A comparative study of the Anti-Synthetase Syndrome;

Clinical features, outcome and implications

Thesis by

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UiO : Universitetet i Oslo

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To know what you know and what you do not know, that is true knowledge.

Confucius (551-479 BC)

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List of papers

I. Andersson H, Sem M, Lund M B, Aaløkken T M, Günther A, Walle-Hansen R, Garen T, Molberg Ø. Long-term experience with Rituximab in anti-synthetase syndrome-related interstitial lung disease. Rheumatology (Oxford). 2015 Aug;54(8):1420-8.

II. Andersson H, Aaløkken T M, Günther A, Mynarek G K, Garen T, Lund M B, Molberg Ø. Pulmonary involvement in the antisynthetase syndrome; A comparative cross-sectional study. J Rheumatol. 2016 Jun;43(6):1107-13.

III. Andersson H, Kirkhus E, Garen T, Walle-Hansen R, Merckoll E, Molberg Ø. Comparative analyses of muscle MRI and muscular function in anti-synthetase syndrome patients and matched controls; A cross-sectional study. Arthritis Res Ther. 2017 Jan 25;19(1):17.

IV. Andersson H, Sjaastad I, Schwartz T, Garen T, Aaløkken T M, Molberg Ø. Detecting pulmonary hypertension in the anti-synthetase syndrome; Performance of a simple algorithm. Submitted.

Abbrevations

6MW = six minutes walking distance

aaRS = aminoacyl t-RNA synthetase

ACPA = anticyclic citrullinated peptide

AMP = adenosine monophosphate

ASS = anti-synthetase syndrome

ATP = adenosine triphosphate

ATS = American Thoracic Society

Aza = Azathioprin

BAFF = B-cell activating factor

CK = creatinine kinase

COPD = chronic obstructive pulmonary disease

CRP = C reactive protein

CTD = connective tissue disease

CyA = Cyclosporin A

Cyc = Cyclophosphamide

DAD = diffuse alveolar damage

DLCO = diffusion capacity for carbon monoxide of the lungs

DM = dermatomyositis

DOI = definition of improvement

ECG = electrocardiography

Echo = echocardiography

ERS = European Respiratory Society

ESC = European Society of Cardiology

estPAP = estimated pulmonary arterial pressure

FEV1 = forced expiratory volume in one second

FI2 = functional index test 2

FVC = forced vital capacity

HAQ = health assessment questionnaire

HLA = human leukocyte antigen

HRCT = high resolution computerized tomography

IIM = idiopathic inflammatory myopathy

IIP = idiopathic interstitial pneumonias

ILD = interstitial lung disease

IMNM = immune mediated necrotizing immunopathy

IP = interstitial pneumonia

IPF = idiopathic pulmonary fibrosis

IPFA = interstitial pneumonia with autoimmune features

IVIG = Intravenous Immunoglobulin

LVDD = left ventricular diastolic dysfunction

MAA = myositis associated antibodies

MDAAT = myositis disease activity assessment tool

MDI = myositis damage index

MH = mechanic's hands

MHC = major histocompatibility complex

MI = myocardial infarction

MMF= mycophenolate mofetil

MMT = manual muscle test

MRI = magnetic resonance imaging

MSA = myositis specific antibodies

Mtx = Methotrexate

NET = neutrophil extracellular traps

NK = natural killer

NOSVAR = Norwegian systemic connective tissue diseases and vasculitis registry

NPV = negative predictive value

NSIP = non specific interstitial pneumonia

OUH = Oslo University Hospital

OP = organizing pneumonia

PAd = Pulmonary Artery diameter

PAH = pulmonary arterial hypertension

PBMC = peripheral blood mononuclear cells

PFT = pulmonary function test

PH= pulmonary hypertension

PM = polymyositis

pSS = primary Sjögrens Syndrome

RA = rheumatoid arthritis

RCT = randomized controlled trial

RHC = right heart catheterization

RIM = Rituximab in myositis

Rtx = Rituximab

SAE = serious adverse events

SF36 = short form 36

SSc = systemic sclerosis

STIR = short T1 inversion recovery

Tac = Tacrolimus

TCR = T-cell receptor

TLR = Toll like rececptor

UCTD = undifferentiated connective tissue disease

UIP = usual interstitial pneumonia

VA = alveolar volume

Summary

The Anti-synthetase syndrome (ASS) is a rare systemic auto-immune disease defined by distinct clinical features and the presence of a specific anti-synthetase antibody (aaRS), most commonly anti-Jo1. The major clinical features are myositis and interstitial lung disease (ILD), others are arthritis, Raynaud's, mechanic's hands and fever (1). Patients with ASS appear to have increased morbidity and mortality compared to general population, mostly due to the pulmonary component of the disease (2-4).

Our aim for this study was to assess long term outcome of the pulmonary and muscular manifestations in a well-defined ASS cohort followed at our department at Oslo University Hospital (OUH). Patients with a positive serological test of an anti-aaRS antibody, diagnosed with ILD according to the American Thoracic Society (ATS) (5) *and/or* myositis according to Bohan and Peter criteria (6) were invited to participate in the study (N=70). For comparisons we invited healthy controls, one age and gender matched control for each patient, randomly collected from the National People Register of Norway. Additionally, we wanted to retrospectively evaluate anti-CD20 therapy in ASS-related ILD and to evaluate different screening methods for pulmonary hypertension (PH) in the ASS.

We found that the ASS patients, with median disease duration of 71 months, had significantly reduced pulmonary function (measured by pulmonary function test, PFT) compared to the controls. The ILD extent assessed in HRCT of the lungs were in the patients median 20% of total lung volume, and correlated with forced vital capacity (FVC) and the lung diffusion capacity of Carbon Monoxide (DLCO). Twenty-four ASS patients had been treated with Rituximab (Rtx) due to severe lung involvement. With median disease duration of 52 months the patients demonstrated significant improvement in PFT after Rtx therapy. 5/24 were diagnosed with infections, all five > six months after therapy.

The patients scored significantly less in muscle strength and endurance compared to controls. Muscle MRI revealed significantly higher scores for muscle edema, fascie edema, fatty replacement and muscle volume reduction in patients compared to controls. 23% of the patients with normal CK values had a muscle MRI activity score (max 42p) of >18p, and the muscle tests did not correlate with MRI activity score but with damage score. 26 % of the patients had concomitant muscle MRI changes of activity and damage.

The PH analyses showed that a Pulmonary Artery diameter (PAd) > 30 mm on lung HRCT was a very sensitive PH marker (100% sensitivity), but specificity was low (68%). None of the 43 patients who had PAd < 30 mm and an estimated Pulmonary Artery Pressure (estPAP) < 45 mmHg by Echocardiography were diagnosed with PH.

ASS has a major impact on pulmonary and muscular function in patients with long standing disease. It is therefore important with effective treatment where anti-CD20 therapy could be an option in patients with pulmonary involvement. The need for thorough clinical assessment is also evident; Here muscle MRI evaluating the myositis component and measurement of PAd as a complementary tool in PH screening could be helpful.

1 The anti-synthetase syndrome

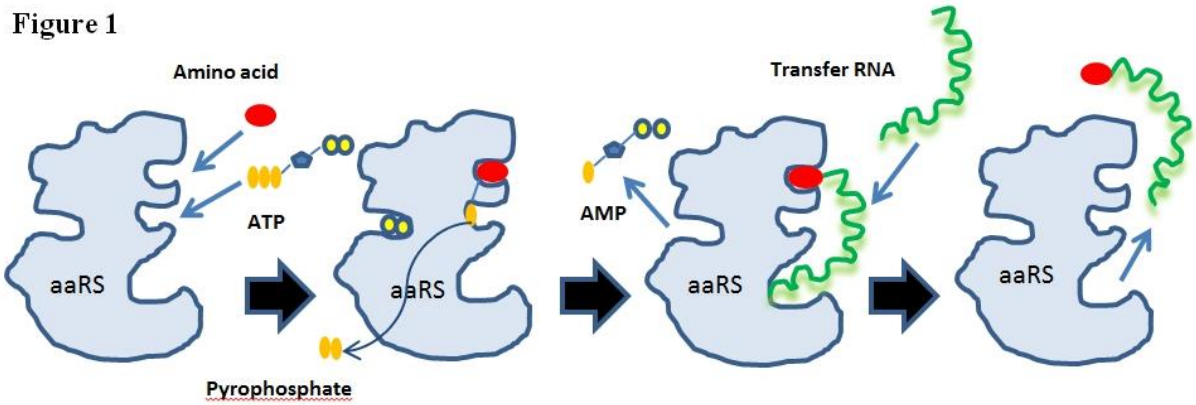
1.1 Introduction

In 1976 a male patient was referred to the Emergency Room with three weeks history of dry cough, progressive respiratory failure, fever and muscle pain (7). An open lung biopsy revealed interstitial pneumonitis including fibrosis. At first there was no suspicion of a specific connective tissue disease, but due to increasing myalgia and proximal muscle weakness the question of polymyositis (PM) with interstitial lung disease (ILD) was raised and eventually diagnosed. The patient was treated with variable doses of steroids and effect until he unfortunately died of acute myocardial infarction in 1980. Investigation of the patient's serum with immune diffusion detected a novel antibody, later also found in other patients with polymyositis (8). The antibody was given the name anti-Jo1, after the patient's first name, John. Further analyses of the immune precipitate revealed the Jo1 antigen as histidyl t-RNA synthetase; Thus the anti-Jo1 antibody was the first detected antibody against an aminoacyl t-RNA synthetase (aaRS) (9).

1.2 Aminoacyl t-RNA synthetases

The aminoacyl t-RNA synthetases (aaRS) are enzymes that catalyze the attachment of an amino acid to its correspondent t-RNA chain and act therefore as a very important basic step in the protein synthesis (10), se Figure 1. There is one specific aaRS for each amino acid. When the attachment is done, made by an ester binding that requires energy from the conversion of adenosintriphosphate (ATP) to adenosinmonophosphate (AMP), the ribosome can transfer the amino acid from the tRNA to a growing peptide and, finally, protein.

Figure 1



A specific Amino acid is enfolded together with ATP into its' correspondent synthetase. An attachment between the Amino acid and Transfer RNA is made possible by the conversion of ATP to AMP and is thereafter released from the synthetase.

To date, eight anti aaRS-antibodies contributing to the clinical entity ***anti-synthetase syndrome*** (ASS) are known and believed to be mutually exclusive.

Table 1; Known Anti aaRS-antibodies in the ASS

ANTIBODY	ANTIGEN	PREVALENCE IN MYOSITIS (%)
Jo-1	histidyl	25-30
PL-7	threonyl	2-5
PL-12	alanyl	2-5
EJ	glycyl	1
OJ	isoleucyl	1
KS	asparaginyl	1
Zo	phenylalanyl	1
Tyr	tyrosyl	1

Adapted from Hervier and Benveniste (11)

The description of the syndrome was first made in 1990 by Marguerie et al who described the clinical picture of 29 British patients with positivity of one of three anti-aaRS antibodies (anti-Jo1, PL-7 and PL-12), followed up by a US study by Love et al in 1991 (1, 12).

1.3 Demographics

The incidence and prevalence of ASS is somewhat difficult to estimate due to the syndrome's rarity and the lack of established classification criteria. Since ASS-associated myositis is

reported to be 25-30 % of all PM/DM, the annual incidence of ASS-associated myositis is estimated to 1.25-2.5/million with a prevalence of 1/100000 (13). In a study of 131 ASS myositis patients the onset of disease had a seasonal peak in March-April (14). Reported female:male ratios are typically 2:1. ASS affects any age, but most commonly between 40-55 years (11).

1.4 Clinical and histological features of the anti-synthetase syndrome

1.4.1 Myositis

Myositis is one of the major component of the ASS found in 57-79 % of the patients, to a lesser degree at time of diagnosis but can develop during disease course (2, 3, 15-19). The frequency depends on which of the anti-aaRS is present; Myositis is found more often in anti-Jo1 and PL-7 subsets than in PL-12 subsets (2, 19-21). The most common myositis phenotype in ASS is generally described as polymyositis (PM), although there are a few cohorts with dermatomyositis (DM) as the dominant type (1, 8, 18, 22, 23).

The severity of myositis seems to differ between anti-Jo1 positive and negative subsets with more muscle involvement in the anti-Jo1 positive patients (2, 18, 19). However, heterogeneity in clinical muscle activity are seen not only in different ASS-subsets but also in the same subgroups and individually during disease course (11).

1.4.1.1 Muscle histology in the ASS

The muscle histology of the ASS was first described as an immune myopathy with perifascicular pathology (IMPP) by Pestronk in 2011 (24). Recently, the ASS muscle pathology was described as a distinct entity compared to the pathology of PM and especially DM. It comprises perifascicular distribution of HLA-DR expression in the myofibers, C5b-9 deposition on sarcolemma of perifascicular fibers, myo-nuclear actin filament inclusions, perifascicular/ perimysial necrosis and, to a minor extent, perifascicular atrophy (25-27).

1.4.2 Interstitial lung disease (ILD)

ILD is the other major component of the ASS with a frequency of 69-94 % depending on which aaRS-antibody present and study population (2, 3, 15-19). The type of ILD-onset in myositis, as well as in ASS, could clinically be divided into acute and gradual form with poorer prognosis in the acute onset group (28-30).

The most frequent ILD pattern on lung-CT scan in the ASS is described with peripheral, basal ground-glass attenuation, traction bronchiectasis and consolidation (2, 4, 19, 31-36). According to the classification of idiopathic interstitial pneumonias (IIPs) by the American Thoracic Society (ATS) and the European Respiratory Society (ERS), this pattern is classified as "non-specific interstitial pneumonia" (NSIP) (Table 2) (37, 38). Other common CT patterns seen in the ASS are "organizing pneumonia" (OP), a "mixture" of NSIP/OP and "usual interstitial pneumonia" (UIP) (2, 4, 19, 31-35). UIP is the CT- pattern of idiopathic pulmonary fibrosis and is, compared to the NSIP pattern, less responsive to treatment (37, 39). Although rare, a CT pattern of diffuse alveolar damage (DAD) is also described in the ASS (40). DAD is classified as an acute IIP and both UIP and DAD have in ASS been associated with increased mortality (4, 40).

Table 2; Lung CT-patterns described in the ASS

INTERSTITIAL PNEUMONIA (IP)	**CT PATTERN**	**CT DISTRIBUTION**	**CT FINDINGS**
Chronic fibrosing IP	NSIP	peripheral, basal, symmetric	ground-glass opacity, linear opacities,traction bronchiectasis, consolidation
	UIP	peripheral, basal, subpleural	reticular opacities, honeycombing, traction bronchiectasis, focal ground-glass opacity
Acute/subacute IP	OP	subpleural or peribronchial	patchy consolidations or nodules, central ground-glass opacity
	DAD	diffuse or patchy	ground-glass opacity, extensive consolidations, traction bronchiectasis

NSIP=non specific interstitial pneumonia, UIP=usual interstitial pneumonia, OP=organizing pneumonia, DAD=diffuse alveolar damage. Adapted after Sverzelatti et al (37)

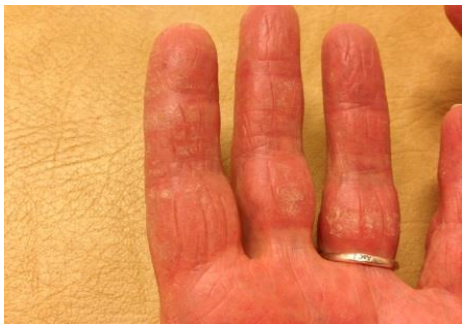
1.4.2.1 Pulmonary histology of the ASS

Due to the rarity of the disease and the fact that lung biopsy is not a common procedure in the diagnosis of ASS-related ILD, most of the studies of pulmonary histology in the ASS are described with few cases (4, 31, 33, 34, 40-50). The histology patterns are classified in the same way as the CT-patterns including NSIP, UIP, OP and DAD. Correspondingly to the CT-patterns, NSIP is the most frequent histological pattern in ASS, but inconsistency between corresponding CT- and histological pattern exist. DAD is an example of this since the pattern of DAD is more often described in lung biopsies than in CT-images (4, 32, 34, 40).

1.4.3 Skin manifestations

Mechanic's hands (MH) is a classic dermatological feature of ASS with hyperkeratosis, erythema and fissures on the palmar and lateral aspects of the hand and digits (11, 51). Initially believed to be a specific feature of the ASS, but have been diagnosed in other forms of myositis and CTD as well (52, 53). The frequency varies in different studies, between 16-60 %, also here depending on which ASS subset is present (2, 16-21, 47, 54). Generally, the frequency is higher in Jo1- compared to non Jo-1 subsets (55). Usually mechanic's hands coexist with other features of the ASS and are more prominent in flares of the disease (11, 51). Other skin manifestations described in ASS are those seen in classical DM with Gottron's papules, heliotrope and cutaneous rash (56). There are also case reports on ASS patients with scleroderma-like skin manifestations including sclerodactyly, calcinosis and teleangiectasia (56, 57).

Figure 2

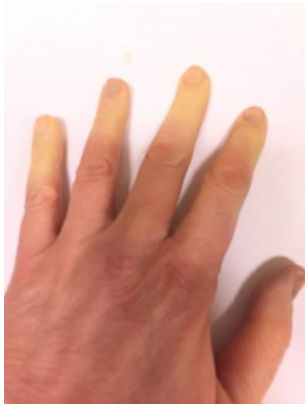


Mechanic's hands; Courtesy of the patient

1.4.4 Raynaud's phenomenom

Raynaud's phenomenom is described in the ASS with a frequency of 24-65 %, higher frequency found in the PL-7 and PL-12 subsets (2, 3, 16, 18, 20, 21). There are no studies that specifically investigate findings of nail-fold capillaroscopy in the ASS, but one study have found correlation between anti-Jo1 positivity and reduced capillary density (58). Digital ulcers, similar to those of systemic sclerosis, are rare but exist in the ASS, most in the anti-Jo1negative subsets (55, 59).

Figure 3

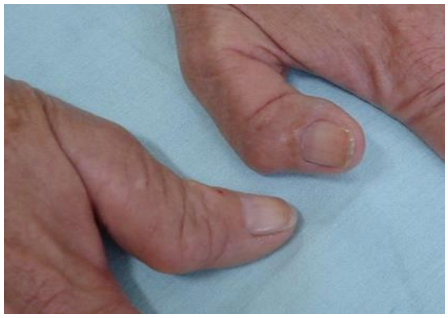


Raynaud's; Courtesy of the author

1.4.5 Arthralgia/arthritis

Joint involvement in the ASS is common with 20-77 % frequency, arthralgia more frequent than arthritis, and more in anti-Jo1 subsets (2, 15, 16, 18, 19, 55). Interestingly, a specific form of subluxing, deforming arthropathy which affects the interphalangeal joints, especially the thumbs (called "floppy thumbs"), has been seen with the syndrome, described as early as in 1976 (60). Some authors divide the ASS arthropathy into 3 groups; 1) subluxing arthropathy, 2) erosive or no erosive polyarthritis and 3) isolated arthralgia (61, 62). Anti-cyclic citrullinated peptide (ACPA) antibodies, as seen in rheumatoid arthritis (RA), are not uncommon in ASS patients with erosive polyarthritis and are associated with severe disease (63-65). Arthritis could also be the first symptom of ASS, with onset of other clinical features later on (66-68).

Figure 4



"Floppy thumbs"; Reprinted from Rev.Bras.Revmatol. (69).

1.4.6 Fever

Fever is a systemic feature of the ASS with a frequency of 26-38 % (2, 3, 16-18). It is more frequent in patients with ILD and active disease (28). Since ILD is more common in anti-Jo1 negative subsets, fever is more frequent in these subsets (55).

1.4.7 Cardiovascular manifestations

Myocarditis, especially related to active myositis, is a known feature of the ASS (70-72). In a French retrospective study of 352 ASS patients, myocarditis at any time during disease course was present in 12 patients (3.4%), evaluated by Troponin I/T and cardiac-MRI or endocardial biopsy (73). As in other inflammatory myopathies the myocarditis could be subclinical and eventually lead to cardiomyopathy, congestive heart failure and arrhythmia (74, 75).

Pericarditis is not extensively investigated but described in the ASS. Interestingly, it has been found with a 50 % frequency in a study of 18 PL-7 positive ASS patients (21).

Another cardiovascular manifestation of the ASS is precapillary pulmonary hypertension (PH). There are case reports on ASS patients with precapillary PH caused by pulmonary arterial hypertension (PAH), but precapillary PH secondary to ILD is more frequent (76-81). A PH frequency of 7.3 % was recently reported in a study of 203 ASS patients, all evaluated as PH secondary to ILD. In this study three years survival of the PH patients was 58 % (80).

Little is known about the incidence of cardiovascular events in myositis and ASS. A Canadian population based study identified 774 cases with PM/DM and found an incidence rate for myocardial infarction (MI) of 22.52 per 1000 person-years in PM patients, compared to 5.50 per 1000 person-years in the general population. The incidence rate of stroke was in PM patients 10.15 per 1000 person-years, for the general population 5.58 per 1000 person-years.

The same tendency was seen for patients with DM (82). In this study no data on myositis specific antibodies (MSA) was reported. Diederichsen et al demonstrated an increase in left ventricular diastolic dysfunction (LVDD) in 76 myositis patients compared to healthy controls (83). LVDD is associated with an increased risk of all-cause mortality (84). Interestingly, the LVDD was associated with myositis specific/associated antibodies where 35/76 patients were positive for an anti-aaRS antibody (83).

1.4.8 Gastrointestinal manifestations

As in other forms of myositis, dysphagia and gastrointestinal reflux are reported in ASS (11, 45). One study with 86 anti-Jo1 positive patients found a 21% frequency of dysmotility in oesophagus (85). There is also one cohort study of 15 anti-PL-7 patients which reports pseudo-intestinal obstruction in 2/15 patients (86).

At the end of year 2011, the ASS cohort of Oslo University Hospital (OUH) included 96 patients. The clinical features of this cohort are seen in Table 3. In Table 4 recent major ASS cohorts including cohorts of specific anti-aaRS antibodies and theirs clinical features are presented.

Table 3; Clinical characteristics of OUH's ASS cohort (1994-2011) with 96 patients

CLINICAL CHARACTERISTICS	TOTAL COHORT (%)	Anti-Jo1 (%)	PL-7 / PL-12 (%)	p
Female/male	67/29	56/23	11/6	
Jo1/PL7/PL12	79/7/10	79	17	
Myositis	73/96 (76)	68/79 (86)	5/17 (29)	<0.001
ILD	90/96 (94)	73/79 (92)	17/17 (100)	n.s
Acute onset of ILD	18/90 (20)	13/73 (18)	5/17 (30)	n.s
>65 years at diagnosis	15/96 (16)	14/79 (18)	1/17 (6)	n.s
SS-A positivity	69/96 (72)	56/79 (70)	13/17 (76)	n.s
Skin manifestations	42/96 (44)	37/79 (47)	5/17 (29)	n.s
Mechanical hands	42/96 (44)	35/79 (44)	7/17 (41)	n.s
Raynaud's	49/92 (53)	38/75 (51)	11/17 (65)	n.s
Arthralgia/arthritis	71/96 (74)	61/79 (77)	10/17 (59)	n.s

n.s = no significance

Table 4; Recent major ASS cohorts inclusive specific minor cohorts

	No of patients	Sex (F/M) Age at diagnosis	aaRS-antibodies (N)	Muscle spt / myositis (%)	PM/DM (%)	ILD (%)	Arthralgi / arthritis (%)	Raynaud (%)	Mechanic hands (%)	Fever (%)
Hervier et al 2012	**233**	**165/68** **48**	**Jo1/PL7/PL12 (160/25/48)**	**133 (57)**	**NA**	**179 (77)**	**146/47 (63/20)**	**99 (42)**	**45 (19)**	**66 (28)**
Marie et al 2012	**95**	**59/36** **56**	**Jo1/PL7/PL12 (75 + 20)[a]**	**60 (63)**	**39/21 (65/35)**	**69 (73)**	**55 (58)**	**43 (45)**	**28 (29)**	**NA**
Aggarwal et al 2014	**202**	**138/64** **48**	**Jo1/PL7/PL12/EJ/ OJ/KS (122 + 80)[a]**	**152 (75)**	**NA**	**136 (76)[b]**	**128 (63)**	**NA**	**NA**	**NA**
Hamaguchi et al 2013	**165**	**130/35** **53**	**Jo1/PL7/PL12/EJ/ OJ/KS (59 + 106)[a]**	**95 (58)**	**36/55 (22/55)[c]**	**155 (94)**	**61 (37)**	**40 (24)**	**67 (41)**	**51 (31)**
Dugar et al 2011	**42**	**30/12** **53**	**JO1/PL7/PL12 (37+4+1)**	**28 (67)**	**14/5 (50/18)[c]**	**29 (69)**	**25 (59)**	**13 (31)**	**9 (21)**	**16 (38)**
Trallero-Aragas et al 2016	**148**	**90/58** **51**	**Jo1**	**123 (83)**	**61/62 (49.5/50.5)**	**121 (82)**	**103 (70)**	**50 (34)**	**66 (45)**	**50 (34)**
Cavagna et al 2015	**225**	**167/58** **53**	**Jo1**	**177 (79)**	**NA**	**189 (84)**	**144 (64)**	**52 (24)[d]**	**42 (19)[d]**	**57 (26)[d]**
Stanciu et al 2012	**48**	**NA** **43[e]**	**Jo1**	**47 (98)**	**NA**	**34 (92)[b]**	**37 (77)**	**23 (48)**	**10 (21)**	**15 (31)**
Kalluri et al 2009	**31**	**25/6** **51**	**PL12**	**21 (68)**	**10/6 (48/29)[c]**	**28 (90)**	**18 (58)**	**20 (65)**	**5 (16)**	**14 (45)**
Sato et al 2007	**7**	**4/3** **53**	**OJ**	**4 (57)**	**4/0 (100/0)**	**7 (100)**	**4 (57)**	**0 (0)**	**NA**	**3 (43)**
Targoff et al 1992	**5**	**3/2** **21-60[f]**	**EJ**	**5 (100)**	**0/5 (0/100)**	**5 (100)**	**4 (80)**	**3 (60)**	**NA**	**NA**
Labirua-Iturburu 2012	**18**	**15/3** **53**	**PL7**	**17 (94)**	**12/5 (71/29)**	**10 (56)**	**12 (67)**	**11 (61)**	**5 (28)**	**10 (56)**
Schneider et al 2015	**5**	**4/1** **49**	**KS**	**1 (20)**	**NA**	**4 (80)**	**1 (20)**	**2 (40)**	**3 (60)**	**NA**

[a]Divided in Jo1/non Jo 1 subsets,[b]Evaluated by CT scans, [c]Exclusion of amyopatic and overlap myositis,
[d]Evaluated in 217-22pts, [e]Age at onset, [f]Range

1.5 Classification criteria

Two set of ASS classification criteria have been proposed, both comprising a positive anti-aaRs antibody as an obligate item (41, 87). The proposed criteria from Connors et al from 2010 (41) demand one additionally positive clinical item, not explained by other causes, of myositis, ILD, arthritis, Raynaud's, mechanic hands' and fever. In the proposed criteria from Solomon et al in 2011 (87), the clinical features are divided into major and minor items. The criteria demand either two major items ***or*** one major item with two minor.

Table 5; Proposed classification criteria for the ASS

	Connors et al, 2010	**Solomon et al, 2011**
Required	Positivity of an aaRS-antibody	Positivity of an aaRs-antibody
Additionally	***One of the following;*** Myositis by Bohan & Peter criteria (6) ILD by ATS criteria (5) Arthritis (clinical, radiological or self-reported) Mechanic's hands Raynaud's Unexplained persistent fever	***Two major or one major and two minor items;*** *Major;* Myositis by Bohan & Peter criteria (6) ILD of no other etiology *Minor;* Arthritis Mechanic's hands Raynaud's

aaRS=aminoacyl RNA synthetase, ILD=interstitial lung disease, ATS=American Thoracic Society

1.6 Pathogenesis

1.6.1 Immune-pathogenesis

The pathogenesis of ASS is still largely unknown. However, in the last two decades there have been increasing knowledge of the immune-pathological role of the ^{his}tRNA synthetase (Jo1) antigen, involving both innate and adaptive immunity in the ASS (88).

1.6.1.1 Adaptive immunity

The clinical correlation between anti-Jo1 antibodies and disease activity indicates a central role for ^{his}tRNA synthetase in the pathogenesis of ASS, possibly mediated by the adaptive immunity (23, 89). The discovery of ^{his}tRNA synthetase as an autoantigen capable to induce anti-Jo1 antibody responses with class switching (from IgM to IgG_1), spectrotype broadening (from oligoclonal to polyclonal) and affinity maturation points towards an involvement by adaptive immunity (90). Especially the fact that anti-Jo1 antibodies undergo affinity maturation over time points towards an antigen-driven process, possible mediated by ^{his}tRNA synthetase specific T-cells (88). Such Jo1 specific T-cells have been found in peripheral blood of ASS patients (91). Interestingly, a conformation of ^{his}tRNA synthetase with a specific epitope of a granzyme B-cleavable form is enriched in the alveolar epithelium (92).

Data from murine model systems supports the notion that adaptive immunity has a role in the pathogenesis of ASS. Specifically, it was demonstrated that immunization of mice with murine ^{his}tRNA synthetase was followed by a direct B and T cell recognition of this antigen in combination with clinical features of myositis and ILD (93).

1.6.1.2 Innate immunity

Direct activation of the innate immunity by ^{his}tRNA synthetase was recently demonstrated. In this model intramuscular immunization with recombinant ^{his}tRNA synthetase without any additional exogenous adjuvant resulted in Toll-like receptor (TLR) and T-cell receptor (TCR) signaling and revealed specific lymphocytic infiltration in muscle tissue (94). Further work on TLR2/4 signaling and the signal pathways of protein MYD88 indicated these three as major components contributing to the development of myositis (95, 96). MYD88 upregulates the transcriptional activator NF-κB, a central key protein in the immune response (88). Importantly, the immunization also caused an IgG autoantibody response towards the ^{his}tRNA synthetase, reinforcing the view that the pathogenesis of ASS is driven by adaptive processes (94).

It has been demonstrated that ^{tyr}tRNA- , ^{his}tRNA and ^{asp}tRNA synthetases all have cytokine- and chemokine-like properties that make them capable of stimulating T-lymphocytes, activated monocytes and immature dendritic cells (97, 98). To note, these specific properties are not detected in synthetases without connection to ASS (88).

Innate immunity could be active in connection with stimulation of the peripheral blood mononuclear cells (PBMC). This can possibly be done by complexes between anti-Jo 1 antibodies and necrotic cell debris (for example found in neutrophil extracellular traps, NETs) (99). Stimulated PBMC have the possibility to upregulate type1 interferon production which is not only seen in ASS-related ILD but also expressed in myositis muscle tissues (88).

A recently published study on natural killer (NK) cells and a NK receptor (NKp30), revealed that ASS patients with active disease had a different NK cell profile and decrease in the NKp30 compared to ASS patients with inactive disease (100). This decrease was correlated with a dysfunctionality of the NK cells. In addition, ASS patients had significantly higher infiltration of NK cells in lung tissue compared to healthy subjects. Hence, the findings from this study indicate a possible role of the innate immunity and NK-NKp30 cells in the pathogenesis of ASS (100).

1.6.2 Immuno-genetics

In the last decade there has been increasing knowledge of immuno-genetics in myositis, including the ASS (101, 102). Collectively, the studies have demonstrated that the major risk genes for Caucasians are located within the major histocompatibility complexes (MHC) on chromosome 6 (103-106). Myositis patients with ILD and myositis specific antibodies (MSA) are not regulated by the myositis subtype, but by the HLA class II haplotype (107). It appears that the major HLA risk alleles for myositis are part of the 8.1 ancestral haplotype. Especially the allele DRB1*0103 is associated with anti-Jo1 positivity, and is also seen in PL-7- and PL-12-positive patients (103, 105).

Outside the 8.1 ancestral haplotype, but in the MHC region, is the HLA-DPB1gene located. The finding of a strong association between the HLA-DPB1*0101 and anti-Jo1 positivity indicates this gene as a possible contributor to the pathogenesis of ASS (108).

1.6.3 Environmental factors

An association between myositis patients with DRB1*0103 positivity, anti-Jo1 and smoking is demonstrated; Anti-Jo1 frequency was higher in DRB1*0103 positive patients who were smokers compared to DRB1*0103 positive patients who were non-smokers. Since the Jo1antigen is expressed in the lungs (see above) it is possible that an interaction between DRB1*0103 and smoking can induce anti-Jo1 positivity (109). There are also theories on

occupational (dust, gases, fumes) and household (mold, birds) exposure by inhalation as trigging factors of anti-Jo1production locally in the lungs (110-112).

1.7 Biomarkers

Several studies have demonstrated a linkage between specific biomarkers, anti-Jo1 antibodies and clinical manifestations such as ILD (88); One study has shown that the chemokines CXCL9 and CXCL10 are increased in ASS-related ILD compared to patients with idiopathic pulmonary fibrosis (IPF) (40). Furthermore, ICAM1, a pro-inflammatory cell surface glycoprotein, is upregulated in lung endothelial cells of anti-Jo1 positive patients (113) and BAFF, an IFN-α inducible cytokine, are associated with anti-Jo1 positivity and ILD (114).

1.8 Treatment of the ASS

1.8.1 Treatment of the myositis component

Due to the rarity and heterogeneity of the inflammatory myopathies there are very few randomized controlled trials (RCT;s) in myositis, even more so for the ASS. In ASS, the first-line treatment for myositis is the same as for PM and DM (11). The basic induction therapy in myositis is still corticosteroids, usually Prednisone, often in combination with Azathioprine (Aza) and/or Methotrexate (Mtx), as steroid-reducing agents (115-120). Interestingly, a study with 113 myositis patients, including 34 with ASS, demonstrated only a partial response in the majority (84%) of the ASS patients with Prednisone alone. Additional therapy with either Aza or Mtx favored Mtx in these 34 patients (116).

Second line agents; In myositis cases who only respond partially or are refractory to first line agents, optional treatment choices are cyclosporin A (CyA), mycophenolate (MMF), immunoglobulins (IVIG) and/or Rituximab (Rtx) (121-126).

CyA is a calcineurin inhibitor as is Tacrolimus (Tac). Both drugs inhibit T-cell activation which is a part of the immune pathogenesis in ASS (see above). A small prospective non-randomized study with nine refractory myositis patients, all without ILD and anti-Jo1 antibodies, demonstrated efficacy of Tac in terms of increased muscle strength, reduced CK levels and doses of Prednisone (127). CyA, MMF and Tac are especially preferred in connection with ILD (see below) (117, 119).

The “Rituximab In Myositis” (RIM) study, the largest RCT with 200 refractory myositis patients (32 with ASS) evaluated the efficacy of Rtx (128). In this study, the patients were randomized in two groups, one group received Rtx upfront, while the other was treated with steroids for eight weeks before they also received Rtx. Although there was no statistical significance between the upfront and delayed Rtx groups in primary and secondary endpoints at the end of the six month study period, 83 % of the patients included met the study-specific definition of improvement (DOI) (128). Furthermore, predictor analyses of clinical improvement in these patients demonstrated a shorter time to achieve DOI in aaRS-positive patients compared to patients without myositis specific antibodies (MSA) (129). Another prospective study with 10 ASS patients indicated an effect in muscle strength including reduction in CK levels and steroid use with Rituximab (130).

1.8.2 Treatment of the ILD component

ILD is often a dominant clinical feature of ASS and, as earlier described, may often be acute and severe. Both MMF and CyA have in small retrospective, serial studies demonstrated efficacy in myositis related ILD (123, 124, 131-134).Tac has in retrospective studies demonstrated a positive effect on myositis associated ILD, including the ASS (135-138). In one of these studies, on 49 myositis associated ILD patients, Tac was even evaluated as superior to conventional treatment with CyA, Cyclophosphamid (Cyc) and Prednisone in terms of event- and disease-free survival (135).

Efficacy of Cyc, usually administered intravenously, for both the ILD- and myositis component has been reported but are mostly used with pulmonary indication (139-141). In ASS, Yamasaki and coworkers demonstrated a positive effect with Cyc on pulmonary function and reduction of ILD changes on HRCT in the majority of the patients (142).

Rituximab seems to be increasingly used in the treatment of myositis associated ILD, perhaps especially in the ASS. Several retrospective studies and case reports have demonstrated a beneficial effect of this drug with stabilization, or even improvement in pulmonary function and reduced ILD changes on HRCT in ASS patients (143-150). Some concerns regarding serious adverse events (SAE:s) with infections, including Pneumocystis Jirovechii, have been raised with this therapy (143, 146).

With the theory of aaRS-antibodies playing a direct role in the pathogenesis, treatment with plasmapheresis could be of benefit in the ASS. A positive effect on lung outcome with this treatment has been reported (151, 152).

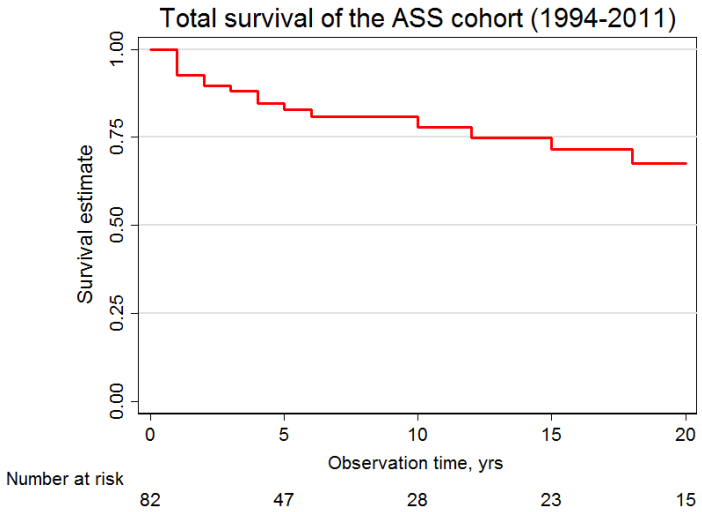
1.9 Outcome and prognosis of the ASS

1.9.1 Survival

ILD is a major factor with impact on morbidity and mortality in the inflammatory myopathies, including the ASS (3, 4, 18, 19, 153-157). In ASS, the reported 5-years survival rates variate in different studies between 75-93%, 10-years survival between 61-78% (2-4, 15). The survival rates appear to be lower in PL-7/PL-12 positive ASS subsets- compared to Jo-1- probably due to higher ILD frequency/intensity and, possibly, also longer diagnostic delay (2, 15). A cumulative 5-years survival of 90 % was found in Jo-1 positive patients compared to 70% in PL-7/PL-12 positive patients with the correspondent 10-years survival of 75% and 47%, respectively (15).

In the ASS cohort of Oslo University Hospital (OUH) 96 patients are registered between 1994 and 2011. Of these 96 patients 24 (25%) were deceased by 31st of December 2011. Manual chart review revealed causes of death as follows; Respiratory failure (N=7), malignancy (N=7), heart failure (N=4), infection (N=2), brain hemorrhage (N=1) and unknown (N=3). The overall survival of the ASS cohort of OUH is seen below.

Figure 5



1.9.2 Prognostic factors

Several risk factors for reduced survival in the ASS have been identified in different cohort studies; Diagnostic delay and age over >50 are two of these. Male gender predicted in one study (only Jo-1 patients) increased mortality (4), in another study (Jo-1 and non Jo-1 patients) gender was not associated with survival (15).

Identified ILD-related ASS mortality risk factors include presence of ILD *per se*, mono-organ disease with ILD as a single manifestation of the syndrome, grade of respiratory involvement at diagnosis and histological type/CT pattern of the ILD (2-4, 15, 19).

Anti-Ro52 positivity is associated with ILD in several CTDs, including myositis and ASS (158-160). The association between anti-Ro52 and ILD in ASS appears to be present across all aaRS subsets (161). Several studies have demonstrated a poorer ILD outcome in ASS patients with anti-Ro52 (162-164). In addition, one study found an association between the myositis component and presence of anti-Ro52 in anti-Jo1 positive patients with worse outcome (163). In the same study the survival was significantly higher for the negative compared to the positive anti-Ro52 patients (163).

1.9.3 Clinical outcome

In ASS, there are few studies with quantitative data evaluating pulmonary and muscular outcome simultaneously. In the large French cohort with 233 ASS patients and a mean follow up of > 6 years, 27 % demonstrated a worsening in the pulmonary and/or muscle component of the disease and 16 % developed PH (2). Marie et al demonstrated in 86 ASS patients with median follow-up of 45 months clinical remission, improvement and worsening in 15%, 64 % and 21% of the patients, respectively (85).

1.9.3.1 Pulmonary and muscular outcome

Paradoxically, even though ILD is associated with increased mortality, it appears that the pulmonary function seems to be rather stable, or decrease slightly from baseline to follow-up in the majority of the ASS patients (3, 4, 19, 85). In the study from Zamora et al, with 103 anti-Jo1 positive patients and median follow-up time of 9.5 years, the yearly decline in predicted FVC and DLCO was median -0.3 % and -0.5 %, respectively (4). In their study on 90 ASS patients from France, Marie et al demonstrated stabilization/improvement in 60 % of the patients (19). In this study there was a significant difference in the pulmonary remission rate between the Jo-1 and Jo-1 negative subsets; 29% compared to 6 % ($p<0.05$) (19).

Regarding the myositis component of the syndrome, the work from Marie et al revealed improvement in > 80 % of the patients with a minimum follow up time of 18 months (19).

1.9.3.2 Disease course

In the earlier mentioned study from Marie et al, the disease course was in 2/3 of the patients chronical continuous, in 1/3 the course was relapsing-remitting. Only one patient had a

monocyclic course (85). Additionally, the different clinical features of the ASS seem to appear at different times during disease course, where the majority of the patients have 1-3 of the major features (myositis, ILD, arthritis) at diagnosis but develop new features over time (2, 3, 16).

1.10 Malignancy and ASS

In myositis there is an increased frequency of malignancies compared to the general population, especially in DM (165-169). The most common types of malignancies in Caucasian IIM patients appears to be lung, ovarian, breast, gastrointestinal and non-Hodgkin lymphoma (166, 170). In Asian patients the tumor type is dominated by nasopharyngeal carcinoma and pulmonary cancer (170, 171). The myositis specific antibodies TIF1γ and NXP2 are associated with malignancy, and there are case reports on malignancy in MI2 positive DM (172, 173). Factors associated with increased risk of malignancy in PM/DM are; Older age, male gender, dysphagia, cutaneous necrosis/vasculitis and elevated levels of ESR, CRP and CK (169, 174-177). The opposite is true for arthritis and Raynaud's, which are associated with a reduced cancer risk (176-178).

Maybe surprisingly, since patients with idiopathic pulmonary fibrosis (IPF) have increased risk of pulmonary cancer (179, 180), myositis associated ILD appears to be associated with a reduced risk of malignancy (176-178, 181, 182). Hence, the ASS syndrome which comprises ILD, arthritis and Raynaud "ought" to have a reduced cancer risk, but here the data is divergent and inconclusive; Studies report both increased, reduced and similar incidence of malignancy compared to the general population (2, 3, 18, 174, 183, 184). Interestingly, one study comparing anti-Jo1with anti-PL-7/PL-12 positive subsets found higher cancer incidence in the Jo-1 positive group (19). In the small anti-OJ- positive cohort of Hamaguchi et al cancer was frequent, with 2/8 patients diagnosed with malignancy (18).

2 Aims of the study

2.1 General aim

The overall aim of this cross-sectional study was to assess long term outcome regarding lung- and muscle function in our ASS cohort compared to the lung- and muscle function of healthy age and gender matched controls.

2.2 Specific aims

1) To retrospectively evaluate effects of Rituximab treatment on pulmonary outcome in ASS.
2) To evaluate possible differences in pulmonary outcome in subsets of ASS, and assess possible correlations between pulmonary function test (PFT) and ILD changes on HRCT images.
3) To describe and compare possible muscle-MRI changes in ASS patients and controls and evaluate possible specific muscle-MRI patterns for the entity ASS.
4) To evaluate long-term muscle outcome in terms of CK levels, MRI changes and muscle strength in the ASS cohort.
5) To compare different screening methods and perform a clinical algorithm for detecting ILD related PH in the ASS.

3 Material and methods

3.1 Study design

Basically, this study had a cross-sectional design where each ASS patient had different disease duration when the investigations were performed (Paper II and III). Additionally, the cross-sectional design was also comparative since each patient had a correspondent age and gender matched healthy control (Paper II and III).

The study of Rtx therapy (Paper I) had a retrospective design, where ASS patients from the OUH cohort treated with Rtx on pulmonary indication were evaluated after given treatment.

The comparison of different screening methods and performance of an algorithm for detecting PH did also apply a retrospective design. The PH study included the patients from the cross-sectional study, and all the additional ASS patients from the total OUH cohort who had performed right heart catheterization (RHC) (Paper IV).

3.2 Study population and organization of data collection

3.2.1 The patient group

For this study, we applied a very simple definition of ASS (Box 1). This definition was used as inclusion criterion in the OUH cohort. ;

Box 1 Definition of ASS applied in the OUH cohort study

1) A positive serum test for one of the aaRS-antibodies
2) ILD defined according to the ATS criteria (5) not readily explained by other CTD;s ***and/or***
3) Myositis according to Bohan & Peter criteria (6)

The Department of Rheumatology at OUH is a tertiary referral center for CTD in Norway. Additionally, the Department has primary responsibility for CTD for the population of Oslo (N = 600 000). ASS patients diagnosed/assessed/treated/followed at OUH are asked to register in our patient registry called NOSVAR (NOrsk Systemisk bindevevssykdom og VAskulitt Register in Norwegian, or Norwegian Systemic Connective Tissue Diseases and Vasculitis Registry in English). Hence, in this register habitants from all over Norway are represented, but the majority comes from Oslo and its' surroundings. Per 31st of December 2011, 96 patients meeting our definition of ASS had ever been registered in NOSVAR, and 72 of these were alive.

The 72 living ASS patients in NOSVAR were asked to participate in the study either by direct contact or regular mail. Two of the 72 patients unfortunately died before inclusion. 68/70 patients were positive to participate, two declined participation. Patients were admitted to our daily-care unit for 2 following days.

The study protocol included the following items;

- Medical history, including current and former medical treatment, family- and occupational history, allergy, smoking and alcohol consumption.
- Height and weight
- Blood/serum samples; ESR, CRP, haematological parameters, creatinine, electrolytes, CK, LD, ASAT, ALAT, immunoglobulins, ANA with subgroups, MSA;s.
- Clinical examination, including the myositis disease activity assessment tool (MDAAT) (185) and myositis damage index (MDI) (185).
- Pulmonary function test (PFT)
- HRCT of the lungs
- Six minutes walking distance (6MWD)
- Electrocardiography (ECG)
- Echocardiography (Echo)
- Magnetic Resonance Images (MRI) of the thigh muscles
- Test of muscle strength and endurance by manual muscle test of 14 muscles (MMT14) and functional index 2 test (FI2) (186, 187).
- Capillaroscopy
- Self-reported health status with the HAQ and SF36 questionnaires (188, 189)

3.2.2 The control group

We aimed for a control group consisting of one control person individually matched to an ASS case regarding gender and age (i.e cases and matched controls in a 1:1 proportion). For practical reasons, we decided that the eligible control population should be limited to individuals with a registered home address in Oslo or the surrounding county Akershus by 1st of January 2013 (total denominator population of approx. 1.1 million). With help from the Norwegian tax authorities using the National People Register of Norway we received a randomly collected list of 700 eligible controls, i.e. 10 controls for each ASS case. Out of these 10, one control was randomly chosen either by the principal investigator, the study nurse or the coordinator of NOSVAR. The controls were contacted by regular mail. If a positive response to participate, they were called up and asked specifically about medical history, physical status and current medications.

Controls were excluded if they; (A) were not able to walk > 1 km without any discomfort, (B) had a history of cardiovascular disease and (C) had malignant disease declared as in remission for ≤ 5 years. Primary prevention for cardiovascular disease and hormone replacement therapy were allowed.

A first positive response rate of ca 60 % was received, but we were not able to find a correspondent healthy female control born in 1933. Hence, the control group consisted of 67 participants. The controls performed the same examinations as the patients except for the HRCT of the lungs. The examinations, except for the muscle-MRI were done in one day. Time between the muscle-MRI and the other examinations was in the majority of the controls no more than four weeks.

The study inclusion started in September 2011 and ended in June 2014. All controls and all patients but one was examined at the Department of Rheumatology at OUH. The last patient was examined in the local hospital due to the patient's physical status.

3.3 Specific examinations

3.3.1 Pulmonary Function Test, PFT

PFT included dynamic spirometry (forced vital capacity and gas diffusion capacity, using the Vmax V6200 automated systems (SensorMedics, VIASYS Respiratory Care inc, Yorba Linda, CA, USA) Recorded variables were in Paper 1 Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1), and the transfer capacity of carbon monoxide (DLCO). In paper II the DLCO divided by alveolar volume (VA) (DLCO/VA), was added. Values were expressed in absolute figures and as percentage of predicted normal values. In paper IV the ratio between FVC/DLCO for each patient were calculated, as a method to detect possible PH. All tests were performed according to the guidelines of ATS-European Respiratory Society (ERS) (190, 191). Reference values for both patients and controls were those recommended by the ERS, except for the FVC/DLCO ratio used in paper IV (192).

3.3.2 HRCT of the lungs

Volumetric multi detector CT was performed in the supine position during breath-holding and deep inspiration with supplementary scans in the prone position when necessary. The images were reconstructed both at 1-1.25 mm section thickness with 10 mm intervals and at 2.5 mm section thickness reformatted in three planes. No intravenous contrast material was used.

In Paper I, the ILD extent % was retrospectively evaluated by volume of total lung parenchyma affected. This was also done in Paper II, including description of specific findings as reticular pattern, ground-glass opacities, airspace consolidations, parenchymal bands, subpleural curvilinear lines and traction bronchiectasis (193). Reticular pattern was classified into three grades; 1) A fine intralobular pattern without evident cysts, 2) A pattern with predominant small cysts involving air spaces smaller than or equal to 4 mm in diameter and 3) A pattern with larger cysts involving air spaces above 4 mm (194).

The ILD changes were reviewed by two different methods; (A) Area measurements were done by drawing a freehand region of interest on the screen to score overall extent of ILD on ten CT sections evenly spaced throughout the lung, and relate this to the total lung volume. The results were expressed as percent ILD of total lung volume (195). (B) Total ILD score was determined by assessing abnormalities consistent with ILD in eight zones, four for each lung. The extent of involvement was evaluated independently for each zone, and each zone was assigned a score (0-10) based on the percentage of lung parenchyma that showed evidence of ILD. Total ILD scores were calculated by adding up scores from the eight lung zones for each patient; giving a maximum score of 80 (195).

The measurement of the Pulmonary artery diameter (PAd), a method we used in Paper IV to assess suspect PH was retrospectively measured in lung CT images at the plane of its bifurcation, at a right angle to its long axis just lateral to the ascending aorta.

3.3.3 The six minutes walking distance, 6MWD

The 6MWD was performed on a flat walking-lane of 35 m and according to ATS guidelines (196), used in Paper II for comparison between ASS patients and controls including assessment of possible correlations with pulmonary function.

3.3.4 Muscle MRI of the thighs

The muscle MRI of the thighs was evaluated in patients and controls in Paper III. The MRI was performed using a 1.5 Tesla MR scanner (Avanto Syngo B19) with coronal and axial T1-weighted spin echo and short T1 inversion recovery (STIR) sequences of the thigh muscles. The muscles were evaluated by two experienced muscle radiologists in the anterior, posterior and medial compartment without information on the participant's status. The evaluation included extent and intensity of muscle edema (grading scale 0-3), presence of fascial edema, presence of muscle volume reduction and grade of fatty replacement (grading scale 0-5) (197). For extent of edema grade 0 was defined as no edema, grade 1 as < 33.3 % (minor extent),

grade 2 as > 33.3 % < 66.6 % (moderate extent) and grade 3 as > 66.6 % (major extent). The signal intensity of the edema was graded as 0= no signal, 1 = low, 2 = moderate and 3 = high signal intensity. Fatty replacement was scored according to the grading system suggested by Gouttalier (198); 0 = no fat, 1 = fatty streaks, 2 = muscle greater to fat, 3 = muscle equal to fat, 4 = muscle less to fat and 5 = muscle totally replaced by fat. A Gouttalier score ≥ 2 was considered pathological.

Fascial edema was defined as abnormal thick deep fascial layer with increased STIR signal. Muscle volume reduction was only noted when there were obvious findings; Asymmetry and/or disproportionate muscle compartments. Two arbitrary scores were derived from this assessment; (A) a total edema score with a maximum score of 42 consisting of three components; edema extent (18), edema intensity (18) and presence of fascial edema (6); and (B) a total damage score (maximum score 36) including fatty replacement (30) and presence of muscle volume reduction (6). Adding A and B gave a total MRI score of 42+36 = 78.

3.3.5 Test of muscle strength and endurance

Muscle strength were evaluated pre and post Rtx treatment by manual muscle test ad modum Kendall on eight muscles (MMT8) in Paper I (187). Each muscle/muscle group tested in MMT is graded with muscle strength 0-10 and was performed on the patients' dominant side. In Paper II 14 muscles were tested (MMT14) for the possible association between muscle strength and FVC %. MMT14 was also assessed in Paper III, for comparison with healthy controls and possible correlations between CK-levels and muscle-MRI findings. In this paper we made a revised MMT (MMT4) with four muscle groups (hip flexion/abduction, knee flexion/extension) for direct comparison with the muscle MRI of the thighs.

Test of muscle endurance was made by Functional Index 2 test (FI2) which consisted of repetitive exercises for head-shoulder-hip-knee-and calf muscles (186). The FI2 was assessed in Paper III, for the same reasons as mentioned above. It was slightly revised with maximum 30 head-lifts instead of 60. Table 6 demonstrates the14 muscles/muscle groups tested in MMT14, including the different endurance exercises in the FI2 test used in this study. Table 7 shows the definition of the muscle strength grading in the MMT.

Table 6; The manual muscle test and Functional Index test 2 used in this study

Manual muscle test, MMT (187)	Functional index test 2, FI2 (186)
Neck flexion / extension (0-10 / 0-10) Shoulder abduction (0-10) Shoulder elevation (0-10) Elbow flexion (0-10) Wrist flexion / extension (0-10 / 0-10) Hip flexion / extension (0-10 / 0-10) Hip abduction (0-10) Knee flexion / extension (0-10 / 0-10) Ankle flexion / extension (0-10 / 0-10)	Head-lift from lying position, 0-30 rep Shoulder flexion, 0-60 rep Hip flexion, 0-60 rep Step test, 0-60 rep Heel raises, 0-120 rep
Max points; 140	**Max points; 330**

rep = repetitions

Table 7 The grading of muscle strength in the MMT, according to Kendall (187)

MANUAL MUSCLE TESTING PROCEDURES

Key to Muscle Grading

	Function of the Muscle	Grade		
No Movement	No contractions felt in the muscle	0	0	Zero
	Tendon becomes prominent or feeble contraction felt in the muscle, but no visible movement of the part	T	1	Trace
Test Movement	**MOVEMENT IN HORIZONTAL PLANE**			
	Moves through partial range of motion	1	2-	Poor-
	Moves through complete range of motion	2	2	Poor
	ANTIGRAVITY POSITION	3	2+	
	Moves through partial range of motion			
Test Position	*Gradual* release from test position	4	3-	Fair-
	Holds test position (no added pressure)	5	3	Fair
	Holds test position against slight pressure	6	3+	Fair+
	Holds test position against slight to moderate pressure	7	4-	Good-
	Holds test position against moderate pressure	8	4	Good
	Holds test position against moderate to strong pressure	9	4+	Good+
	Holds test position against strong pressure	10	5	Normal

Modified from 1993 Florence P. Kendall. Author grants permission to reproduce this chart

3.3.6 Echocardiography (echo)

Echo was assessed in paper IV to estimate the pulmonary arterial pressure (estPAP), the method most commonly used in the clinic for PH screening (199). We utilized a Vivid E9 ultrasound scanner (GE - Vingmed Ultrasound, Horten, Norway) to perform two dimensional and doppler echo. A minimum of three cardiac cycles were recorded, analysed and averaged. Transtricuspidal antegrade and retrograde flow velocity were obtained. Peak right ventricular pressure was assumed to be the sum of transmitral gradient during systole, and right atrial pressure estimated by vena cava inferior diameter (> or < 2 cm) and variation in diameter during respiration (55-35% reduction in vena cava diameter during inspiration). Analyses were performed blinded to clinical information.

3.4 Statistics

Statistical Package of Social science (SPSS) was used for statistical analyses; In Paper I version 21.0 was used, in Paper II-IV we used version 22.0. In all four papers statistical significance was defined as $p<0.05$.

3.4.1 Descriptive data

Descriptive data are presented as mean with standard deviations (SD) in normal distributed variables (Paper II-IV). Skewed distributed variables are in Paper I-IV presented as median with range, in Paper III also with 25^{th}, 75^{th} percentile. Dichotomous variables are presented in total number and percentage.

3.4.2 Test for comparisons

In Paper I PFT, ILD extent, MMT, CK and anti-Jo1 levels before and after Rtx treatment were compared by Wilcoxon signed-rank test. The comparisons between the ASS patients and the controls in Paper II and III were in normally distributed continuous variables evaluated by Student's t-test using paired samples t-test, in skewed distributed variables with Wilcoxon signed-rank test/ Mann-Whitney U-test. For subgroup analyses in the same papers, including the group analyses in Paper IV, the Mann-Whitney U-test was used. The Chi-square test evaluated dichotomous variables and was used in paper II-IV.

3.4.3 Correlations

The possible clinical correlations regarding the different pulmonary and muscular outcomes in patients and controls were tested by Pearson correlation in Paper II and III. In Paper III possible correlations between different MRI scores and subsets of ASS groups were tested by Spearman rank correlation.

3.4.4 Multivariable analysis

In paper II multivariable analysis were assessed by linear regression analysis with FVC%, DLCO% and extent of ILD % as dependent variables and disease duration, ASS subgroup, SS-A positivity and acute onset of ILD as independent variables. In paper III the dependent variables were total edema score and total damage score with age, gender, disease duration, SS-A positivity, anti-Jo1 positivity, CK level at diagnosis and ongoing treatment as independent variables.

3.5 Ethical aspects

All participants of this study (N=135) were informed of the study's purposes before inclusion and provided a written consent. The study was approved by the regional committee of health and medical research ethics of Southeast Norway in May 2011 (2011/895).

4 Summary of results

4.1 Paper I

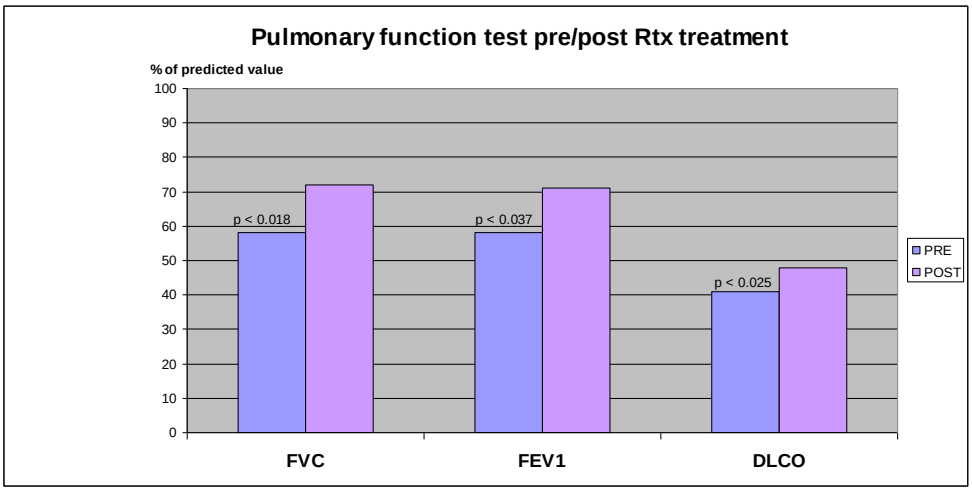
Long-term experience with rituximab in anti-synthetase syndrome-related interstitial lung disease

The primary objectives for this study were to retrospectively evaluate the efficacy and safety of Rtx treatment in ASS patients with severe ILD. Secondary objectives were to evaluate muscle strength, CK levels and anti-Jo1 titers before and after Rtx.
In total 34 ASS patients had been treated with Rtx, 24/34 with indication severe ILD and with a follow-up post Rtx treatment of >12 months (median 52 months). In these 24 patients, median % of predicted FVC, FEV1 and DLCO increased 24 %, 22% and 17 % post-Rtx, respectively. Seven patients (all with disease duration <12 months and/or acute onset/exacerbation of ILD) had >30% improvement in all three PFTs. HRCT analyses demonstrated a median 34% reduction in ILD extent post-Rtx treatment.

MMT8 increased from 93% to 98% of maximum score after treatment with Rtx ($p<0.05$). Median CK value was before Rtx 990 U/l and after Rtx 88 U/l ($p<0.002$). 9/17 patients had available anti-Jo1 titers pre and post treatment; All nine had a reduction in the titers with a median decrease of 33 % ($p<0.008$).

In total 7/34 ASS patients (21%) died during follow-up. 6/7 deaths were possible related to infections, all but one occurring > 6 months after Rtx-treatment. Non-fatal SAE;s were reported in seven patients, six of these were infections. 4/34 patients had verified infection with P. Jirovechii. Mortality-rate in the Rtx-treated group was comparable to the remaining ASS cohort (25/78 deceased; 32%).

Figure 6



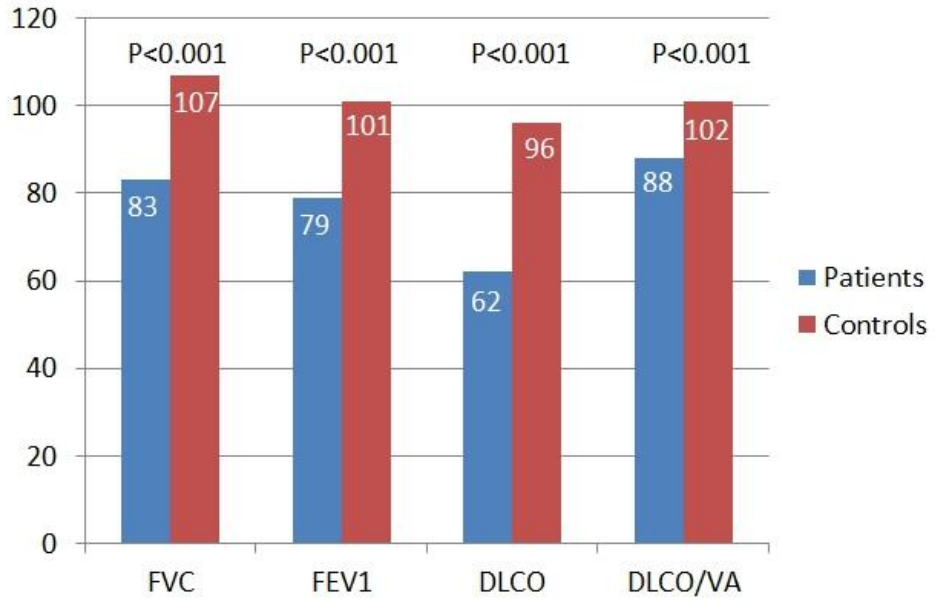
4.2 Paper II

Pulmonary involvement in the Antisynthetase syndrome; A comparative cross-sectional study

In this study, the aims were to 1) Compare pulmonary function tests (PFTs) and six minute walking distance (6MWD) between 68 ASS patients with median 71 months disease duration and healthy, sex-, and age matched controls. 2) Evaluate extent of ILD by lung HRCT in the ASS cohort, and assess correlations between PFT measurements, 6MWD and ILD extent. 3) Evaluate any possible differences in pulmonary outcome between the Jo-1 and non Jo-1 subsets.

The measurements of FVC, FEV1 and DLCO were mean 28%, 27% and 53 % lower in the ASS patients compared to the controls ($p<0.001$). A significant difference in 6MWD was demonstrated, with controls walking mean 116 m further compared to the patients ($p<0.001$). In the patients, 6MWD correlated significantly with FVC ($p<0.01$) and DLCO ($p<0.01$). The ILD extent was median 20 % of total lung volume and median total ILD score (maximum score 80) was 16 in the ASS cohort. The ILD extent was negatively correlated to FVC% (r-0.348, $p<0.01$) and DLCO% (r-0.539, $p<0.01$). No significant differences in FVC, FEV1, DLCO, DLCO/VA, ILD extent % or total ILD score were demonstrated between the Jo1 positive and negative subsets. In the patients there was a slight increase in FVC%, FEV1% and DLCO% from baseline to follow-up, however not significant.

Figure 7 Pulmonary Function in ASS patients and healthy controls, % of expected values



4.3 Paper III

Comparative analyses of muscle MRI and muscular function in Anti-Synthetase Syndrome patients and matched controls; A cross-sectional study

Magnetic Resonance Imaging (MRI) of thigh muscles is increasingly used to assess disease activity and damage extent in chronic myositis, but the validity of the findings is not clear. Additionally, little is known about possible muscle MRI changes in healthy population. Here, the primary aim was to describe and compare thigh MRI findings in patients with chronic ASS-associated myositis and matched healthy controls. Secondary aims were to 1) evaluate muscle outcome and potential muscle-MRI patterns in ASS including different subsets of ASS, 2) assess possible correlations between muscle-MRI changes, CK levels and muscle strength in both patients and controls.

The total MRI score evaluating muscle edema, fascial edema, fatty replacement and muscle volume reduction (max 78p) was higher in the ASS patients than in controls, 14.1 p and 3.0 p, respectively ($p<0.001$). Muscle edema was more frequent in ASS than controls (38% vs 10%) as was fatty replacement (42% vs 4%). In the compartment analyses of the ASS group muscle edema was most pronounced anteriorly, while fatty replacement dominated posteriorly. CK levels correlated with total edema score (max 42p) in the patients but not in controls. However, 23% of the patients with normal CK had a total edema score of >18 p.

The ASS group had significantly lower muscle strength scores assessed by MMT14- ($p<0.001$), MMT4- ($p<0.004$), and FI2 ($p<0.001$) tests than the matched controls. In patients, significant correlations were seen between total damage score, MMT14 (r-.340, $p<0.01$), MMT4 (r-.344, $p<0.01$) and the FI2 test (r-.484, $p<0.01$). None of the three muscle strength tests correlated with the total edema score.

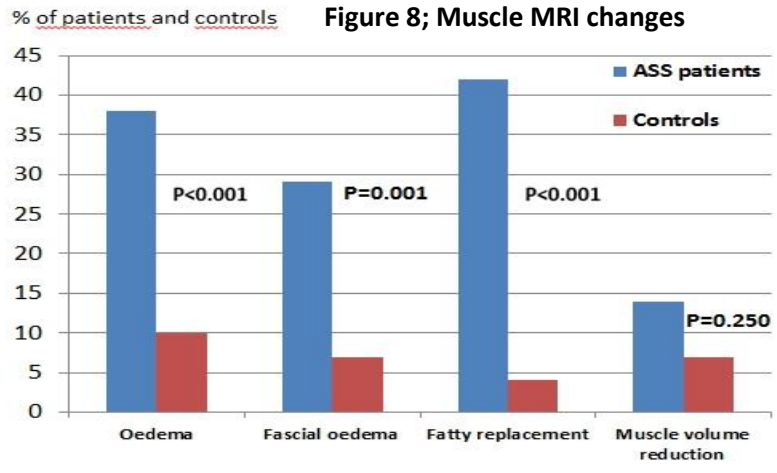


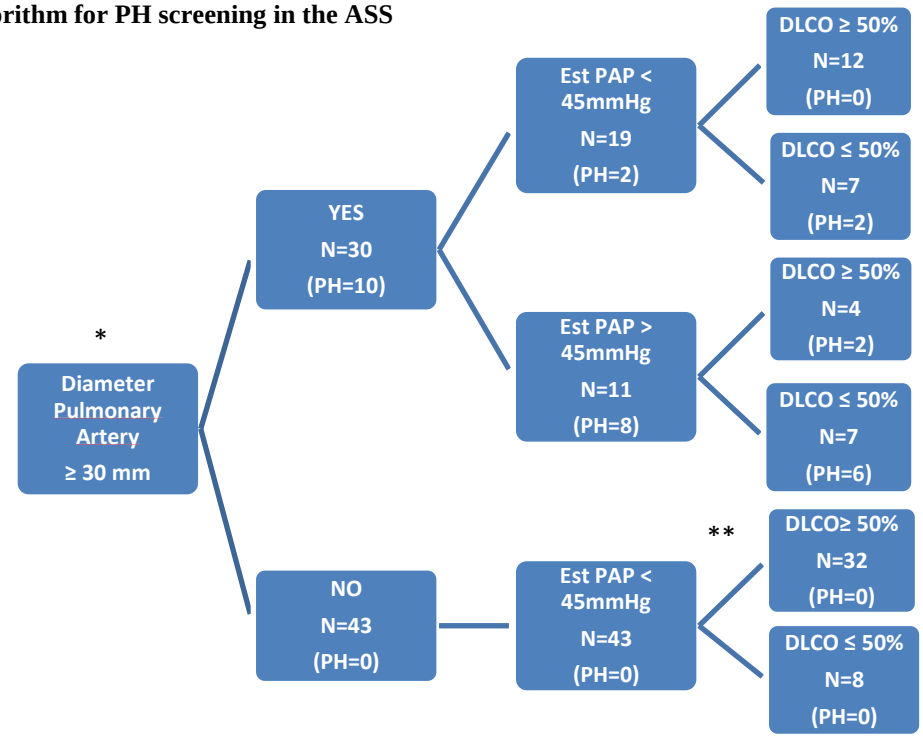
Figure 8; Muscle MRI changes

4.4 Paper IV

Detecting pulmonary hypertension in the Anti-synthetase Syndrome; Performance of a simple clinical algorithm

The aim of this study was to test an algorithm for detection of pulmonary hypertension (PH) in ASS, useful in daily clinical practice by evaluating four different methods commonly used in PH screening; (1) the diameter of the Pulmonary Artery (PAd) in lung HRCT (cut off value 30 mm), (2) the estimated pulmonary artery pressure (estPAP), cut off value 45mmHG, by echocardiography, (3) gas diffusion capacity (DLCO) with cut off value 50 % and (4) ratio of forced vital capacity (FVC) to DLCO, with cut off value of 1.6, by pulmonary function test. All algorithm parameters, except FVC/DLCO ratio, differed significantly between the patients with confirmed PH (n=11) and the remaining cohort (n=63) (Table 1). All patients with confirmed PH had positive cut-off values for the PAd (sensitivity 100%) while the method estPAP had a specificity of 95%. Combining the measurements of PAd, estPAP and DLCO predicted PH in 86% of cases. None of the patients with negative values of PAd and estPAP (n=43) had confirmed PH (negative predictive value of 100%).

Figure 9
Algorithm for PH screening in the ASS



* Measurements of the Pulmonary Artery diameter (PAd) were available in 73 out of total 74 patients.
** DLCO measurements in patients with negative cut offs values for PAd and estPAP were available in 40 out of total 43 patients.

5 Discussion

In this study we wanted to evaluate the total impact ASS has on patients with established disease. A similar approach has been used in previous retrospective multi-center studies as well (2, 17-19). However, this study is, to our knowledge, the first to apply a cross-sectional, comparative design. When performing this type of clinical study several aspects have to be considered;

5.1 Methodological aspects

5.1.1 Definition of ASS

How to define/classify ASS? Two proposed classification criteria of ASS exist (41, 87), but they are not universally accepted, nor widely used. In this study, we selected for a pragmatic ASS definition in which a patient was defined as having ASS if he or she had a positive test of one of the eight known aaRS-antibodies, ILD *and/or* myositis (Box 1). We believe that application of the proposed criteria by Connors et al, with the required positive aaRS-antibody but only one additionally clinical item would have resulted in a more heterogeneous study cohort. The criteria proposed by Solomon et al are more stringent; They do not just require one or two major clinical items, but also at least one minor clinical item. Since several authors describe development of new clinical features during the clinical course of ASS (2, 3, 16) we believe there would have been a possibility of missing cases using the Solomon classification criteria for this study.

Additionally, one could discuss *if* ASS is a distinct clinical entity. We know that especially anti-Jo1 positivity is occasionally seen in patients with diagnosed seropositive rheumatoid arthritis (RA), systemic sclerosis (SSc) and primary Sjögrens syndrome (pSS). Each one of these diseases can have common features with ASS, for example arthritis, sclerodactily and ILD (15, 66, 200). Since no widely accepted ASS criteria exist and the fact that ASS in itself is heterogenous (11, 18), these patients could be defined as “overlap” or perhaps undifferentiated connective tissue disease (UCTD). If ILD is a major feature in these patients, Fischer et al have suggested classification criteria for ”interstitial pneumonia of autoimmune features” (IPAF) (201). Several clinical features of ASS are included in these criteria (201). However, the recent years “discoveries” in the genetics, immunology and pathology of the ASS (25, 27, 88), make us believe that ASS should be defined as a distinct clinical entity.

5.1.2 Study design

Since ASS is an uncommon disease it is difficult to include a relevant number of patients in a limited period of time. This is the main reason why we chose a cross-sectional design, and not a prospective, for this study (Paper II and III). Our ASS-cohort includes patients with disease from the early 1980:s, hence, the median disease duration is several years, suitable when analyzing long-term outcomes. The limitation with this design is of course that evaluation of repetitive clinical measurements and possible predictive factors not can be assessed.

To emphasize the disease impact on clinical outcomes and daily life we chose to compare each patient with a healthy control. A larger control group would have strengthened the study further, but was restricted due to economic aspects. By including healthy participants the discussion about "what is healthy?" came up; Should a person on blood pressure treatment and stable blood pressure be included? A person who had been treated for malignancy? In this study we have tried to be rather stringent to the definition of "healthy" (see page 23) and we believe that this resulted in a rather homogenous control group. Two controls were excluded from the study due to psychiatric condition and post burn-injuries. Three controls were after the examination referred to further investigations due to positive findings at the echocardiography (N=2) and the MRI examination (N=1).

Paper I and IV have a retrospective design. The preferred study design for these two papers would have been a prospective design; A randomized controlled trial (RCT) evaluating Rtx treatment in Paper I and a prospective study with all screening methods for PH, right heart catheterization (RHC) included, in Paper IV. To do this in the ASS, within a respectable period of time, you would most probably have to do a multi-center study to reach an acceptable number of participants. Well aware of this limitation, we still believe that the results analyzed in these two papers are reliable and clinically useful in the assessment of ASS.

5.1.3 The study population

The majority of the ASS patients come from Oslo and the south-east region of Norway. All participants in the control group come from Oslo and its' surroundings. It would have been an advantage with geographically matched controls as well, but was in this study restricted due to practical reasons. All controls were also examined after the patients; The controls were in mean 14 months older than the patients when the examinations were made. This means that

there is a possibility for underestimated differences between patients and controls both in pulmonary and muscular outcome.

5.1.4 Methods evaluating HRCT images

There exist several methods to evaluate ILD extent and type of lung parenchymal abnormalities in HRCT images (34, 202-205). We used two methods for evaluation of ILD extent, developed from our Department of Radiology; 1) Drawn freehand regions of interest in 10 thin sections related to the total volume of lung parenchyma (Paper I and II). 2) Division of the lungs into eight zones, each zone graded (0-10) in terms of ILD extent, with maximum extent score of 80 (Paper II). We believe that these methods give good accuracy on both total and regional ILD extent compared to other used methods for this purpose (204, 205). In Paper II we also described the frequency, but not the specific CT-pattern, of the different CT-abnormalities according to Fleischner Society (193). It would have been of interest to evaluate specific CT patterns in HRCT images from baseline to follow-up, but due to limited baseline HRCT data at time of the study period this was not done.

5.1.5 Methods used in muscle-MRI score

In Paper III we used a muscle-MRI scoring system with both dichotomous and continuous scales. The continuous scales of muscle edema and fatty replacement allowed us to assess these MRI changes more subtle and precise, an advantage not only in evaluating myositis activity and chronical changes but also in the direct comparison between patients and controls. With our MRI score we were also able to grade both the edema extent and edema signal intensity. Other studies on MRI in myositis have evaluated presence and grade of edema extent, but not, as far as we know, the signal intensity of the edema (206-208). We believe that the signal intensity analyses provide additional information on myositis activity, but this needs to be confirmed in longitudinal studies.

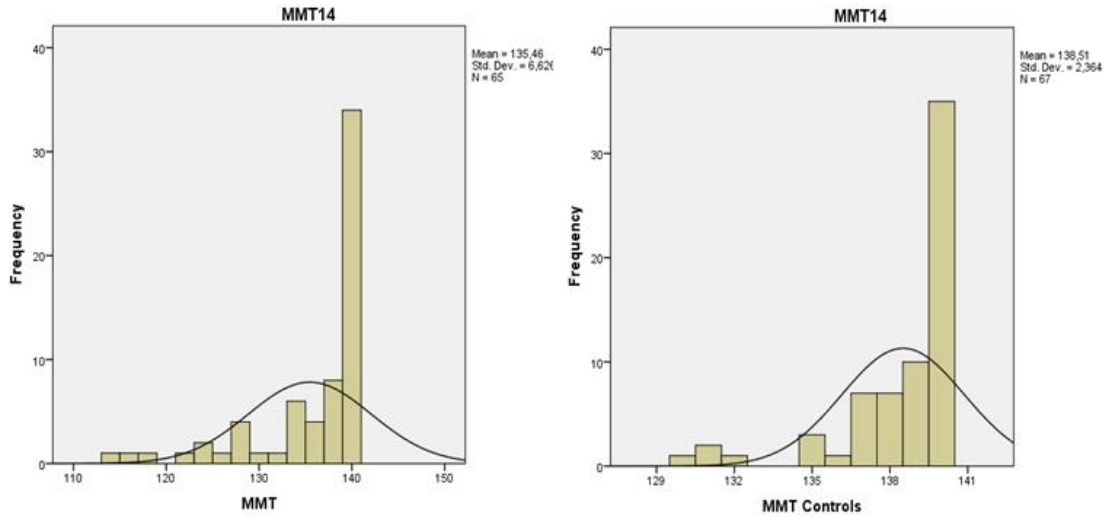
Muscle volume reduction/muscle atrophy is more difficult to assess since no gold standard of normal muscle volume exists. Evaluating minor muscle volume reduction in myositis could be challenging since the atrophy often is symmetric and the comparison with the contralateral muscle would not be reliable (208). We therefore used a dichotomous scale (present/not present) to assess muscle volume reduction with stringent criteria of “present”. With this method it is possible that the presence of muscle volume reduction could be underestimated, as also discussed in Paper III.

5.1.6 Statistical aspects

As seen in Table 1 in Paper III the distribution of the results of the MMT14 and MMT4 test for both patients and controls are (almost) interchangeable , although there is a highly significant difference in both test between the groups. Neither in the patients nor controls are the results of MMT14 and MMT4 normally distributed, exemplified with the MMT14 results below;

Figure 10

Frequency of the MMT14 test in patients and controls



It is possible to transforme the data (by reflect and logarithm) to equalize the skewness and thereafter analyze the results. However, for this study we chose to present the original data as median with the 25^{th} , 75^{th} percentile to discriminate the results better. The comparative analyses of MMT14 and MMT4 were made with the non-parametric Wilcoxon signed-rank test.

5.2 Discussion of the results

5.2.1 Pulmonary outcome in the ASS

Not surprisingly, the pulmonary outcome measured by PFT was significantly worse in the patients compared to the controls. No other study has compared ASS patients with healthy controls and very few studies have reported quantitative follow-up PFT measurements in the ASS. Interestingly, Trallera-Araguas et al demonstrated in their anti-Jo1 positive cohort with

median disease duration of 78 months a mean FVC% of 72%, comparable to our findings with a mean FVC of 83 % (3). 21/68 patients had at study inclusion been treated with Rtx. Analyses of the DLCO% and FVC% in Rtx treated compared to Rtx naïve patients revealed no significant difference, but a trend towards lower DLCO% and FVC% in the Rtx treatment group was found (median DLCO; 81% and 89%, median FVC; 53% and 67%, respectively). However, the ILD extent differed significantly between Rtx treated and Rtx naïve patients; mean 31% and 20%, respectively ($p<0.036$). The difference could be explained by a more severe pulmonary involvement in the Rtx treated patients.

In Paper I, the retrospective analyses of Rtx treatment in ASS patients with severe lung disease (N=24) demonstrated an overall significant increase in PFT and decrease in ILD extent with this treatment. The results are consistent with other retrospective studies evaluating the pulmonary component in Rtx-treated ASS patients (143, 144). Notably, subgroup analyses from our study demonstrated best response with Rtx in patients with disease duration < 12 months and/or acute onset/exacerbtion of ILD. A limitation of our study, as in the study from Bauhammer et al (143), is that in the majority of patients other immuno suppressives were given in close connection with Rtx. However, there are prospective studies with demonstrated efficacy of Rtx monotherapy in ASS related ILD; In a Phase II study, Allenbach et al demonstrated improvement and stabilisation of the pulmonary function in the majority of the patients (130). Post-hoc analyses from the RIM study (128) revealed anti-Jo1 antibody as a predictor of shorter time to response compared to patients with negative MSA;s (129). Additionally, the same study demonstrated a trend towards shorter time to response in patients with interstitial lung disease (129).Taken together, anti-CD 20 therapy could be a treatment choice in ASS related ILD.

The assessment of ILD extent has been suggested as one of two core domains in clinical trials with CTD-related ILD. The other domain is FVC% (209). In our study (Paper II) FVC% correlated with the ILD extent% but we also found a slightly higher correlation between DLCO% and the ILD extent%. Reasons for this could be that our study cohort were rather homogenous in terms of few smokers (9/68) and only 5/68 had signs of emphysema, two known causes with the possibility to reduce DLCO%.

In the ASS cohort we compared baseline FVC%, FEV1% and DLCO% measurements with those at follow-up; A trend of improvement was found in all three measurements. These findings are consistent with other outcome studies in ASS, demonstrating

stabilization/improvement in the majority of the patients (3, 19, 45). In contrast to other studies (2, 18) where pulmonary outcome is worse in anti-Jo1 negative subsets, no significant differences in PFT or ILD extent were found between anti-Jo1 positive/negative subsets. The fact that the Jo-1 negative group had significant less disease duration compared to the anti-Jo1 positive group could have contributed to these results.

In paper II, we did not specifically evaluate treatment history of the ASS patients, neither did we assess specific CT patterns in the HRCT images; Limitations of this study, since both possibly affect the pulmonary outcome in ASS. As earlier described, the CT pattern most commonly found in the ASS is NSIP with predominantly basal distribution (31-36). Interestingly, we found that the ILD changes in > 80 % of the ASS patients were distributed in a specific wedge-shaped pattern, as far as we know not previously described. What this may represent is for us at present time unknown, but would be very interesting to investigate further.

5.2.2. Muscle outcome in the ASS and controls

Significant differences in the muscle MRI, muscle strength and endurance were demonstrated between patients and controls. Of the three muscle tests performed (MMT4, MMT14 and FI2), it was in the latter the difference between patients and controls was most obvious. Interestingly, as far as we know, there are no studys in healthy population on possible MRI changes evaluating what is normal/unnormal. We know that muscle edema increases after physical activity; How much edema do you "need to have" to define it as pathological? Could muscle MRI changes appear/disappear with age as a "natural" course? Our study revealed that all muscle MRI changes assessed (muscle edema, fascial edema, fatty replacement and muscle volume reduction) also were found in the healthy controls. However, only six controls had concurrent MRI-changes, but none had all four variables concomitantly. Importantly, the MRI changes of the controls were also considerably less pronounced compared to the ASS patients.

The frequency of "fatty streaks" (Goutallier score =1) was, maybe surprisingly, higher in controls than in the patients (40% and 33%, respectively). A Gouttalier score =1 is defined as normal; Nevertheless, the fatty replacement score correlated with age both in patients and controls. This indicates that "fatty streaks" could be a natural finding in "aging muscle".

CK levels correlated with total edema score, this tested with both continous and dichotomous CK-scales. This was also demonstrated by Barsotti et al (206) but Pipitone reported a discrepancy between the presence of edema and CK levels in PM/DM patients (208). To some extent the divergent results could be due to unadjusted CK values, i.e. adjusted for gender, age and muscle mass. Another possible cause is different cut-off values for pathological CK. Although we found a correlation between CK levels and total edema score in our study, there was 23% of the ASS patients with normal CK values who had a total edema score of >18 (max points 42). Furthermore, the 13 patients with no myositis diagnosis at study inclusion had all normal CK values but significantly higher MRI scores than the healthy controls. This indicates that muscle MRI could be a useful clinical tool in managing ASS-related myositis.

In the ASS patients, the MMT and FI2 tests did not correlate with total edema score or CK-levels, but with total damage score. As many as 61% of the patients had a fatty replacement score of ≥ 6 (max points 30) and 26% had concomitant signs of muscle damage and activity changes. Hence, muscle MRI can give us complementary information when for example evaluating treatment strategies in the ASS.

Any specific pattern for the different muscle changes in ASS were not found; edema was most frequent anteriorly, fatty replacement posteriorly, as also seen in muscle MRI evaluation of PM, especially immun mediated necrotizing myopathy (IMNM) (207, 210). A limitation of our study is, since we don't have any baseline muscle MRI data, that we do not know if muscle edema preludes fatty replacement in the posterior compartment; To note, the controls with fatty replacement (Gouttalier score >2, N=3) all had their changes in this compartment.

5.2.3 PH screening methods in ASS

The PH frequency of our ASS study cohort was 15 % (11/74). In the total ASS cohort (N=96) the frequency of PH is 11.5 %. The results are consistent with other reported frequencies (7-15%) in the ASS (3, 15, 80). Here, again, the freqeuncy can vary because of small cohorts and different classification of ASS.

Echocardiography is the preferred recommended non-invasive method for PH screening (199). In this study we used evaluation of estPAP as commonly used in other CTD-related PH screening studies (211-213). However, if we only had used this method we would have missed 2/11 patients with PH, although we used a rather strict cut-off value for estPAP (45mmHg). The finding in our study of a 100% sensitivity for PH by increased diameter of

the Pulmonary Artery (PAd) made us try out this clinical algorithm; All patients with a negative PAd test (N=43) also had a negative test of estPAP. None of these patients were diagnosed with PH, i.e a negative predictive value (NPV) of 100 %. Interestingly, in patients who had performed RHC, we found that the increase in PAd over time, measured from baseline to time of RHC, were significantly higher in patients with PH compared to patients without PH. Hence; PAd change over time could be a complementary tool in PH screening in the ASS.

The PAd has earlier been assessed as a single screening method for ILD related PH with a sensitivity of 87% and specificity of 89 % (214). However, with a specificity of 68 % as was demonstrated in our study, the utility of PAd as a single PH screening method in ASS is probably limited.

PAd is included in the PH screening algorithm of Australian Scleroderma Interest Group (ASIG) (213), but not in the "Detection of pulmonary arterial hypertension in Systemic sclerosis" (DETECT) algorithm (215). Both these algorithms include assessment of other clinical variables, but not PAd, before referring to echo, for example NT-proBNP; A possible limitation of our study since NT-proBNP wasn't assessed. Another limitation of our study is the possibility of missing PH cases since not every patient in the study performed RHC. Prospective studies including RHC are therefore highly warranted.

6 Conclusions

6.1 Conclusions of the study

- ASS has a major impact on pulmonary and muscular outcome in long standing disease. Highly significant differences in lung and muscle function between ASS patients and age and gender matched healthy controls were demonstrated, with worse outcome in the patients (Paper II and III).
- No difference in the pulmonary outcome between Jo-1 and non Jo-1 subsets were revealed, but the disease duration differed significantly between the groups and likely interfered with the results (Paper II).
- In the ASS patients, the ILD extent correlated significantly with FVC% and DLCO%. A specific wedge shaped distribution of the CT patterns on HRCT was seen in >80 % of the patients with so far unknown significance (Paper II).
- Anti-CD 20 therapy is a treatment option in ASS-related ILD, especially in patients with disease duration less than one year and/or acute onset/exacerbation of ILD. Both the PFT and ILD extent improved after anti-CD 20 therapy (Paper I).
- With median disease duration of six years, 65% of the patients had muscle MRI abnormalities compatible of muscle damage and/or muscle activity, 26 % of the patients with concomitant changes of damage and activity (Paper III).
- Significant differences in muscle MRI abnormalities were found between Jo1 positive and negative subsets with higher MRI scores in the Jo1 positive group (Paper III).
- Muscle-MRI is a possible complementary tool in the assessment of ASS; CK levels correlated with total MRI score, but 23 % of the patients with normal CK values had signs of active muscle disease on MRI. Patients with no previous diagnosed myositis had significantly higher total MRI score compared to healthy controls. Muscle strength did not correlate with muscle activity in MRI, but with muscle damage (Paper III).
- Evaluation of the PAd on HRCT images could be a useful complementary screening method of PH in the ASS; While 2/11 patients with confirmed PH had negative cut-off value for estPAP but positive cut-off value for PAd, all patients with negative cut-off values for PAd had negative cut-off values for estPAP and no one was diagnosed with PH (N=43) (Paper IV).

6.2 Clinical implications

Although not a RCT, our study indicated beneficial effects of anti-CD20 therapy in ASS associated ILD. Response in muscular outcome was also seen. Anti-CD20 therapy seems to be a reasonable choice as induction and maintenance therapy in ASS patients with acute ILD. It could be a treatment option to the, at present time, more commonly used Cyc in these cases. However, prospective studies are definitely needed to evaluate possible advantages/disadvantages with these two treatments.

ASS has a major impact on lung and muscular function. By demonstrating this we want to emphasize the need for regularly assessment and development of effective treatment strategies in ASS. Muscle MRI has been shown to be a clinical complementary tool in the assessment of ASS. Not only is it useful in differentiating muscle activity from muscle damage, and thereby evaluate possible treatments; It could also be helpful to evaluate the grade of activity since CK levels and test of muscle strength not always correspond to the MRI findings.

Another clinical implication of this study is the use of the PAd as a complementary tool in ASS related PH screening, especially in combination of the estPAP; A negative cut-off value for both PAd and estPAP gives a very low risk of PH. Additionally, it seems that the PAd increase measured over time is associated with PH.

6.3.Future perspectives

As seen in the chapter of methods we have performed several other examinations besides those reported in the current study;

We plan to;

1) Assess possible cardiac abnormalities seen at echocardiography in ASS patients and compare them to controls.
2) Evaluate the capillaroscopy examinations and look for possible correlations with other clinical manifestations in the ASS patients.
3) Evaluate the outcome of the self-reported questionnaires of HAQ and SF-36 and correlate those with other clinical manifestations in the ASS patients.
4) More thoroughly investigate the findings of the wedge-shaped distribution of HRCT changes seen in > 80 % of the patients and compare them with available lung biopsies.

In a larger perspective we want to perform longitudinal evaluations of the HRCT, MRI and echo examinations from baseline to follow-up. These data are now available for analyses in most of the patients, and we believe that they could give us essential information on disease progression, also in relation to treatment and other possible predictive factors.

We are in contact with other (Scandinavian) myositis centers to conduct a RCT on newly diagnosed myositis patients with associated ILD, including the ASS. In this trial we want to evaluate efficacy and safety of anti-CD20 therapy and Cyc.

Finally, we would like to build a prospective cohort study where we have the possibility to include all ASS patients who are and will be diagnosed in Norway. This demands good and reliable interdisciplinary teamwork, which we believe is possible to achieve in our country.

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