with myositis are identical to those in idiopathic pulmonary fibrosis. According to previous studies certain patterns of ILD apparent by HRCT correlate well with the findings of open lung biopsy (Muller NL 1987, Muller NL 1986, Nishimura K 1992, Wells AU 1992). A reticular pattern in HRCT of the lungs correlates with histological findings of fibrosis, whereas a ground-glass pattern suggests a reversible inflammatory disease and a better prognosis. Thus HRCT seems to reduce the prognostic value of lung biopsy and histopathology. In patients with poly-/dermatomyositis-associated ILD different histopathological patterns such as usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP), bronchiolitis obliterans with organizing pneumonia (BOOP) and diffuse alveolar damage (DAD) have been recognized (Tazelaar HD 1990, Marie I 2002, Douglas WW 2001, Tansey D 2004). Myositis-associated ILD tends to exhibit a mixture of histopathological patterns. In a retrospective review of 54 lung biopsies in 37 patients with a variety of connective tissue diseases, the most common pattern were non-specific interstitial pneumonia followed by organizing pneumonia (Tansey D 2004). Thirteen of those 37 patients had a diagnosis of poly-/dermatomyositis. In several biopsies there was more than one histopathological pattern of ILD (Tansey D 2004). Histopathology of lung biopsy has been considered to be of prognostic value in patients with ILD. Patients with BOOP and cellular NSIP tend to have a good response to treatment and the best prognosis. Patients with DAD have the worst prognosis with a high mortality rate while patients with UIP have an intermediate course (Tazalaar HD 1990, Marie I 2002).

The autoantibody pattern in patients with poly-/dermatomyositis is a valuable indicator of the risk of developing ILD. Several studies have reported that myositis-specific autoantibodies are associated with specific clinical features (Love LA 1991, Sauty A 1997). Anti-synthetase antibodies are the most frequent myositis-specific antibodies (Love LA 1991). Among the anti-synthetase antibodies, anti-histidyl tRNA synthetase antibodies (anti-Jo-1 antibody) are the most common and have been recorded in 20-30% of patients with myositis, most often in patients with polymyositis and occasionally in dermatomyositis patients (Hausmanowa-Petrusewicz I 1997, Yoshida S 1983). There is a strong association between occurrence of anti-Jo-1 antibodies and ILD. Anti-Jo-1 antibodies have been detected in 34 to 75% of myositis patients with ILD (Marie I 2002, Schnabel A 2003, Grau JM 1996). The presence of anti-Jo-1 antibody may occur months before the onset of symptomatic myositis (Nash P 1987).

The presence of anti-Jo-1 antibodies in a patient with myositis warrants a close pulmonary follow-up for early detection of ILD.

An elevated serum concentration of KL-6 (Krebs von den Lungen-6), a glycoprotein expressed on type II alveolar pneumocytes and bronchiolar epithelial cells, has been found to correlate with the presence and severity of ILD (Kubo M 2000, Bandoh S 2000). Furthermore, in adult patients with myositis, elevated level of serum KL-6 has been found to correlate with the presence of ILD, and with a decreased diffusing capacity of carbon monoxide and a reduced vital capacity (Kubo M 2000, Bandoh S 2000).

The level of surfactant protein D in serum has also been found to increase in patients with ILD associated with myositis, and the level was inversely correlated with vital capacity and diffusing capacity for carbon monoxide in those patients (Ihn H 2002).

Serum cytokeratin 19 fragment (CK-19), a structural component of bronchial epithelial cells, is also associated with presence of ILD and the activity of disease (Fujita j 1999). Patients who had ILD with histopathological evidence of DAD had a higher level of CK-19 than patients with myositis-associated NSIP.

Neither KL-6, surfactant protein D nor CK-19 analyses are used in clinical practice. These markers are interesting although not specific and they need to be studied over time in lager patient cohorts before they can be used as diagnostic or prognostic signs of myositis-associated ILD.

It has also been claimed that characteristic nail fold capillarscopic microangiopathy in poly-/dermatomyositis and digital infarcts in dermatomyositis suggest a severe pulmonary involvement and a poor prognosis (Tjiu JW 2004).

The natural history of myositis-associated ILD is not known. Well designed longitudinal studies are needed to define long-term clinical outcome of myositis patients with ILD. Careful clinical examination, autoantibody screening, chest radiography and pulmonary function tests are used for detection and diagnosis of myositis-associated ILD and these tools can also be used for assessing the course of the disease. Different histopathological patterns of myositis-ILD may indicate different pathogenetic mechanisms for development of ILD. It is not known if the pathogenesis of the different types of ILD in myositis is the same as in idiopathic pulmonary fibrosis or the mechanisms underlying myositis-associated ILD are the same as in the muscle or skin disease of these patients. It is likely that a complex of host- and disease-specific factors such as myositis-specific autoantibodies are involved in the initiation of the disease and determine its course in lungs, muscle tissues and skin.

## 1.8 TREATMENT

Most authors today recommend treatment of inflammatory myopathies with corticosteroids with or without other immunosuppressive agents. The optimal dosage and duration of treatment with corticosteroids is empiric as placebo-controlled trials of

corticosteroid treatment have never been performed. Most often the initial recommended therapy is prednisolone 0.75 mg/kg/day for 6-8 weeks and subsequent tapering depending on clinical and laboratory assessment. Approximately 30% of the patients with poly-/dermatomyositis do not respond to corticosteroid therapy (Henriksson KG 1982) and a large number of the patients experience side-effects of the treatment (Clarke AE 1995). A variety of drugs are available as corticosteroid-sparing regimens or to maintain remission, but the ideal choice is still unknown. Drugs such as azathioprine, methotrexate, cyclophosphamide and cyclosporine A have been used with different results. The reason why only a limited number of patients respond to these therapies is unknown.

The optimal treatment regime for patients with ILD associated with myositis is also not known. Despite advances in diagnostic techniques, therapy of myositis-associated ILD has remained empiric and controlled trials are lacking. Corticosteroids are often used as a first-line treatment of muscle inflammation and also for treatment of myositis-associated ILD. Also cyclophosphamide, azathioprine, methotrexate and cyclosporine A have been used for treatment of myositis-associated ILD refractory to corticosteroids (Marie I 1998, Marie I 2002, Douglas WW 2001, Cottin V 2003, Grau JM 1996, schnabel A 2003, Maeda K 1997, Nawata Y 1999). The results have been variable. Recent case series suggest that tacrolimus is effective in treatment of polymyositis-associated ILD (Selva-O,Callaghan A 2005, Takada K 2005), resulting in improvement of CT findings and lung function. TNF-α inhibitors are effective in the treatment of many rheumatic diseases, and there are a few studies demonstrating beneficial effects in myositis patients refractory to corticosteroids (Labioche I 2004, Efthimiou P 2006). TNF-α has also been used as induction therapy (Hengstman GJ 2004). It is not clear if any of the patients included in these studies had ILD. Notably, there are reports on a few patients with rheumatoid arthritis who developed a fatal ILD during treatment with TNF-α inhibitor (Ostor AJ 2004, Tengstrand B 2005). In a few cases where a combination of multiple drugs had failed, the disease was successfully controlled by autologous stem-cell transplantation, resulting in improvement of clinical symptoms and diminishment of radiographic changes (Bingham S 2001, Baron F 2000, Oryoji K 2005). Lung transplantation is also a therapeutic option for patients with end-stage lung disease.

Whether tacrolimus, TNF-α inhibitors or the above-mentioned immune-modulating agents may have a role in the treatment of myositis-associated ILD requires confirmation by prospective randomized trials. In addition, not only the choice of drugs, but also when during the course of the disease treatment is initiated might be significant for the outcome. The response rate may be higher if treatment is initiated early in the course of the disease, before irreversible changes have developed.

# 2 AIMS OF THE THESIS

The aims of the thesis were to

- estimate the prevalence and predictors of interstitial lung disease in newly diagnosed polymyositis and dermatomyositis

- estimate characteristics, predictors and long-term outcome of interstitial lung disease in polymyositis and dermatomyositis

- analyze the occurrence of antigen-specific T cell receptor usage in three different compartments, muscle tissue, lung, and peripheral blood which might be involved in patients with idiopathic inflammatory myopathies

- study relation between genetically predetermined production of cytokines and autoantibodies in myositis patients, with focus on development of ILD

# 3 MATERIALS AND METHODS

## 3.1 STUDY POPULATION (I-IV)

Patients included in studies I-III are consecutive patients with a recent onset of myositis who were identified between March 1998 and December 2002 at the Rheumatology Unit at Karolinska University Hospital, Stockholm. Patients with poly-/ dermatomyositis were diagnosed according to the criteria of Bohan and Peter, those with inclusion body myositis according to diagnostic criteria proposed by Griggs. Patients with other defined connective tissue diseases and malignancy were excluded.

In study IV, 65 patients with diagnosed polymyositis or dermatomyositis, according to the criteria of Bohan and Peter, were included. Of these, 15 patients also fulfilled classification criteria for other connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, mixed connective tissue disease, Sjögren´s syndrome). Data from non-related healthy individuals, matched for sex, age and ethnicity, were included for comparison.

All of the patients and healthy controls gave their informed consent and the studies were approved by the Local Ethics Committee at Karolinska University Hospital, Solna.

## 3.2 BRONCHOSCOPY, BRONCHEOALVEOLAR LAVAGE AND PREPARATION OF CELLS (III)

Broncheoalveolar lavage was performed with a fiberoptic flexible bronchoscope. After pre-medication with scopolamin or atropine in combination with morphine or petidine chloride, the mouth, pharynx and lower respiratory tract were anaesthetized topically with lignocaine solution. The bronchoscope was wedged in a middle lobe bronchus and sterile saline solution at 37 °C was instilled in five aliquots of 50 ml each. The fluid was gently suctioned back and collected in a siliconized bottle kept on ice.
The BAL fluid was then strained through a Dacrone net. After centrifuging at 400 G for 5 min at 4 °C, the supernatant was poured off. A total cell count was performed using a Bürker chamber. The viability was evaluated by trypan blue exclusion. A differential cell count was performed after preparation of a centrifuge smear.

## 3.3 PERIPHERAL BLOOD SAMPLES (III)

Blood samples were collected in heparinized tubes. Peripheral blood mononuclear cells (PMNC) were separated within 2 hours after sampling by Ficoll-Hypaque gradient centrifugation. Cells were thereafter washed in PBS and counted using a Bürker chamber, and re-suspended in PBS.

## 3.4 IMMUNOFLUORESCENCE AND FLOW CYTOMETRY (III)

Cells (BAL, PMNC) were incubated for 30 min with unlabeled TCR V-specific antibody, washed twice in RPMI and fluorescein isothiocyanate (FITC)-conjugated F (ab) fragments of rabbit anti-mouse immunoglobulin (Ig) were added for detection of bound antibodies. Normal mouse serum was added in order to block remaining rabbit anti-mouse Ig before adding anti-CD4-RPE-Cy5 and anti-CD8-PE conjugated antibodies. Labelled cells were fixed overnight in paraformaldehyde. Cells were analyzed using a FACSscan flow cytometer (Bectone-Dickinson, Mountain View, CA, USA).

## 3.5 MUSCLE BIOPSY (I-IV)

Biopsies were taken from the *vastus lateralis* muscle under local anaesthesia using a semi-open technique. Cryostat sections were mounted on glass slides and stored unfixed at –80 °C until immunostaining.

## 3.6 IMMUNOHISTOCHEMISTRY / IMMUNOFLUORESCENCE (III)

All TCR V monoclonal antibodies used for the FACS analysis were titrated on peripheral blood monocytes and on tonsil sections to find preferable fixation and optimal concentrations for immunostaining. Optimal staining results were accomplished with fixation in acetone or formaldehyde, respectively. Staining with monoclonal anti-CD3, anti-CD4 and anti-CD8 antibodies was included to determine T cell phenotype.
Double-immunofluorescence labelling was used to determine the phenotype of T cells expressing BV3 TCR.

## 3.7 GENETIC ANALYSIS (III, IV)

HLA-DR was typed by PCR amplification with sequence-specific primers (PCR-SSP). The DNA was extracted from peripheral blood leucocytes using the salting-out technique. The presence or absence of PCR products was visualized by agarose gel electrophoresis. The gels were examined under ultraviolet illumination and documented by photography.
Analysis of G-1087A IL10, G-308A TNFA and G915C TGFB1 gene polymorphisms as well TNFα and MICA microsatellites were performed as described previously (Padyukov L 2001, Hassan AB 2003).

## 3.8 CYTOKINE SERUM LEVELS (IV)

Commercial ELISA kits (human TNF-α/IL-10 Quantikine High Sensivity Kit, R&D System) were used for the quantitative determination of IL-10 and TNF in sera. The minimum detectable concentration for TNF was 0.12 pg/ml and for IL-10 less than 0.5 pg/ml.

# 4 RESULTS AND DISCUSSION

## 4.1 INTERSTITIAL LUNG DISEASE, A COMMON MANIFESTATION OF NEWLY DIAGNOSED POLYMYOSITIS AND DERMATOMYOSITIS (I)

In a prospective study, 17 consecutive patients with newly diagnosed polymyositis or dermatomyositis, regardless of clinical symptoms of pulmonary disease, were investigated with chest radiography/HRCT, pulmonary function tests and biochemical and autoantibody analysis. Eleven of these patients (65%) were diagnosed with ILD at the onset of myositis. The diagnosis of ILD was based on chest radiography/HRCT and/or pulmonary function tests. Cough and dyspnoea were the most reported symptoms, although ILD also occurred in patients without any overt clinical signs of pulmonary involvement. However, a majority of the patients without objective signs of ILD also reported respiratory symptoms. ILD was more common in men than in women. Arthritis and anti-Jo-1 antibody were evident more often in patients with ILD than in those without. There were no statistically significant differences in serum levels of muscle enzymes and the prevalence of autoantibodies other than anti-Jo-1 antibodies between patients with ILD and those without.

ILD frequency was higher in our study compared to in previous studies, despite its restriction to newly diagnosed cases and exclusion of patients with cancer and overlap syndromes (Dickey BF 1984, Benbassat J 1985, Frazier AR 1974, Marie I 1998). The high incidence of ILD in this study could be due to the systematic use of sensitive detection methods such as HRCT and pulmonary function tests. Moreover, all newly diagnosed myositis patients were investigated regardless of clinical lung symptoms, and some asymptomatic patients were detected with signs of ILD using these methods. It is unclear if this group of patients with subclinical ILD will eventually develop clinically significant ILD. Long-term prospective follow-up studies are needed to evaluate the relevance of subclinical ILD in myositis patients.

The present study confirms that there is an association between presence of ILD, arthritis, and anti-Jo-1 antibody (Dickey BF 1984, Marie I 1998, Yoshida S 1983, Schumacher HR 1979, Hoschberg MC 1984). In contrast to previous studies all of our patients with positive anti-Jo-1 antibodies had ILD (Hoschberg MC 1984, Marie I 1998), which could be due to systematic use of HRCT in the diagnosis of ILD. There was a gender difference in the presence of ILD in our patients. This result should be interpreted with caution due to the limited number of patients. Larger study groups are needed to confirm this result.

In conclusion, ILD was frequently evident at the time of diagnosis of myositis and it was not related to the presence of respiratory symptoms such as cough or dyspnoea. We used radiographic examinations and lung function tests for diagnosis of ILD. The reported incidence of ILD will probably increase further with increasing use of other sensitive diagnostic methods such as bronchoalveolar lavage. As ILD is regarded as an unfavourable prognostic factor of myositis, and since the diagnosis of ILD might affect the choice of treatment and monitoring, chest radiograph/HRCT, pulmonary function

tests and analysis of anti-Jo-1 antibodies should be included in the initial investigation of patients with myositis.

## 4.2 INTERSTITIAL LUNG DISEASE IN POLYMYOSITIS AND DERMATOMYOSITIS – LONGITUDINAL EVALUATION BY PULMONARY FUNCTION AND RADIOLOGY (II)

In a prospective study, 23 consecutive patients with newly diagnosed polymyositis or dermatomyositis, regardless of pulmonary symptoms, were investigated with repeated chest radiography, HRCT, pulmonary function tests, biochemical and autoantibody analyses. The activity of the disease and the patient's functional status (according to the physicians' global assessment) was extracted from medical charts. Clinical, radiological, and lung function outcome of each patient was determined based on the last follow-up results. Mean follow-up was 35 months. ILD was diagnosed in 19 patients (79%) on the first evaluation. Patients with ILD had lower values in lung function tests, higher radiological scores, and higher serum creatine kinase levels than those without ILD. During the follow-up period, improvement of TLC was observed in 6/18 (33%) patients, TLC remaining stable in 7/18 (39%) patients and deteriorating in five (28%) patients. Those who improved in lung capacity had a lower $FEV_1$, TLC and residual volume at the initial examination; otherwise there were no differences in initial clinical features between those who improved, remained stable or deteriorated in TLC. Only one patient with isolated reduction of diffusion capacity for carbon monoxide at first examination diminished in TLC to less that 80% of predicted value during the follow-up period. Changes in pulmonary function tests only partially correlated with HRCT score and pattern. Septal lines, ground-glass opacities and peribronchovascular thickening were the most common HRCT abnormalities at the initial and follow-up examinations. The abnormalities of HRCT persisted even after normalizing of pulmonary function.

In the present study the incidence of ILD was higher than in previous studies (Dickey BF 1984, Benbassat J 1985, Frazier AR 1974, Marie I 1998). ILD was in most cases mild, chronic and non-progressive. It does not seem to be possible to predict the course of ILD on the basis of the first examination. Thus all patients need careful evaluation of lung function tests and radiological features during follow-up. Patients with a normal lung function early in the course of the disease seem to have a greater likelihood of maintaining their lung functions if the therapy successfully controls extra-pulmonary manifestations of the disease. In this study there was a good response to immunosuppressive treatment in many cases. Normalized pulmonary function was observed particularly in patients who had received a combination of corticosteroids and other immunosuppressive drugs at an early stage of the disease. Our study was not designed as a treatment intervention trial. Different drugs were used in our patients and changes in therapy were made during follow-up when clinically indicated according to the physicians' decisions. For this reason different treatment effects could not be evaluated. Future treatment intervention trials are needed to determine potential differences in these therapies.

During treatment with immunosuppressive agents signs of ILD on HRCT may remain despite remission of muscle involvement and improvement of lung function. This might be due to technical factors; even HRCT cannot distinguish between alveolitis and very fine fibrosis. Another reason could be that the pathogenetic mechanisms underlying muscle symptoms and ILD might not always be the same.

Although presence of anti-Jo-1 antibodies is associated with ILD it did not correlate with greater pulmonary function loss over time. In contrast, anti-Jo-1 antibodies were only detected in those patients who improved or remained stable and in none of those who deteriorated, although this difference was not statistically significant. This may suggest that anti-Jo-1 antibodies is not only a predictor of ILD but may also have a prognostic value. Prospective studies with a higher number of patients with or without anti-Jo-1 antibodies are needed to allow a definite conclusion.

## 4.3 RESTRICTED TCR BV GENE USAGE IN LUNGS AND MUSCLE TISSUE OF PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES (III)

Nine patients with recent onset of poly-/dermatomysositis or inclusion body myositis, regardless of clinical symptoms that might indicate lung disease, underwent bronchoscopy with bronchoalveolar lavage in addition to muscle biopsy and blood sampling. A panel of 19 TCR beta chain variable (BV)- and alpha chain variable (AV)-specific monoclonal antibodies were used to characterize the TCR profile in $CD4^+$ and $CD8^+$ T cell populations in BAL fluid and peripheral blood by flow cytometry. Muscle biopsies were analyzed by immunohistochemistry and immunofluorescence. The patients were also analyzed for HLA-DRB1 and DRB3 alleles. A total of 17 T cell expansions were detected in BAL fluid, six in the $CD4^+$ T cell population and 11 in the $CD8^+$ T cell population. Four T cell expansions were detected in peripheral blood. Two polymyositis patients who had BAL fluid $BV3^+$ T cell expansions in the $CD4^+$ population, and in whom BV3 was also a prominent TCR V segment in muscle tissue, shared the HLA-DRB1*03 allele and were the only anti-Jo-1 positive patients.

The etiology and pathogenesis of myositis and myositis-associated ILD remains unclear. Affected muscle and lung tissue is characterized histologically by the presence of lymphocytes and macrophages. It is not known which antigen these cells react to. As myositis is a multiorgan disease it is reasonable to presume that the antigen is not only presented on the muscle surface but also in other involved organs such as the lungs. The lungs are chronically exposed to antigens. Inhaled antigens reach the bronchial mucosa at the upper and lower respiratory tract depending on the size of the particles. Macrophages phagocytose antigens and present them to T cells that become activated, clonally expanded and which produce different cytokines. Determining which T cells become activated would not only give us the possibility to understand the pathogenesis of myositis, but also to develop more targeted immunotherapies.

In the present study we have determined a restricted accumulation of T cells expressing selected gene segments in the target organ compartments. The presence of TCR BV3 in

muscle and lungs of patients with myositis in combination with the presence of the DRB1*03 allele and anti-Jo-1 antibodies might suggest a common target antigen in these organs. It might thus be speculated whether the immunological reactions leading to inflammatory muscle diseases are initiated in the lungs.

## 4.4 GENETICALLY DETERMINED IMBALANCE BETWEEN SERUM LEVELS OF TUMOR NECROSIS FACTOR (TNF) AND INTERLEUKIN (IL)-10 IS ASSOCIATED WITH ANTI-JO-1 AND ANTI-RO52 AUTOANTIBODIES IN PATIENTS WITH POLYMYOSITIS AND DERMATOMYOSITIS (IV)

Serum levels and the ratio between TNF and IL-10 were measured in 65 patients with polymyositis or dermatomyositis. In addition, these levels were investigated in relation to G-308A TNFA, G-1087A IL10 and G915C TGFB1 gene polymorphisms, 8.1 ancestral haplotype frequencies, gender, autoantibody profiles and clinical manifestations. Increased serum level of TNF and IL-10 was observed in patients compared to healthy controls. A significantly higher TNF: IL-10 ratio was detected in females carrying the TNF2 allele compared to those with the TNF1/TNF1 genotype; also in the whole group of patients (both genders) this difference was suggestive. The ratio was significantly higher in patients with the extended MICA5.1/TNF2/TNFa2/DRB1*03 haplotype (8.1 ancestral haplotype) compared to patients who were negative for this haplotype. There was a significantly higher TNF: IL-10 ratio in sera of patients with anti-Ro52 antibodies and in women with anti-Jo-1 antibodies. There was no significant difference in –1087 IL10 and 915 TGFB1 genotypes or allele frequencies between patients and controls. An association between the –1087 IL10 polymorphism and ILD was also noted, the frequency of ILD being higher in patients with A-1087 IL10 allele.

These data suggest that a genetically programmed cytokine imbalance exists in patients with polymyositis and dermatomyositis and that this imbalance is related to the presence of disease-associated autoantibodies. This might also determine clinical features, including development of ILD, and seems to be of particular relevance in women with these disorders.

In this study we analyzed not only the absolute serum level of pro-inflammatory TNF and anti-inflammatory IL-10, but also the ratio between these two cytokines in relation to genetic markers. As IL-10 is a potent down-regulator of TNF production and TNF by itself stimulates release of IL-10 from different cells (Van der Poll T 1994), the ratio between these two cytokines can better reflect the balance between pro- and anti-inflammatory stimuli. This ratio was also related to the presence of anti-Ro52 autoantibodies and with anti-Jo-1 autoantibodies. The absence of a significant difference in analyzed parameters in men could be due to the lower number of men in the study. This ratio was also significantly higher in patients with the pro-inflammatory extended ancestral haplotype MICA5.1/TNF2/TNFa2/DRB1*03 compared to patients who were negative for this haplotype. These data suggest that the genetic background of myositis patients, possibly together with other factors regulating TNF and IL-10

production, may directly or indirectly influence the production of autoantibodies associated with the development of ILD.

The TNF: IL-10 ratio has been proposed to be a marker for the prognosis of different diseases (Remoue F 2001, Asai K 2001). Our data demonstrate that this ratio could be of clinical relevance in patients with myositis. Genetic background for higher TNF expression and disease severity were also previously reported for juvenile dermatomyositis patients (Pachman LM 2000). It is possible that treatment which "normalises" the TNF: IL-10 ratio could be beneficial in myositis patients. There are also some reports on the beneficial effect of TNF blockade in patients with myositis (Efthimiou P 2006, Labioche I 2004, Hengstman GJ 2004). Whether these beneficial effects of therapy depend on genetic background, cytokine balance or other factors has not, to our knowledge, been investigated.

We did not find any significant genetic differences in frequency of IL-10 and TGFB1 alleles in patients compared to controls. This could be due to small size of the patients. Studies with lager number of patients are needed to confirm our results.

# 5 CONCLUSIONS

- Interstitial lung disease, detected by means of chest radiography and/or pulmonary function tests, was evident in 79% of patients with recent onset of myositis. Respiratory symptoms were not a valid indicator of ILD and cannot be used for selection of patients who should undergo pulmonary assessment. Arthritis and anti-Jo-1 antibodies were present more often in patients with ILD than in those without ILD. However, as respiratory symptoms are not reliable for detection of ILD in patients with myositis, all myositis patients should, besides complete physical examination, be investigated for early detection of pulmonary involvement. The most important tests to detect ILD are screening for anti-Jo-1 antibodies, pulmonary function tests, chest radiography and HRCT of the lungs.

- Follow-up studies with pulmonary function tests and radiographic examinations suggest that in most cases ILD is mild, chronic and does not progress during immunosuppressive therapy. Pulmonary function may normalize during treatment with immunosuppressive therapy although changes in HRCT may persist. The course of ILD cannot be predicted by the results of the initial examination.

- A restricted accumulation of T cells expressing selected TCR V gene segments in the lung and muscle tissues were identified in patients with myositis. The occurrence of shared TCR gene segment usage in lung and muscle tissue might suggest common target antigens in these organs.

- Patients with myositis had higher serum levels of TNF and IL-10 than did healthy controls. A significantly higher TNF: IL-10 ratio was detected in female patients carrying the TNF2 allele compared to female patients with the TNF1/TNF1 genotype. The ratios were higher in patients with the extended MICA 5.1/TNF2/TNFa2/DRB1*03 haplotype compared to patients lacking this haplotype. A higher TNF: IL-10 ratio was recorded in sera of patients with anti-Ro52 antibodies and in women with anti-Jo-1 antibodies. A higher frequency of ILD was found in patients carrying A-1087 IL10 genotypes compared to those with the GG-1087 IL10 genotype.

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