Cardiac Risk Profiling in Systemic Sclerosis

Thesis for degree of philosophiæ doctor (ph.d.)

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Abbreviations:

A2C = Apical two-chamber view

A4C = Apical four-chamber view

ACA = Anti-centromere antibodies

ASE = American Society of Echocardiography

ATA = Anti-topoisomerase I antibodies

ANGPT = Angiopoietin

APLAX = Apical long-axis view

BSA = Body surface area

CTD = Connective tissue disease

dcSSc = Diffuse cutaneous systemic sclerosis

DD = Diastolic dysfunction

DLCO = Diffusion capacity of the lungs for carbon monoxide

EACVI = European Association of Cardiovascular Imaging

EC = Endothelial cell

EF = Ejection fraction

ELISA = Enzyme-linked immunosorbent assay

FAC = Fractional area change

FS= Fractional shortening

GLS = Global longitudinal strain

HRCT = High resolution computed tomography

ILD = Interstitial lung disease

LA = Left atrium

LAA4C = Left atrial area in apical four-chamber view

LAA2C = Left atrial area in apical two-chamber view

LAVI = Left atrial volume index

LV = Left ventricle

lcSSc = Limited cutaneous systemic sclerosis

mPAP = Mean pulmonary artery pressure

mRSS = Modified Rodnan skin score

OPN = Osteopontin

PE = Pericardial effusion

PH = Pulmonary hypertension

PAH = Pulmonary arterial hypertension

PLAX = Parasternal long-axis view

PSAX = Parasternal short-axis view

RA = Right atrium

RAA4C = Right atrial area in apical four-chamber view

RV = Right ventricle

RVFWLS = Right ventricle free wall longitudinal strain

SD = Systolic dysfunction

SSc = Systemic sclerosis

TAPSE = Tricuspid annular plane systolic excursion

TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand

TRV = Tricuspid regurgitant velocity

VEGF = Vascular endothelial growth factor

List of papers

Paper I

Left Ventricular Diastolic Function Predicts Mortality in Patients With Systemic Sclerosis.
Tennøe AH, Murbræch K, Andreassen JC, Fretheim H, Garen T, Gude E, Andreassen A, Aakhus S, Molberg Ø and Hoffmann-Vold AH.
Journal of the American College of Cardiology, October 2018; 72 (15): 1804-1813.

Paper II

Systolic Dysfunction in Systemic Sclerosis; Prevalence and Prognostic Implications.
Tennøe AH, Murbræch K, Andreassen JC, Fretheim H, Midtvedt Ø, Garen T, Dalen H, Gude E, Andreassen A, Aakhus S, Molberg Ø and Hoffmann-Vold AM.
ACR Open Rheumatology, June 2019.

Paper III

Serum Markers of Cardiac Complications in Systemic Sclerosis
Tennøe AH, Murbræch K, Didriksen H, Belpario JA, Weigt SS, Palchevskiy V, Fretheim H, Ueland T, Aukrust P, Midtvedt Ø, Garen T, Brunborg C, Molberg Ø and Hoffmann-Vold AM.
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Rationale

Systemic sclerosis (SSc) is a severe connective tissue disease (CTD), and a majority of SSc patients are reported to die from disease related causes (1, 2). Cardiac affection is reported a frequent finding in SSc and is together with pulmonary affection a leading cause of death (3).

Most cardiac SSc studies utilize echocardiography for function evaluation. Echocardiography studies on SSc have often investigated various function parameters, making results challenging to unify (4-6). Conclusions are further complicated by dissimilar cohorts applying differing inclusion criteria. Studies on cardiac SSc are also often cross-sectional (7-9). While this may be a relevant design to estimate the prevalence of cardiac disease, cross-sectional studies cannot describe the chronologic progression of cardiac affliction. These limitations hamper the knowledge on how, and to what degree, cardiac function develops in SSc.

Cardiac affliction in SSc often remains subclinical until considerable organ damage is present, and when symptoms of cardiac disease are present, prognosis is considered poor (10). It is therefore of vital importance to develop screening methods that reveal patients at risk in order to start early treatment, consequently improving life quality and survival.

In this project, we aimed to describe the natural course of cardiac affliction in a large, unselected SSc cohort. Serial echocardiographies from SSc patients were analyzed in order to evaluate cardiac systolic and diastolic function in early SSc. Results were compared to healthy controls. For patients with two or more echocardiographies available, follow-up echocardiographies were analyzed in order to describe the evolution of cardiac function over the disease course. Last, serum samples were collected from patients of early SSc. Samples were analyzed for serum proteins known from cardiopulmonary and fibrotic diseases in order to investigate potential biomarkers of cardiac SSc.

1.0 Introduction:

1.1. General introduction and pathogenesis

Systemic sclerosis (SSc) is a disease hallmarked by a triad of vasculopathy, dysregulation of the immune system and extensive fibrosis, replacing functional organ tissue. The etiology of the disease is unknown, but both genetic factors and immunological responses triggered by environmental and microbial stimuli are suspected to participate in the pathophysiology (11). Although SSc is rare, prevalence and incidence vary across the world, with a higher disease burden in Southern Europe and Northern America, opposed to Northern Europe and Japan (12). In Norway, the prevalence of SSc is reported around 10/100.000 (13).

SSc displays the highest mortality among the connective tissue diseases (CTDs) (2, 14, 15) with a standardized mortality ratio around 3 (2, 14). The prognosis is however highly heterogenic and depends on organ involvement. Today, the most frequent causes of SSc-related mortality are affection of the cardiovascular and/or respiratory system (1). In 2017, a European multicenter study on > 11.000 patients revealed primary heart disease as the main cause of mortality in SSc, highlighting the need for disclosure of cardiac affection at an early stage (3). Combined, interstitial lung disease (ILD), pulmonary arterial hypertension (PAH) and cardiac affection account for almost 90 % of SSc-related mortality (1).

1.2. Pathophysiology and management

The chronology of how immune dysregulation and vasculopathy develop in early SSc is poorly understood. The immunological component of the disease is confirmed by the frequent presence of antibodies directed against nuclear antigens. Vasculopathy leads to luminal narrowing and obliteration of microvasculature harming blood supply (16) in early SSc, while tissue fibrosis is considered a consequence of protracted vasculopathy. Although potentially affecting all organs, SSc most often affects skin, gastro-intestinal tract, lungs, heart and kidneys.

There exists to date no cure for SSc. Treatment is directed against the individual organ manifestations, resulting in numerous treatment options. As mentioned, SSc is hallmarked by a dysregulated immune system, vasculopathy and fibrosis. Conversely, therapeutic strategies include anti-inflammatory agents, vasodilators and anti-fibrotic medication. In recent years, autologous stem cell transplantation (ASCT) has risen as a potential treatment for the disease. This is however a life-threatening procedure with an early treatment mortality reaching up to 10 % (17-19). ASCT is therefore only considered in a small subset of patients presenting poor prognosis for survival, and only before severe organ manifestation has evolved.

1.3. Disease progression

Studies show that severe organ affliction, hereunder cardiopulmonary manifestations, presents already in the early stages of SSc (20, 21). Including patients of early disease in clinical studies is challenging as disease onset is normally in advance of diagnosis, and as the heterogeneous clinical presentation of SSc may cause both patient's- and doctor's delay. Consequently, patients may present with internal organ manifestations at initial clinical evaluation. Categorizing patient phenotypes at risk of organ involvement is therefore a prioritized research subject. However, definitions of organ involvement are not standardized and often differ between studies, making it difficult to compare results (4-6). Today, sex, extent of skin involvement and serum antibodies may aid the physician in predicting risk of organ involvement (21).

In SSc, manifestations of different organs show unequal advancement. Skin fibrosis develops early, affects nearly all patients and regresses over the disease course. ILD and PAH affect only subsets of patients; though when first evident, progress without spontaneous improvement. While PAH may develop at any stage of the disease (22), ILD is reported to progress solely in patients presenting pulmonary fibrosis in early disease (23). It is necessary to map the natural disease course and progressive potential of organ manifestations in order to know when intervention can be of benefit. If cardiac affection were shown to be progressive throughout the disease course, therapeutic interventions may be appropriate. Intervention will however be of

less value if cardiac affliction mainly develops in advance of diagnosis and stabilizes. As mentioned, studies on cardiac SSc have often been cross sectional (7-9), and more research is needed to describe the natural course of cardiac affliction in SSc.

1.4. Outcome measures

In order to define abnormalities, it is vital to establish standardized outcome measures that display organ dysfunction. Outcome measures define the patient's current status and may be used as cut-offs to segregate normal and abnormal organ function and/or structure. In cardiac research, echocardiographic parameters are often used for evaluation of cardiac function. However, mere associations between investigated parameters and outcome measures do not necessarily reflect a pathophysiologic role. It is important to adjust analyses for multiple parameters in order to exclude confounding. Further, prospective studies are needed to evaluate the predictive ability of clinical, seral and imaging markers.

1.5. Organ manifestations of SSc

1.5.1. Raynaud's phenomenon

The initial clinical sign of SSc is Raynaud's phenomenon (16). Raynaud's phenomenon involves spasms of the microvasculature, leading to transient ischemia of surrounding tissue. As the tissue suffers from hypoxia-reperfusion injury, inflammation promotes fibrosis and vital tissue is lost (24). Episodes of Raynaud's phenomenon are apparent in distal extremities as fingers and toes, but altered vasoreactivity is also considered to drive internal organ fibrosis (24).

1.5.2. Skin disease

The skin is the most frequently affected organ in SSc (25), and only approximately 2 % of patients present without skin involvement (26). Based on extent of skin involvement, patients are divided into limited cutaneous (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc) (27). These subsets show clinical relevance as they present differing risk profiles with respect to inner organ manifestation and prognosis (25). LcSSc includes skin affection of the face and extremities

distal to elbow and or knees, while dcSSc in addition comprises skin lesions of proximal extremities and truncus. Patients with dcSSc more often develop ILD, and lcSSc presents higher frequency of PAH, yet a favorable prognosis (1) . Apart from thickening of the skin; digital ulcerations, telangiectasias and calcinosis are common skin complications of SSc. Skin affection is mainly treated by methotrexate and mycophenolate mofetil (28, 29).

1.5.3. Gastrointestinal disease

Next to skin involvement, the gastrointestinal (GI) tract is the most commonly affected organ system in SSc (30, 31). Gastroesophageal reflux, dysphagia, bacterial overgrowth, diarrhea and fecal incontinence are common complications (30), highly reducing the wellbeing and quality of life of SSc patients (32). Treatment differs between the different complications. In addition, GI bleeding is a well-recognized complication of SSc (33), presenting a fatal potential (34).

1.5.4. Interstitial lung disease

ILD is characterized by inflammation and/or fibrosis of lung parenchyma, leading to a restrictive ventilatory defect and impaired gas exchange (35). Patients with pulmonary affection present highly heterogeneous disease courses, ranging from mildly symptomatic to rapid evolving with respiratory failure. Pulmonary fibrosis by HRCT is reported present in up to 50 % of patients, and both lcSSc and dcSSc patients are frequently affected (36-39). Patients with ILD often die from respiratory failure (39). The ventilator defect of ILD makes alveolar regions hypoxic. In order to maintain an acceptable ventilation/perfusion ratio, adjacent small arteries constrict, guiding blood to pulmonary tissue with higher oxygen concentration and better capacity of alveoli-blood gas exchange. As increasing numbers of pulmonary vessels contract, vascular resistance elevates and pulmonary artery pressure increases, potentially promoting pulmonary hypertension secondary to ILD (PH-ILD). Hence ILD patients may also die from heart failure. Although PH-ILD is reported less frequent than PAH, PH-ILD displays an even worse prognosis (40). Treatment of pulmonary fibrosis is still a priority in SSc research (41) as immunosuppressive therapy by cyclophosphamide and mycophenolate has been shown to be

only modestly beneficial (42, 43). Recently, the tyrosine kinase inhibitor nintedanib was shown to reduce the rate of progression of ILD in a broad population of patients with SSc-ILD (44).

1.5.5. Pulmonary hypertension

PH is defined as increased blood pressure of the pulmonary arteries. As the pulmonary circulation is a low pressure system, the right ventricle (RV) is lean and vulnerable to large increases in afterload. By gradual increase of afterload, the RV eventually fails (45, 46) , reducing blood volume through the pulmonary circulation and equivalently lowering left ventricular preload.

PH is categorized into five groups according to etiology (47). In SSc, the most common groups are group I (PAH), group II (PH due to left heart disease) and group III (PH due to pulmonary disease). While pulmonary disease and pulmonary congestion from diastolic dysfunction (DD) are the most common causes of PH in the general population (48), PAH is considered the most frequent cause of PH in SSc (49). PAH is expected to develop in around 10-15 % of SSc patients (50, 51). Dysfunction of endothelial cells (ECs) lining pulmonary vessels is an important pathophysiologic factor of PAH. Endothelial dysfunction is suggested to cause increased production of the vasoconstrictor endothelin-1, as well as reduced production of vasodilating nitric oxide (52). Further, EC apoptosis leads to remodeling with capillary destruction and vessel occlusion. Morphologically, PAH presents thickened microvascular vessel walls, potentially obliterating vessel lumen. As vasoconstriction and remodeling progress, pulmonary pressures increase in order to pump sufficient amounts of blood through the shrinking vessel bed. Patients with SSc-PAH show a worse prognosis opposed to patients of idiopathic PAH (iPAH) (53). Why these subsets show different mortality rates is yet to be revealed.

An early diagnosis of SSc-PAH, with consequent initiation of treatment, is thought beneficial to limit organ dysfunction and improve prognosis. Patients at a symptomatic stage hold poor prognosis, and much effort is put into screening and diagnosing PH at even a preclinical stage. Today, annual echocardiographies are advised for screening of PAH (54). Further, the recently

developed DETECT calculator (55) is a novel non-invasive tool for evaluation of pulmonary vessel pathology, basing likelihood of PH on results from echocardiography, electrocardiography, pulmonary function tests, clinical signs and serum markers (56).

PH is suspected in patients presenting symptoms of RV heart failure, e.g. breathlessness, fatigue or syncope. The gold standard for diagnosing PH is right heart catheterization (RHC), an invasive method measuring pulmonary vasculature pressures. PH is defined as an increased mean pulmonary artery pressure (mPAP) ≥25 mmHg at rest. The World Symposium on Pulmonary Hypertension Task Force has recently suggested lowering the cutoff to mPAP ≥21 mm Hg (57). In addition, a pulmonary vascular resistance >3 Wood units has been suggested necessary for a diagnosis of precapillary PH. These suggestions are however not yet included in formal guidelines. In order to differentiate precapillary (group I and III) from postcapillary (group II) PH, backflow pressure from pulmonary capillaries (pulmonary artery wedge pressure, PAWP) is measured. Elevated capillary pressures are considered to reflect elevated left atrial pressure and pulmonary venous congestion from left heart disease (57).

Treatment regimens of the respective PH groups differ substantially as they target heterogeneous pathophysiologies. For group II and III, therapeutic possibilities are limited. Optimization of volume status and treatment of underlying heart disease is recommended in group II, while hypoxic group III patients have shown profit from oxygen therapy. In PAH, the use of pulmonary vasodilators has improved survival substantially, and a recent study reports a five year survival for 63 % of patients (58). While pulmonary vasodilators are shown beneficial in PAH, such treatment may even be harmful to patients of group II (59) and III (60). The fear is that vasodilator therapy may increase blood flow to the failing left ventricle or hypoxic regions of the lung, respectively. It is therefore of high importance to classify PH patients correctly.

There are studies indicating that postcapillary PH patients may be misclassified as PAH. In 2009, Halpern and Taichman showed poor calibration between PAWP and LV end-diastolic pressure (LVEDP) in a study on four thousand PH patients, uncovering raised LVEDP in 54 % of all

patients with normal PAWP (61). In 2013, Fox et al performed fluid-challenge during RHC on SSc patients (62). After re-categorizing PAH patients who presented occult DD to PH group II, group II became the most frequent cause of SSc-PH. Similar findings have also been reported in cohorts of PAH patients without SSc (63). Further, fluid challenge is reported to reveal a higher frequency of occult left heart disease in SSc patients without PH, compared to controls (64). These studies suggest that left heart affection may be an underappreciated complication of SSc. If this is factual, SSc patients treated by pulmonary vasodilators may be at risk of unintentional and harmful therapeutic complications.

1.5.6. Primary cardiac involvement

Primary cardiac involvement is assumed to follow from the same pathophysiology as other organ manifestations, and coronary microcirculation is shown to display abnormal vasoreactivity in SSc (24). Cardiac affection seems to follow a non-coronary distribution (65), supporting the theory of a transitory vasospastic pathophysiology (24) and/or inflammatory myocarditis (65). The tissue is thought to suffer from hypoxia-reperfusion injury from oxidative stress as blood flow returns (66). This may in turn drive inflammation, promoting fibrosis. Fibrosis may replace myocardium, conductive tissue, valves and/or pericardium (67). Autopsy studies have shown cardiac fibrosis in 44-70 % of patients (68, 69). Fibrosis of myocardial tissue may weaken the contracting (70) and filling ability of the heart (71), fibrosis of conductive tissue may promote arrhythmic vulnerability (72), and affection of the pericardium may restrict ventricular filling during diastole (73). SSc related mortality from primary heart affection is mainly attributed to general heart failure or arrhythmias, and a 2010 EUSTAR study estimated heart failure and arrhythmias to account for 13 % and 11 % of SSc related deaths, respectively (1). Pericarditis was reported to account for 1.6 %. Some studies further propose ischemic heart disease (IHD) to be a feature of SSc (74-76); however affection of the coronary arteries and increased frequency of IHD in SSc is still a matter of debate (77). The treatment of SSc related arrhythmias and HF is equal to treatment of general cardiac disease (28).

1.6. Heart failure

Heart failure (HF) is a disease state in which one or both ventricles are unable to pump sufficient amounts of blood to accommodate the body's demands, or only able to do so by elevated ventricular filling pressures (78). Primary HF may present with i.e. dyspnea, peripheral edema or fatigue; symptoms and findings similar to those of PH and ILD (79-82). Many etiologies may account for HF; hereunder hypertension, coronary ischemic disease, valvular disease, myocarditis, arrhythmias, pulmonary vasculature disease and others (83). The etiology of HF in SSc is challenging as HF may represent either a primary complication of SSc, non-SSc cardiac disease as in the general population or advanced precapillary PH (84, 85). Although precapillary PH and primary cardiac affection are distinct complications, HF is a common end point of cardiac affection, advanced PAH, and in patients with advanced PH-ILD. HF may therefore be regarded as a common focus for treatment, reflecting poor prognosis unless properly managed.

A failing heart may display difficulties with either (i) ventricular filling during diastole, so called diastolic dysfunction (DD), or (ii) pumping blood out from the ventricle during systole, so called systolic dysfunction (SD) (86) . The Frank-Starling mechanism explains that increased stretch of the myocardium during diastolic filling will increase myocardial contraction during systole (87). In order to maintain cardiac output in SD, higher end diastolic filling pressure is therefore present. Hence, SD is generally followed by DD.

HF symptoms represent either congestion of the venous system (88) or inadequate filling of the arterial system and may present as e.g. dyspnea, syncope or lightheadedness (89, 90). Syncope is a symptom of SD, explained by reduced perfusion of oxygenated blood to the brain (91). Dyspnea is a sign of DD; as ventricular filling pressures increases, blood is congested backwards into the pulmonary venous circulation. As the hydrostatic pressure of the capillaries increases, fluid leaks into the alveoli and hampers gas exchange (92). At the symptomatic stage, HF may be categorized into the New York Heart Association clinical classification (NYHA I-IV) (93), where advancing classes represent increasing symptoms and worse prognosis.

HF may also be categorized in stages, based on severity. A healthy individual presenting solely risk factors for HF is categorized stage A HF (94). In pre-clinical stages of HF, it is normal to present cardiac dysfunction on cardiac imaging in absence of subjective symptoms. This is referred to as stage B HF. Natriuretic peptides are released from atrial and/or ventricular tissue as elevated chamber pressures and blood congestion strain myocardial tissue (95). A diagnosis of clinical HF requires that the patient presents either increased serum concentrations of natriuretic peptides or symptoms/findings of HF, in addition to cardiac dysfunction on imaging modalities (stage C/D).

HF is divided into two main types: heart failure with reduced (HFrEF) or preserved ejection fraction (HFpEF), defined by an EF <40 % or ≥50 %, respectively (78). EF is the percentage of end diastolic volume that is ejected during systole. Recent guidelines define EF values between 40-50 % as heart failure with mid-range EF (HFmrEF) (78). HFpEF is characterized by isolated DD, while HFrEF is characterized by SD. Both HFpEF and HFrEF present equal morbidity and mortality (96, 97), but HFpEF is considered more frequent among elder and female HF patients (98). To date, it is still a matter of debate whether HFpEF and HFrEF represent separate disease entities with differing pathophysiology, or if they reflect two stages of the same disease (99).

Treatment for HF is challenging as HFrEF and HFpEF show different response to treatment. Angiotensin converting enzyme-inhibitors, mineralocorticoid receptor-antagonists and beta-blockers are advised in HFrEF unless contraindicated, as they are shown to improve survival (78). While HFpEF patients often receive the same medications as HFrEF patients, no treatment has yet been shown to improve survival (78).

Echocardiography is a leading instrument of cardiac function evaluation. By ultrasound, dimensions, structure, flow and tissue velocities, as well as chamber pressures, may be estimated. Pathology on echocardiography is defined to reflect cardiac dysfunction rather than heart failure as the latter is a clinical diagnosis (78). The echocardiographic equivalents of HFrEF and HFpEF are SD and DD, respectively.

1.6.1. Systolic dysfunction

EF has for decades served as the leading parameter of systolic function (100). It is calculated from two projections in order to estimate three-dimensional volume, opposed to two-dimensional area. In recent guidelines, EF<52 % in men and <54 % in women is considered pathologic (101). Fractional shortening (FS) is an alternative marker of systolic function. However, as FS only measures two-dimensional contraction along a radial axis, it is considered inferior to EF (101). Global longitudinal strain (GLS) is a new systolic parameter, utilizing speckle tracking (102). In speckle tracking, the software follows speckles of myocardial tissue during contraction, estimating the contractile ability the myocardium (103). GLS is reported as the percentage shortening of the myocardium along the longitudinal axis. GLS has proved superior to EF with respect to reproducibility (104, 105) and prediction of cardiovascular outcomes (106, 107).

Most studies on SSc cohorts declare SD to be a rare finding (9, 108); while some report lower EF in SSc patients, opposed to controls (109). However, previous small and medium sized studies agree that SSc patients present deviant GLS values, compared to healthy controls (7, 8, 110, 111). One study further proposed continuing deterioration of GLS in a small cohort followed for two years (112). The burden of SD in SSc patients may therefore be considered unclear, and further studies are warranted.

1.6.2. Diastolic dysfunction

For decades, the diagnostic algorithm of DD has involved several parameters (113, 114) and proven more complex than assessment of systolic function (115). Recommendations for the evaluation of diastolic parameters have therefore been altered several times (113, 116). As DD has been somewhat challenging to assess, evaluating diastolic function in HF has historically been somewhat neglected opposed to systolic function (117). The pathophysiology of DD may reflect reduced ventricular relaxation or myocardial compliance, limiting inflow during early diastole (118). To ensure adequate ventricular filling, the heart will increase atrial contraction in

end-diastole, making increased ventricular filling pressure a marker of DD (116). Invasive measurements by cardiac catheterization may estimate left atrial pressure. However, these procedures are both time and cost consuming and come with a risk of complication (119).

Echocardiography is a more widely used surrogate for evaluation of diastolic function. Although ultrasound is not able to measure pressures directly, calculations of pressure differences are performed from measurements of blood flow velocities (101). Diastolic function has historically been evaluated by numerous echocardiographic parameters and algorithms. In 2016, the American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI) published new guidelines to standardize and simplify echocardiographic diastolic evaluation (116). DD is opposed to SD accepted as a frequent complication of SSc compared to controls (4-6, 120-123). Fibrosis is thought to weaken the stretching ability of the myocardium, hence calling for a higher filling pressure to assure adequate ventricular filling.

1.7. Echocardiography for prediction of cardiovascular outcome in SSc

Echocardiography is a widely used screening method for PH as one may estimate RV pressure during systole, which in absence of pulmonary valve disease is considered to resemble pulmonary artery pressures (101). Annual echocardiographies from PH screening are valuable to SSc cardiac research as they facilitate development of large prospective echocardiography databases. Historically, substantial effort has been made in order to discover echocardiographic parameters with outcome predicting abilities (124-126), of which EF is perhaps the most renowned predictor. Reduced EF is a predictor of unfavorable cardiovascular outcome (127). Recent studies suggest that GLS shows predictive abilities for cardiovascular outcomes that may be superior to EF in the general population (107, 128-131). However, as different diseases display heterogeneous pathophysiology, one must appreciate that results may not be generalizable to other cardiac diseases or patient subgroups. In example, while a Danish study found GLS to be a marker of outcome in the male general population, this was not proven significant in the female subset (128).

The predictive role of echocardiographic abnormalities in SSc on mortality is inadequately addressed. Two previous studies on 34 and 153 patients have reported the diastolic parameter e' to predict mortality in SSc (6, 132). However, according to the 2016 recommendations (116), diastolic function should not be assessed by individual parameters in isolation. RV systolic function has also been reported a predictor of cardiovascular outcome and/or mortality in two studies on 50 and 103 SSc patients, respectively (133, 134).

1.8. Serum markers for prediction of cardiovascular outcome in SSc

A biomarker may be defined as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (135). Serum (bio-)markers are biological molecules extracted from blood that relate with a physiologic or pathophysiologic state. They are of great importance, aiding the physician in diagnosis and monitoring of several disease states (136, 137). Serum markers may be i) diagnostic, ii) prognostic, evaluating disease extent and severity, or iii) predictive for development of disease complication or responsiveness to therapeutic intervention (138, 139). In SSc, serum antibodies help the physician categorize patients into clinical subgroups with differing clinical course and prognosis (140).

Serum markers are a prioritized subject of research in SSc (141, 142), aiming to categorize patients at time of diagnosis as likely or unlikely to develop organ complications. To date there are numerous serum markers showing association with PAH and pulmonary fibrotic disease (143-145).

Cardiac involvement is recognized as both a frequent complication and a negative predictive factor in SSc (10), yet serum markers of SSc cardiac pathology have not been in focus (146). However, there exist numerous studies on potential serum markers for cardiovascular disease in the general population (147-150). Troponins are a cornerstone in diagnosis of myocardial infarction (151), while NT-proBNP is of resembling importance in diagnosis of heart failure (152). Studies focusing exclusively on SSc patients are of interest as the pathophysiology of

cardiac SSc is assumed dissimilar from common etiologies of cardiovascular disease states. Standardizing evaluation for RV, LV, systolic and diastolic functions may clarify the burden of cardiac involvement and facilitate research on serum markers of SSc cardiac involvement.

Serum markers of cardiac disease in SSc may serve two important roles: i) Patients at high risk may be exposed, allowing close monitoring for disclosure of complications and initiation of treatment at an early stage. ii) As the pathophysiology of SSc is inadequately understood (11) and treatment of complications is suboptimal (153), knowledge on mechanistic pathways may suggest novel treatment strategies, improving SSc prognosis.

2.0 Aims of the thesis:

2.1 General objective:

To map echocardiographic features of a large and unselcted SSc cohort and investigate serum markers as predictors of cardiac dysfunction in SSc.

2.2 Specific objectives:

Paper 1:

To investigate the frequency of DD in patients with SSc, and to further evaluate the impact of DD on mortality.

Paper 2:

To investigate the frequency of SD in patients with SSc, at baseline and follow-up echocardiography.

Paper 3:

To evaluate serum levels of pro-fibrotic, inflammatory and cardiac related serum markers in SSc subsets, stratify for groups of cardiac dysfunction, and evaluate the impact of these serum markers on mortality.

3.0 Patients and Methods:

3.1 Patient cohort and clinical data:

The study cohort was exclusively recruited from the Rheumatology Unit at Oslo University Hospital (OUH), Rikshopitalet. The Rheumatology Unit at OUH specializes on CTDs and systemic diseases. All SSc patients followed at the Rheumatology Unit are included in the prospective Oslo SSc cohort. The cohort consists of the denominational population of OUH, together with national SSc patients referred to OUH.

At initial visit, patients are examined by a rheumatologist and registered in the Norwegian Systemic Connective Tissue Disease and Vasculitis Registry (NOSVAR). Data on demographic, clinical, laboratory and imaging parameters are registered in NOSVAR.

During the first years of disease, patients are followed annually with comprehensive clinical assessments, echocardiography and pulmonary function tests. A high resolution computer tomography (HRCT) is performed on all patients at diagnosis. Sera are collected in all patients at time of diagnosis and at every annual visit.

3.2 Inclusion criteria

Inclusion criteria for the study were i) fulfillment of the 2013 European League Against Rheumatism/American College of Rheumatology criteria and/or the 1980 American College of Rheumatology criteria for SSc (154, 155). For the echocardiography study, an additional inclusion criterion was iia) at least one protocol echocardiography performed between 2003-2016 at OUH, available for evaluation. For patients with ≥2 echocardiograms, the earliest was considered as baseline echocardiography and the most recent as follow-up. For the serum marker study, an additional criterion was iib) an available serum sample acquired between 2003-2016 for analyzation. Demographic and clinical data on included subjects are shown in Table 1.

Table 1: Demographic and clinical variables of the SSc echocardiography cohort

	Echocardiography cohort (n=333)
Age at baseline, years	58 (14)
Disease duration at baseline, years	1.6 (0.3-7.4)
Observation period, years	4.9 (2.1-7.2)
Female, n (%)	275 (83)
lcSSc, n (%)	237 (71)
Anti-centromere antibodies, n (%)	170 (51)
Anti-topoisomerase I antibodies, n (%)	57 (17)
Modified Rodnan skin score	6.0 (3.0-14.0)
Digital ulcers, n (%)	140 (42)
Calcinosis, n (%)	112 (34)
Pulmonary fibrosis, n (%)	183 (55)
Interstitial lung disease, n (%)	87 (26)
Ischemic heart disease, n (%)	54 (16)
Heart failure, n (%)	25 (8)
Precapillary PH, n (%)	84 (25)
Hypertension, n (%)	38 (11)
Atrial fibrillation, n (%)	35 (11)
Body mass index, kg/m^2	24 (4)
History of smoking, n (%)	186 (56)
NT-proBNP at baseline, pmol/l	16 (8-62)
Creatinine at baseline, µmol/l	66 (47-80)
Mortality, n (%)	98 (29)
Calcium channel blockers, n (%)	260 (78)
ACE inhibitors/ARBs, n (%)	124 (37)
Anticoagulants, n (%)	89 (27)
Beta blockers, n (%)	105 (32)
Statins, n (%)	121 (36)
Diuretics, n (%)	141 (42)
Acetylsalicylic acid, n (%)	124 (37)

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; lcSSc, limited cutaneous systemic sclerosis; NT-proBNP, N-terminal pro-brain natriuretic peptide; PH, pulmonary hypertension.

3.3. Clinical assessment

3.3.1. Skin and joints

Clinical examinations were performed routinely by a rheumatologist. Examinations included evaluation of modified Rodnan skin score (mRSS) with respect to skin affection, digital ulcers, pitting scars, calcinosis, tendon friction rubs and telangiectasias.

3.3.2. ILD

Patients underwent pulmonary function tests and HRCTs at baseline in order to screen for ILD. Extent of involvement was evaluated on thin-section images. Area affected was related to total volume of lung parenchyma (23). Reticular pattern and super-imposed ground-glass opacities were defined as fibrotic tissue. Presence of significant ILD was defined as i) >10 % pulmonary fibrosis on HRCT or ii) two consecutive pulmonary function tests with forced vital capacity < 70 % of predicted value, in the absence of HRCT. Fibrosis affecting 0.1%–10 % of pulmonary tissue did not qualify for ILD diagnosis.

3.3.3. Pulmonary hypertension

Suspicion of PH was based on clinical symptoms, echocardiography or the DETECT calculator. Patients suspected of PH underwent RHC for diagnosis. In RHC, a Swan-Ganz catheter is guided through the venous system, right atrium and ventricle into the pulmonary arteries measuring blood pressures of the respective compartments. When the catheter can no longer proceed through narrow arteries, a balloon is inflected to obstruct the artery. Backflow pressure from the capillaries is measured and reported as PAWP. RHC PH was in paper I defined as mPAP >25 mmHg, according to guidelines (47). PH was defined pre- or postcapillary based on postcapillary wedge pressure >15 or ≤15 mm Hg, respectively. PH-ILD and PAH was defined as precapillary PH with presence or absence of ILD, respectively. In paper II and III, new suggestions from the 6th World Symposium on PH were utilized, defining PH as mPAP ≥ 21 mmHg and requiring a pulmonary vascular resistance >3.0 Wood Units for diagnosis of precapillary PH (57).

3.3.4. Echocardiographic examinations:

All examinations were performed at a single center, Oslo University Hospital (OUH), Rikshospitalet. Echocardiographic recordings were obtained using GE Vivid 7 or Vivid E9 (GE Vingmed Ultrasound, Horten, Norway). Software analyses were performed using EchoPAC, version 201 (GE Healthcare). Examinations were executed with the patient in the left decubitus position. Every examination was reanalyzed for the present study between September 2016 and July 2017 by the same investigator (A.H.T.), blinded for patient clinical status. A second investigator (J.C.A.) analyzed 43 examinations to assure adequate inter-observer variability. In addition, 19 examinations were reanalyzed by A.H.T. to assure adequate intra-observer variability. Echocardiographic parameters evaluated are shown in Table 2.

Left ventricular dimensions at end-diastole and end-systole were measured by M-mode from the parasternal long (PLAX) and short (PSAX) axis-views (101).

LV systolic function was evaluated by three parameters:

(i) FS from PLAX (101).

(ii) GLS from apical four chamber- (A4C), apical two chamber- (A2C) and apical long axis-view (APLAX) using -17.0 % as a cutoff for abnormal values (156). Adequate image of all three views was necessary for evaluation. Image quality of the individual projections was considered acceptable if not more than two of six segments were untraceable, and not more than three segments in total.

(iii) EF from the Simpson biplane method of disks from A4C (Figure 1) and A2C (157), based on end-diastolic and end-systolic volumes.

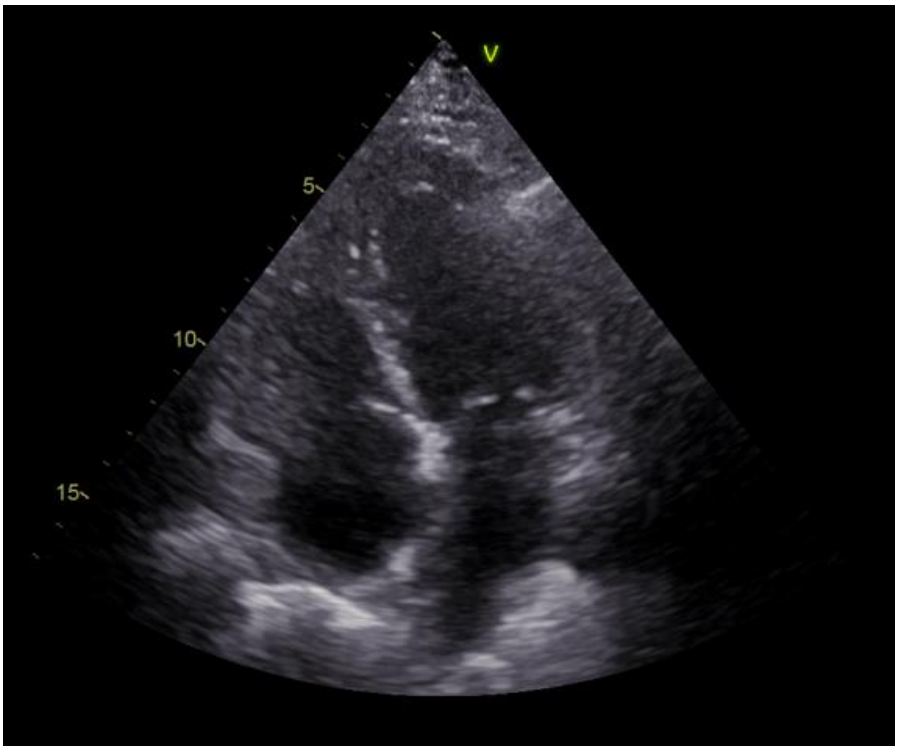

Figure 1: Apical four chamber view of the ventricles and atria.

Diastolic function was assessed according to 2016 recommendations (116) by measuring:

1. Tissue Doppler displacement of the septal mitral annulus during early diastole (e' and E/e').

2. Transmitral flow pattern by pulsed Doppler (E and E/e') (Figure 2).

3. Body surface indexed left atrial volumes by the biplane area length method (LAVI). The shortest length from A4C and A2C was defined as atrial length.

4. Tricuspid regurgitant maximum velocity (TRV) by continuous Doppler of the transtricuspid flow during systole (Figure 3).

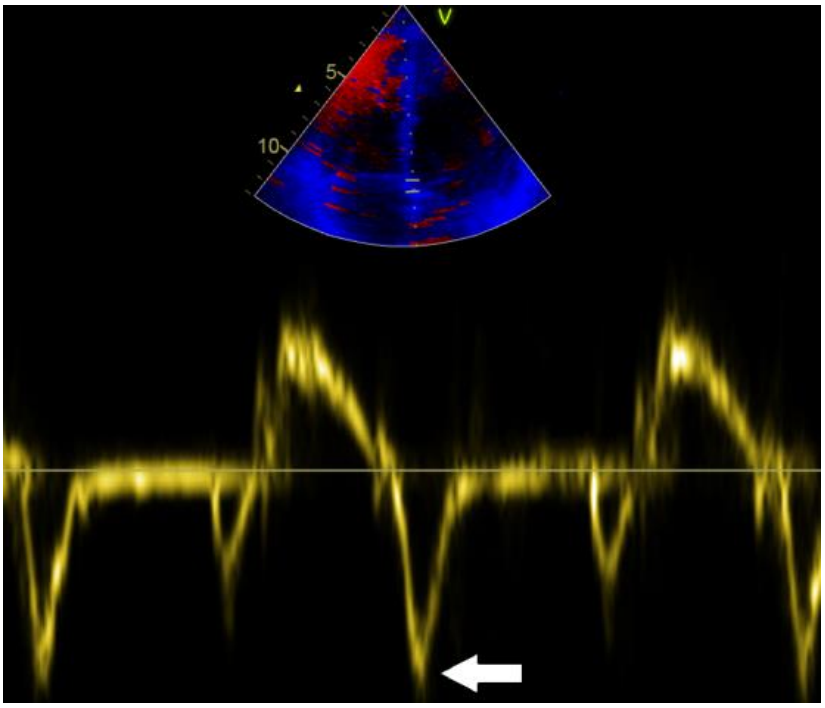

Figure 2: Tissue Doppler measurements of mitral annular displacement velocity (e'). Arrow marking e' value.

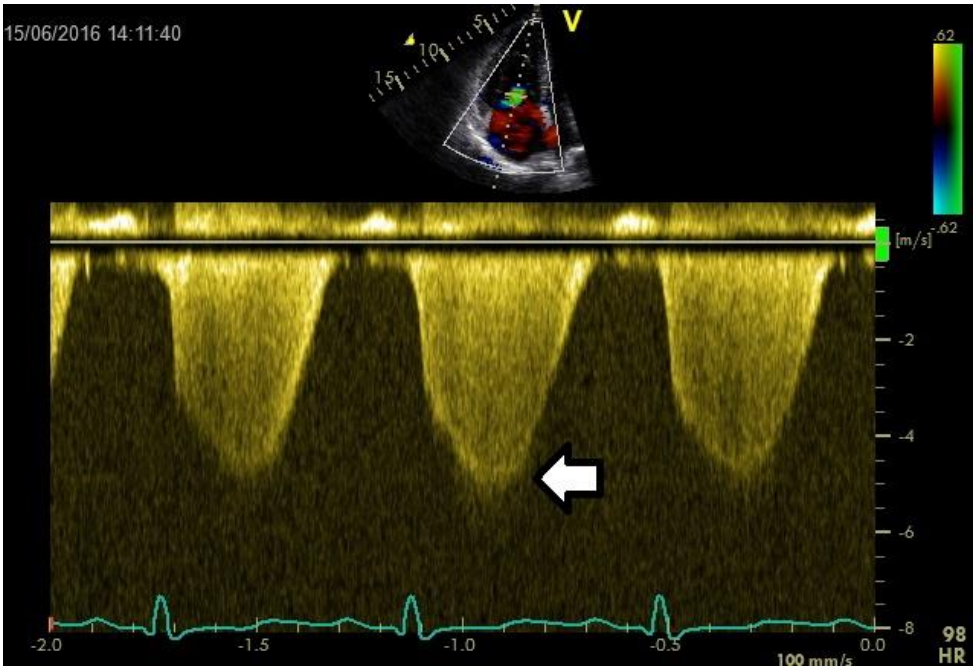

Figure 3: Continuous Doppler measurement of tricuspid regurgitant jet in systole. Arrow marking velocity in meter/second.

The new ASE/EACVI general guidelines were applied to evaluate diastolic function as a composite outcome (116). All four parameters were utilized for evaluation of diastolic function in the entire cohort; including TRV in patients with precapillary PH and LAVI in patients with mitral regurgitation. DD was defined as abnormality in more than 50 % of the available parameters, while abnormality in less than 50 % indicated normal diastolic function. In cases of 50 % abnormal parameters, status was considered inconclusive. We applied these guidelines on all patients with ≥2 evaluable parameters. Patients with normal or inconclusive diastolic function at baseline, and a DD diagnosis at the follow-up echo, were defined as new onset DD. DD was segregated into three severity grades; grade I, II and III, using early and late transmitral flow velocities and number of abnormal diastolic parameters, as recommended by the guidelines (116).

Systolic RV parameters as tricuspid annular plane systolic excursion (TAPSE), fractional area change and RV free wall longitudinal strain, RV dimensions and right atrial area were all evaluated in the A4C view (158). Mitral regurgitation was categorized as mild, moderate or severe based on visual estimation (159) in combination with color Doppler echocardiography (160). Pericardial effusion was categorized as mild (<5 mm), moderate (5-15 mm) or severe (≥ 15 mm) (161).

Table 2: Echocardiographic parameters included in protocol analyses

Structural parameters	**LV systolic parameters**	**LV diastolic parameters**	**RV systolic function**	**Other**
IVSd	Global longitudinal strain	E wave	Tricuspid annular plane systolic excursion	Pericardial effusion
LVIDd	Ejection fraction	A wave	Fractional area change	
RV basal diameter	Fractional shortening	E/A ratio	RV free wall strain	
		Deceleration time		
		e’		
		E/e’		
		LA area from A4C and A2C		
		LA volume index		
		Tricuspid regurgitant velocity		

A wave, late transmitral filling velocity; E wave, early transmitral filling velocity; e’, early displacement velocity of the mitral valve; IVSd, interventricular septal thickness at end-diastole; LAA4C, left atrial area, apical four-chamber view; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole; RV, right ventricle.

3.4. Serum marker analyses

At OUH, serum samples are collected from patients at baseline visit using a standardized protocol for analyzation following the European League for Scleroderma Trial and Research (EUSTAR) recommendations for biobanking (162). Samples are centrifuged at room temperature within 30 minutes and stored at -70 °C until analyzation. The biobank contains serum samples from NOSVAR patients and was approved by the Regional Committee of Health and Medical Research Ethics in South-East Norway, protocol No. 2016/119.

3.4.1. Luminex assay

Twenty-nine serum markers were analyzed by the Luminex method (Table 3). In Luminex serum marker analysis, the analytes are added to a mixture of color coded beads (163). The beads are pre-coated by antibodies with high analyte specificity. Analytes and antibodies bind. Sequently, a second detection antibody is added to the solution, forming an antibody-antigen sandwich. Phycoerythrin-conjugated streptavidin is added to the solution, binding the detection antibody. The analyte is then analyzed by a dual-laser flow-based detection instrument with one laser detecting the bead and classifying the analyte, while the other laser quantifies the analyte from the magnitude of the streptavidin signal.

3.4.2. ELISA assay

Fourteen serum markers were analyzed by the enzyme-linked immunosorbent assay (ELISA) method (R&D Systems) (Table 2). In sandwich ELISA, the plate is covered with capture antibodies specific to the analyte of interest. As the analyte solution is added, antibody-antigen bonds are formed. Next, a detection antibody is added, binding the analyte at a second site, "sandwiching" the analyte (164). The detection antibody-antigen creates an enzyme conjugated color or electrochemical signal, in which stronger signal reflect higher analyte concentration.

Table 3: Luminex and ELISA serum markers analyzed.

Luminex markers	**ELISA markers**
Angiopoietin 2	Activin A
CCL2	CCL17
CCL3	CCL18
CCL4	CCL19
CCL5	CCL21
CCL8	CD166 antigen
CCL11	CX_3CL1
CCL13	CXCL10
CCL22	DKK1
CCL24	Endostatin
CXCL13	Lipocalin-2
Fibroblast growth factor 1	Osteoprotegerin
Fibroblast growth factor 2	Osteopontin
Hepatocyte growth factor	Pentraxin-related protein PTX3
IL-1 receptor antagonist	
IL-1β	
IL-6	
IL-8	
IL-10	
IL-12p70	
IL-17a	
IL 23	
IL 33	
Placental growth factor	
Thymic stromal lymphopoietin	
Tumor necrosis factor-related apoptosis-inducing ligand	
VEGF A	
VEGF C	
VEGF D	

CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CXCL, chemokine (C-X-C motif) ligand; DKK1, Dickkopf-related protein 1; IL, interleukin; VEGF, vascular endothelial growth factor.

3.5 Control cohorts/material

3.5.1 Echocardiography controls

Study controls were collected from the Nord-Trøndelag Health Study (HUNT). HUNT was initiated in the 1980s as a population based health study on people living in Nord-Trøndelag County (165). Echocardiographies were performed between 2006-2008 during the third wave of HUNT (166) of 1,296 participants without recognized hypertension, cardiovascular disease or diabetes mellitus. Among these, 30 were excluded due to significant pathology, requiring clinical follow-up. In total, 65 controls were matched to the SSc patients with respect to age, sex, systolic blood pressure and body mass index at time of baseline echo.

3.5.2 Serum sample controls

One hundred blood bank donors served as controls for the serum marker study (paper III). The same controls were utilized as controls for both panel A and panel B. Only healthy individuals > 18 years of age are allowed to serve as donors. According to Norwegian law, only individuals without cardiovascular disease, infectious diseases, immunodeficiencies or other chronic diseases are allowed to donate blood (143).

3.6 Statistical analyses

Statistical analyses were performed using SPSS, versions 23 and 25 (IBM, Armonk, New York, USA), and STATA, versions 14 and 15 (StataCorp LLC, College Station, Texas, USA). Subgroups were compared by independent t-tests, non-parametric tests and chi-square tests as appropriate for continuous and categorical data. T-test and Mann-Whitney U tests were applied for normal or skewed distributions, respectively. P-values < 0.05 were considered to reflect statistical significant differences. In article I, parameters of more than two variables were compared by one way-ANOVA, including post-hoc tests.

For all three articles, clinical, serological and echocardiographic parameters of relevance, as evaluated by experts, were included in analyses. Association analyses were performed using logistic regression, reporting odds ratios and 95 % confidence intervals. Multivariable models were evaluated by area under the receiver operating curve, and models presenting values >0.7 were considered acceptable. Mortality predicting analyses were performed using Cox regression, reporting hazard ratios and 95 % confidence intervals. Multivariable models were preceded by correlation analyses, and variables presenting correlation >0.5 were not included in common models in order to avoid multicollinearity. Pearson or Spearman coefficients were applied as appropriate for correlation analyses of continuous and ordinal variables, respectively. Parameters presenting significance levels <0.2 at a univariable level were included in multivariable analyses. A manual backward stepwise elimination procedure was utilized to identify independent risk factors of multivariable models. Parameters presenting significance levels $p>0.05$ in multivariable models were excluded. The quality of multivariable models was compared by C-indexes, with values > 0.7 representing adequate models. Schoenfeld's test was applied for affirmation of proportional hazards.

For article I, cumulative survival rates were presented and compared in Kaplan-Meier plots, using log-rank test for evaluation of significance.

4.0 Summary of results

4.1 Paper I:

Left Ventricular Diastolic Function Predicts Mortality in Patients With Systemic Sclerosis.

In total, 333 consecutive SSc patients with baseline echocardiographies were included. Patients presented a mean age of 58 ± 14 years, 83% of the cohort was female and 71% had limited cutaneous SSc. Median disease duration from diagnosis to baseline echocardiography was 1.6 years (0.3-7.4 IQR).

Diastolic function was evaluable on baseline echocardiography of 275/333 patients (83 %). DD was present in 46 patients (17 %), 195 had normal diastolic function (71 %), while 34 patients had inconclusive diastolic function. Four controls (6 %) fulfilled DD criteria.

DD patients were older, had higher frequency of hypertension, ischemic heart disease and precapillary PH, compared to patients with normal diastolic function. DD at baseline echocardiography was associated with calcinosis (OR 5.86, 95%CI 1.74-19.71, p=0.004).

During the median 4.9 years (IQR 2.1-7.2) observation period, 98/333 patients (29 %) died. Among DD patients, 57 % died, compared to 13 % of patients with normal diastolic function. Deceased DD patients showed a numerically *lower* age than DD survivors (66 vs 69 years, p=0.293). Among the subset with precapillary PH at baseline, 60 % were dead at study end. In the final multivariable model for prediction of mortality, DD was a strong independent predictor (HR 3.71, 95 % CI 1.69-8.14, p=0.001). While patients with precapillary PH showed a higher mortality than DD patients, the best mortality predicting model including precapillary PH showed inferior predictive ability compared to the DD model.

Follow-up echocardiography was available in 207 patients (62 %). Median time from baseline to follow-up echocardiography was 3.4 years. DD was present in 54 patients (29 %) at follow-up. Patients with new onset DD showed a near significant higher mortality than patients with normal diastolic function at follow-up (31 % vs 11 %, p=0.055).

4.2 Paper II:

Systolic Dysfunction in Systemic Sclerosis; Prevalence and Prognostic Implications.

In total, 277 SSc patients with baseline echocardiographies evaluable for systolic function were included. Patients presented a mean age of 57 ± 13 years. Median disease duration was 1.7 years (0.3-7.6 inter quartile range). Women accounted for 82 % (227/275) of the cohort, and 194 patients (70 %) presented with lcSSc. Baseline images evaluable for EF, GLS and TAPSE were available in 258 (93 %), 194 (70 %) and 251 (91 %) patients, respectively. Median observation period was 5.1 years (IQR 2.2-7.2).

Patients displayed lower EF (58 % vs 62 %, p<0.001), GLS (-18.4 % vs -20.3 %, p<0.001) and TAPSE (23.1 mm vs 25.2 mm, p=0.005) at baseline, compared to healthy controls. A reduced EF (<50 %) was found in 30/258 (12 %), a reduced GLS (>-17.0 %) in 47/192 (24 %), while a reduced TAPSE (<17 mm) was observed in 24/251 (10 %). Reduced EF, GLS and TAPSE was found in 0 %, 5 % and 0 %, respectively, of healthy controls.

Low GLS was associated with smoking, DLCO and NT-proBNP when adjusted for age and sex. Male sex was also associated with low GLS when adjusted for age. Low TAPSE was associated with PH, DLCO, forced vital capacity, pulmonary fibrosis and GLS, when adjusted for age and gender.

A follow-up echocardiography was available in 173 patients (62 %) after a median of 3.3 years (IQR 1.5-5.6) from baseline. LV systolic function by EF and GLS remained stable while RV systolic function by TAPSE deteriorated from 23.1 mm to 21.7 mm (p=0.005) during the follow up period. Patients with PH presented a decline in TAPSE, while patients without PH showed only a non-significant trend. During the observation period, 73 patients died (26 %). Baseline GLS was an independent predictor of mortality when adjusted for age and gender, while EF was not. When adjusted for TAPSE, GLS lost its predictive ability, while TAPSE remained significant. One millimeter increase in TAPSE equaled a mortality risk reduction of 9 %,

4.3 Paper III:

Serum Markers of Cardiac Complications in Systemic Sclerosis

In total, 371 patients and 100 healthy controls were included in the study. Serum samples from all patients were analyzed by Luminex (panel A), while additional ELISA samples (panel B) were available from 255 patients of the cohort (69 %). Women accounted for 84 % (n=313) of all patients, and 269 (73 %) patients presented with lcSSc. Average age at serum sampling was 57 ± 14 years. Median disease duration was 2.5 years (IQR 0.7-8.1). Echocardiographic data on GLS, DD and/or TAPSE within three years of serum sampling was available for 188 patients (51 %).

Among the 51 % of patients with available echocardiograms, GLS was evaluable in 111 (59 %), DD in 135 (72 %) and TAPSE in 167 (89 %) individuals.

LV systolic dysfunction by GLS >-17.0 % was present in 30/111 (27 %) of patients with evaluable GLS. These patients were more often male, and showed a numerical higher mortality. Logistic regression revealed an association between LV SD and the serum markers angiopoietin 2 (ANGPT2) and osteopontin (OPN).

LV diastolic dysfunction was present in 37/135 patients (27 %). These patients were of older age, and presented higher mortality. Adjusted for age and gender, DD was associated to TNF-related apoptosis-inducing ligand (TRAIL), while showing a numerical association to ANGPT2.

RV SD by TAPSE <17 mm was present in 29/167 patients (17 %). These patients presented numerical higher age and higher mortality (72 % vs 26 %, p<0.001). Adjusting for age and gender, RV SD was associated to ANGPT2, endostatin, OPN and TRAIL.

During the observation period, 103/371 (28 %) patients died. ANGPT2, OPN and TRAIL were strong independent predictors of mortality in multivariable analyses.

5.0 Discussion:

5.1 Prevalence of systolic and diastolic dysfunction in SSc

Several earlier echocardiography studies on SSc have presented non-significant trends of lower systolic function in patients compared to controls (122, 167-169). We report that 12 % of SSc patients presented reduced EF, opposed to none of the controls, while 24 % of patients presented with deteriorated GLS, opposed to 5 % of controls. However, many studies on cardiac SSc have excluded patients with established cardiac disease (9, 169, 170), and one must appreciate the possibility that such selection may skew results towards healthier values and disguise abnormal systolic function. At the same time, it is important to be aware that not all SSc patients of South East Norway with minor organ manifestations are referred to OUH for a second opinion. It is therefore vital to acknowledge that our cohort may present minor skewness towards patients of more serious affliction.

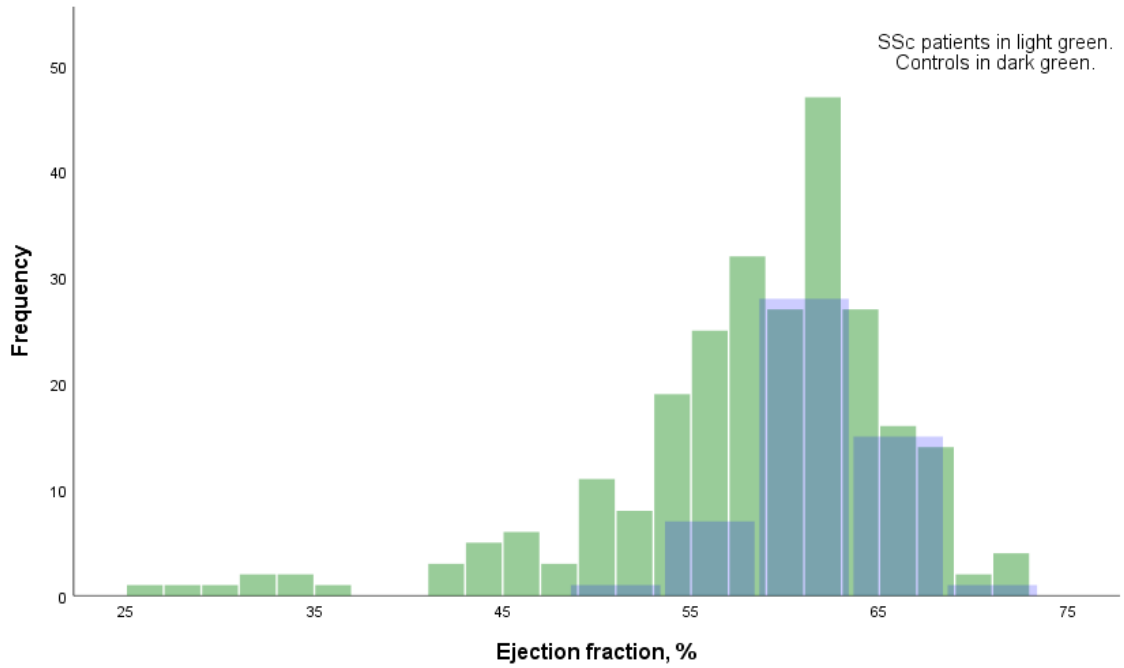

Figure 4: Distribution of ejection fraction in SSc patients and controls.

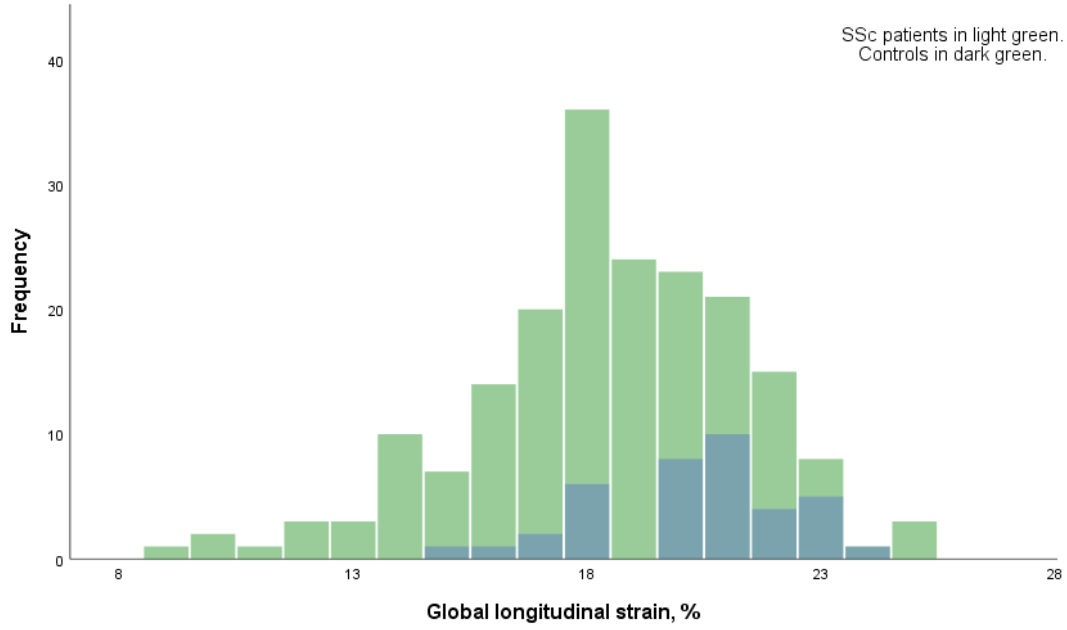

Figure 5: Distribution of global longitudinal strain in SSc patients and controls.

Figure 4 shows the EF distribution in SSc patients and controls. At first glance it may seem that a subset of patients are responsible for the low average EF in SSc. However, when excluding the 30 patients with SD, SSc patients still present lower EF opposed to controls (59.8 % vs 61.8 %, p=0.005).

The SSc EF distribution is skewed towards lower values, while GLS seems rather normally distributed. This may suggest that SSc patients with low EF are either i) a subset affected substantially by SSc pathophysiology or ii) affected by a non-SSc etiology of cardiac dysfunction. However, while we did not possess data on diabetes, we could not reveal a higher frequency of cardiovascular risk factors as hypertension or ischemic heart disease in the low EF subset.

The higher burden of systolic dysfunction by GLS is expected as GLS is considered more sensitive than EF (171). GLS describes ventricular contraction along the longitudinal axis. It is interesting to note that longitudinal contraction is mostly accounted by fibers of the subendocardium (172). The subendocardial layer is especially vulnerable to ischemia (173, 174). This may in part explain why SSc patients, who are thought to suffer from Raynaud 's phenomenon of cardiac vasculature, show considerably, reduced GLS. Global *longitudinal* strain is thought to impact EF

less than global *circumferential* strain. Circumferential strain is accounted for by circumferential fibers of the midwall and, when afflicted, implies a more protruding ischemic state (175).

The image qualities of ultrasound instruments are constantly improving, and high quality images are necessary for assessment of GLS. As part of our patients presented baseline echocardiography close to 2003, only two-thirds of our cohort was evaluable with respect to GLS. Further, it is vital to acknowledge that older images presenting low, yet acceptable quality are at risk of allowing inaccurate measurements. This calls for a caution when attributing our results to all patients with SSc. Patients with evaluable GLS had a lower mortality than patients without GLS measurements (22 % vs 36 %, p=0.016). This may partly be explained by a numerical lower age at study end (61.8 vs 64.5 years, p=0.11). Patients with EF < 50 % tended to present GLS less frequently (53 % vs 70 %, p=0.07) which may explain why EF seems more skewed in SSc patients than GLS. There was no difference with respect to PH, ILD or DD between patients with adequate and inadequate images for GLS evaluation. We cannot rule out that the low analysis percentage may have limited the mortality predicting abilities of GLS in our analyses. However, apart from these limitations we believe our results are representative and patients with inadequate images were excluded from analyses. Interobserver variability for GLS was also well within the acceptable range (interclass correlation coefficient 0.88). As the prognostic burden of lower GLS in SSc is uncertain, more research on the burden of systolic dysfunction in SSc is in demand.

DD is, opposed to SD, appreciated as a common feature of SSc, and numerous studies have shown higher burden of DD in patients with SSc, compared to controls (4, 110, 121). Still, the clinical implication of DD has been unclear. In the OUH cohort, 17 % of patients presented with DD at baseline, opposed to 6 % of controls. This may be overestimated as TRV is not validated as a parameter of diastolic function in patients with pulmonary vascular disease (116). When excluding TRV as a parameter in patients with precapillary PH at time of echo, 12 % of patients presented DD. Whether the frequency is twice or thrice that of the general population is not

vital; it is however necessary to appreciate that SSc patients are at higher risk of developing cardiac dysfunction. Contrary, the considerable rise of DD frequency from 17 % to 29 % after 3.3 years of follow-up may suggest an underestimation at baseline.

Cardiac fibrosis affects both systolic and diastolic function (70, 71). Compared to controls, our cohort presented reduced systolic and diastolic function at baseline. This is not surprising as much of organ involvement is thought to develop early in the disease course (20, 21). While time from diagnosis to echocardiography did not exceed two years, it is important to note that at time of diagnosis, the disease process is likely to have been present for months or years. Organ manifestations may therefore be present already at time of diagnosis, and one must appreciate the possibility of reduced cardiac function already at baseline echocardiography.

Irrespective of the absolute frequencies of SD and DD, such afflictions must have an impact on patient wellbeing or survival to be of clinical relevance. If future studies can confirm an increased cardiac dysfunction in SSc, an SSc-specific cardiac pathophysiology is likely. If this is further assumed clinically relevant, studies on prophylactic or therapeutic strategies are in demand.

5.2 Progression of cardiac dysfunction

It is of interest that LV systolic function was stabile over the follow-up period. This may support the notion that cardiac affection develops during the first years of the disease, or even before the patient is diagnosed. A recent Dutch study on a large SSc cohort, using the same ultrasound vendor as OUH, reported GLS to deteriorate from -20.9 % to -19.3 % after a median of 2.3 years (176), equaling a yearly deterioration of 0.7 %. A continuous yearly GLS deterioration of 0.7 % would after few years lead the majority of SSc patients into systolic dysfunction defined by GLS >-17.0 %. It is therefore possible that such deterioration will stagnate. This cohort presented longer disease duration than the OUH cohort, however an average age five years younger (52 vs 57 years) than the Norwegians. It is natural to question whether the Norwegian cohort presented at a later and more advanced stage of the disease, while the Dutch cohort captured the

cardiac deterioration of early SSc. Alternatively, it is possible that unrecognized underlying dissimilarities between the two cohorts, in example demographic or treatment-related, may account for the differing results.

Evidence of cardiac dysfunction in SSc initiates medical treatment equal to patients of cardiac disease from other etiologies. However, the calcium channel blocker (CCB) nifedipine is prescribed against Raynaud's phenomenon to a high fraction of patients. Nifedipine has been proposed to increase myocardial vascular perfusion (177) and CCBs are shown negatively associated to LV systolic dysfunction (108). It is worth questioning whether nifedipine may partly counterbalance pathologic vascular perfusion of cardiac SSc and impede progression of cardiac dysfunction. In the Dutch cohort, 44 % of patients were prescribed CCBs, compared to 78 % of the OUH cohort.

In our cohort, patients presented deteriorating diastolic function through the observation period. Yet, while both e' and E/e' worsened in the total cohort, only deterioration of e' was significant in the subset without precapillary PH. E' is a parameter with known age-dependency (178). To what degree myocardial fibrosis from SSc is responsible for further deterioration of diastolic function requires further investigation. It is likely that the increased DD frequency is in part due to increasing age and precapillary PH. For comparison with the SSc cohort, it would have been of great interest to present follow-up data of healthy controls. This was however not feasible. The cause of DD in SSc patients is of vital importance as it may potentially call for different treatment strategies.

5.3 DD vs PH

In this work, we suggest DD to be a more significant predictor of prognosis than presence of precapillary PH. It is important to appreciate that DD and PH are not mutually exclusive. Severe PH may cause DD, and DD may cause PH. The notion that DD predicts mortality at a stronger level than PH does not suggest that PH is any less important than earlier assumed. It does however suggest that estimating diastolic function by echocardiography may reveal patients at a

particularly high risk of mortality. In our material, patients with DD without PH showed a higher mortality than patients with PH without DD. However, these groups were not ideally comparable as the DD subset presented DD at an earlier date than the PH group presented PH, and therefore had a longer observation period.

Cardiac catheterization studies suggest that subclinical LV DD may be underappreciated in SSc, but also in general PAH patients (61, 62, 64, 179). Fox et al reported fluid challenge during RHC to reveal a substantial number of SSc patients misclassified with PAH instead of occult PH due to left heart disease (62). However, there exist to date no guidelines on the diagnosis of pulmonary hypertension after fluid challenge (47), and it is therefore not evident whether these patients presented true occult DD or a normal physiologic response to fluid loading. If PAH patients are misclassified, covering a postcapillary component or a pure postcapillary PH, one would expect these patients to be treated suboptimally, possible even harmfully. As we show DD to be a frequent complication of SSc, one may further postulate whether such maltreatment could be partly responsible for the increased mortality in SSc-PAH, opposed to iPAH. In addition, categorizing patients into correct subgroups is vital for therapeutical efficacy. Patients misclassified with PAH do not necessarily respond to PAH treatment and may therefore introduce type two errors, masking the significance of purposeful medications. The abovementioned literature by Fox (62) and D'Alto (64) used fluid challenge to reveal occult LV DD. In our opinion, larger studies on SSc-PH evaluated by fluid challenge, together with research on treatment and outcome, are requested.

5.4 Implementing evaluation of diastolic dysfunction in routine echocardiography

Our study shows a strong association between DD and mortality. We believe screening for DD may be a useful approach to improve survival among patients with SSc, given that treatment regimens improve. As annual echocardiographies are already recommended in SSc, this addition

should not be cost-consuming. However, this was only an observational study, and more studies on the subject are in demand to validate the prognostic value of DD.

It is important to remember that DD may be either primary or secondary to other disease states; in example PH and ischemic heart disease. Although diagnosis and treatment of primary DD may be highly essential with regards to prognosis, it is not evident that a patient with LV DD due to severe PH would profit from DD treatment. However, half of all DD patients in our cohort presented no sign of PH, neither before nor after DD diagnosis. We therefore believe that many DD cases may be due to intrinsic SSc cardiac disease.

The frequency of abnormal cardiac functional parameters is irrelevant unless one can reveal an effect of such parameters on patient wellbeing or prognosis. At the present time, a major challenge is that therapies improving survival among patients with DD or HFpEF are lacking. While angiotensin converting enzyme-inhibitors and beta blockers are shown to reduce mortality in HFrEF patients, such medications are not shown beneficial in HFpEF (78). However, one may suggest that in patients with DD or inconclusive diastolic function, the physician should focus on minimalizing the pathophysiologic contribution from other risk factors of DD as hypertension, ischemic heart disease, PH, obesity and diabetes mellitus.

5.5 Implementing GLS in routine echocardiography

Assessment of systolic function has been a cornerstone of cardiovascular ultrasound. The question is whether assessment of GLS will bring additional information to the established EF. Significantly reduced systolic function is generally known as a predictive factor of poor prognosis. Although we could not show GLS to be of value as an independent predictor of mortality in SSc, it is important to remember that there are several factors that may have diluted the mortality predicting ability of GLS in our study:

i) A substantial part of SSc related mortality is related to pulmonary fibrosis, not necessarily impeding GLS. We did not possess data on which patients died from cardiovascular causes. If we were to look at the subset dying from cardiovascular causes, it is not unthinkable

that GLS would have regained its predicting ability.

ii) While DD may be present in isolation; presence of SD is generally accompanied by DD. Analyses adjusted for DD may therefore have hazed the significance of SD. The fulfillment of today's criteria for DD may describe an advanced stage of cardiac affection. In our cohort, the majority of patients with reduced GLS presented normal diastolic function. GLS may still be a valuable predictor of cardiovascular outcomes in patients of milder cardiac disease, who have not yet developed DD.

iii) Evaluation of RV systolic function applies both arguments mentioned above. RV systolic function is reduced in patients with pulmonary vascular disease, who account for a substantial part of SSc related mortality. Further, as severe LV heart failure leads to RV heart failure, adjusting for RV systolic function has probably diluted the predictive ability of GLS. GLS lost its predictive ability when adjusted for RV systolic function.

iv) Not all patients of the total cohort had an evaluable GLS. Patients without a baseline GLS estimation presented higher mortality than patients with evaluable GLS. This may have caused a lack of association between GLS and mortality.

It is however important to remember that GLS was in fact a predictor of mortality, even when adjusted for age and sex. Patients who have not yet developed DD or RV SD may be in a state where GLS is a valuable predictor of outcome. GLS is likely in the dawn of its clinical significance. As strain analysis software is improving, and as clinicians develop instrumental experience, analyses may also become more accurate and reflect the factual myocardial strain to an even higher degree. This will probably enhance the predictive ability of GLS.

We could not show deterioration of any LV systolic parameter at follow-up. This may reflect a true lack of prognostic ability in our cohort. It is however more likely that the significance of GLS was diluted from other comorbidities. EF did not prove an independent predictor of mortality. However, when patients were categorized as EF<50 or not, EF<50 proved an independent predictor of mortality adjusted for age and sex (OR 1.85, 95%CI 1.04-3.27, p=0.035).

In summary, although we could not prove LV systolic function by GLS to be an independent predictor of mortality, we believe it is too early, and possibly even misleading, to reject the validity of GLS as an outcome predictor in SSc.

5.6 Implementing TAPSE in routine echocardiography

TAPSE proved to be an independent predictor of mortality, even when adjusted for DD. It is however a commonly applied parameter which is already known for its clinical value (133, 158). In our study, 62.5 % of patients with TAPSE<17 mm at baseline died, opposed to 21.1 % of patients with normal TAPSE. We believe TAPSE is a marker of pulmonary hypertension and/or cardiac disease, bringing significant prognostic information to clinicians. With respect to therapeutic strategies, it would be of interest to display whether isolated RV failure (in absence of both LV disease and PH) is a common feature of SSc. However, more research on treatment of cardiac SSc is needed if deterioration of TAPSE, with normal pulmonary pressures, should have implications for monitoring and treatment.

5.7 Primary or secondary cardiac dysfunction?

PH is known to affect LV DD and RV SD (46, 180, 181). While reports also indicate reduced LV SD by GLS in PAH patients (182), our cohort did not show differing GLS values in patients segregated by PAH status. It is therefore uncertain whether echocardiographic dysfunction represents primary cardiac dysfunction or influence from PAH. While our cohort included data on PAH status, we did not possess simultaneous data on echocardiography and RHCs as necessary to evaluate the interrelation between PAH and cardiac dysfunction.

5.8 Potential serum markers of cardiac SSc

Limited data on cardiac serum markers in SSc may partly be due to the lack of consistency of how cardiac involvement is categorized, and to the lack of data on the predictive abilities of cardiac function. A biomarker of cardiac dysfunction is only of interest if it can show relation to a clinical outcome. We investigated serum markers with earlier shown associations to pro-fibrotic, inflammatory and cardiopulmonary disease. Among these, we found interest in serum markers

showing association to cardiac dysfunction and predictive ability for mortality in our cohort. A lack of statistical association between clinical outcome and serum markers may result from study limitations and does not allow excluding a marker from lack of significance. It is therefore important to note that we do not exclude any of the investigated markers as insignificant. As an association does not imply a causative mechanism, predictive ability is of even greater interest. While we performed mortality predicting analyses, multivariable analyses for prediction of cardiac dysfunction will require a larger cohort as cardiac dysfunction is a feature of a minority of SSc patients.

In our cohort, ANGPT2 showed association with LV SD, RV SD and a numerical association with LV DD. ANGPT2 is an antagonist to angiopoietin 1 (ANGPT1), an anti-inflammatory protein binding to the Tie2-receptor. ANGPT1 is constantly active and stimulates vascular integrity and homeostasis in healthy individuals (183, 184). ANGPT2 expression is contrary tightly regulated and is produced mainly by endothelial cells (184). When vascular endothelial growth factor (VEGF) is present, ANGPT2 is reported to induce vascular sprouting, while in absence of VEGF, ANGPT2 induces endothelial apoptosis (185). ANGPT2 is considered pro-inflammatory as it may sensitize Tie2 towards inflammatory stimuli such as TNF-alfa, further inducing leukocyte adhesion to endothelial cells (184). It is reported that ANGPT2 sensitizes the endothelium for structural and functional changes mediated by e.g. VEGF A and TNF-alfa (186). Beyond the autocrine effect, ANGPT2 is also suggested to act as a chemoattractant for monocytes and leukocytes (187, 188). Rodent experiments have shown injection of ANGPT2 to promote cardiac fibrosis, while overexpression of ANGPT1 increased capillary density and reduced myocardial fibrosis (150).

OPN is a glycoprotein known to recruit T-cells and macrophages (189) to site of inflammation, and altered levels are reported in several disease states, in example cancer, wound healing and diabetes (190). Although altered levels of OPN is reported even in various cardiovascular disease states, its physiology, or pathophysiology, in cardiac disease is still unclear (190). OPN

has been suggested a marker of both cardiovascular disease and HF. Coskun et al showed that plasma OPN levels were elevated in patients with unstable angina or non-ST-segment elevation myocardial infarction (191). Trueblood et al reported decreased collagen accumulation and greater LV dilation in OPN knock-out mice after myocardial infarction, suggesting a protective role maintaining cardiac structure (192). OPN levels are shown increased in rodent models of vessel damage (193). As SSc is hallmarked by vasculopathy and obliterated blood vessels, increased OPN levels among SSc patients seems legitimate. OPN has also been suggested a marker of HF as basal expression is low in normal rodent and human hearts, while increasing with cardiac remodeling (194). Matsui et al showed that angiotensin II increased blood pressure and stimulated cardiac fibrosis in wildtype mice opposed to OPN deficient mice, suggesting a role for OPN in development of angiotensin II-induced fibrosis (149).

Tumor necrosis factor-related apoptosis-inducing ligand was in our material negatively associated with LV DD and RV SD. While many TNF family members have highly regulated expression, TRAIL is a transmembrane protein constitutively expressed in various tissues (195). The primary recognized function of TRAIL is induction of apoptosis in cancer cells and other transformed cells by binding death receptors (196). It has therefore been suggested as a potential agent for cancer therapy (197). Further, TRAIL is demonstrated to inhibit autoantigen-specific T-cells (196) and TRAIL-deficient mice are reported to develop heightened autoimmune responses (198). TRAIL is reported to promote survival and proliferation of endothelial cells (199). In acute coronary syndrome, TRAIL levels are reduced (196), and low serum values are reported to predict unfavorable prognosis independently of other cardiovascular disease parameters (148).

In our cohort, ANGPT2, OPN and TRAIL were significantly associated to cardiac dysfunction and proved strong independent predictors of mortality. As all three serum markers showed association to all SSc subsets (lcSSc, dcSSc, ACA, ATA), the relations could represent underlying disease rather than cardiac dysfunction. Increasingly abnormal values may reflect severe

disease, resulting in more cardiac involvement and higher mortality from both cardiac and non-cardiac causes. However, patients with cardiac dysfunction showed significant deviating values compared to patients with normal cardiac function.

Endothelial dysfunction is a common feature of SSc and general cardiovascular disease (200), however the pathophysiologic function of ANGPT2, OPN and TRAIL is complex and inadequately understood. Both ANGPT2 and TRAIL may interact with ECs and angiogenesis (201, 202). The aim of this study was not to elucidate the mechanism of these three markers, but to assess their clinical significance in cardiac SSc. Our data suggest a strong association between these markers and prognosis. Their functions as prognostic markers is however only of interest if i) it becomes evident what organ manifestations altered levels reflect and ii) such organ manifestation may be prevented or treated when abnormal serum marker levels are recognized. These serum markers may be of use if they achieve to take one of two roles: i) a marker of diagnostic value; as troponins for ischemic heart disease, or ii) as a therapeutic target, as angiotensin converting enzyme in heart failure. However, these findings need to be confirmed from other cohorts, ideally collecting serum samples and performing echocardiographies simultaneously.

6.0 Methodological considerations:

6.1 Study cohort:

The current work is designed as a cohort study. We have followed a cohort of patients diagnosed with SSc and compared them to healthy controls. We believe this is a relevant design to evaluate the given hypotheses. A cohort study may suggest strong associations between risk factors and outcome, however its ability to estimate causality is generally considered inferior to that of randomized controlled studies (203, 204). As this is a study on suspected negative prognostic factors from SSc on disease outcome (mortality), randomizing patients to such prognostic factors is neither methodologically possible, nor ethically acceptable. A cohort study design was relevant as we wished to investigate the difference of numerous parameters between patients and controls. A case-control study would have been relevant if we were to investigate risk factors for a specific outcome. However, our study included several outcomes, which would require several case and control groups. Still, analyses on risk factors and outcomes within the SSc cohorts are mostly performed as logistic regression on groups divided according to risk factor status, which resembles analyses of a case-control study.

The three papers focused on different exposures, and cohort sizes were therefore of unequal sizes. All three papers included around 300 patients with SSc and a control group. Being a rare disease, these SSc cohorts are considered relatively large. Yet, as some outcomes were infrequent, certain multivariable analyses were not feasible.

SSc may present numerous symptoms with varying severity, of which many are unspecific. This makes early suspicion challenging, and both patient's and doctor's delay may postpone time of diagnosis. Time of disease onset is therefore often estimated retrospectively, and disease duration may be several years already at initial examination. As a consequence, characterizing common features of SSc patients at baseline is difficult. Disease duration is often defined as time from either i) Raynaud's phenomenon, ii) a non-Raynaud SSc complication or iii) diagnosis. In our papers, we have defined disease duration as time from diagnosis to echocardiography or

serum sampling. The median disease duration was less than two years at echocardiography, but showed high heterogeneity. One may argue that the disease durations of our papers are artificially low, however as serum samples were collected at initial visit and echocardiographies evaluated were the earliest available, we consider the definition of baseline echocardiography and serum sampling at baseline as a truthful characterization.

The ideal study population would include a non-selected cohort, referred to our hospital at time of SSc suspicion, or at time of diagnosis. We consider our cohort relatively unselected, as most patients diagnosed with SSc in South-East Norway, together with a considerable amount of patients from other regions, are referred to OUH for a second opinion at an early stage. In addition, age, sex and SSc subtype distribution are in concordance with other SSc cohorts (9, 176). There may be skewness towards more severe organ manifestations in our cohort as complicated patients are referred more often than patients with mild presentation. However, we do not believe our cohort suffers from greater selection bias compared to international cohorts.

6.2 Echocardiographies

The earliest available echocardiography was defined baseline echocardiography. However, there was a large variability in disease duration at time of echocardiography. These examinations were not necessarily from the initial visit.

The most recent echocardiography was defined follow-up echocardiography. Unfortunately, follow-up echocardiographies were often performed at local cardiology departments and were therefore not available for study analyses. Due to different dates of diagnosis, time of follow-up varied considerably between patients. However, as prediction analyses by Cox regression account for time elapsed, we do not suspect that large variation in observation time have biased the results.

6.3 Control group

6.3.1 Echocardiography controls

Echocardiography controls were collected from the Nord-Trøndelag Health Study (HUNT 3), including echocardiographies of 1,266 individuals from the general population without recognized hypertension, cardiovascular disease, or diabetes mellitus. We have no data claiming that the Nord-Trøndelag population is more or less healthy than the general Norwegian population. However, we know that 30 controls were excluded from the normal material due to discovery of pathology. It is therefore possible that the selected control group is skewed towards healthier individuals, overestimating the dissimilarity between patients and background population.

6.3.2. Serum marker controls

Serum marker controls were collected from blood bank donors. These individuals are healthy and likely resemble the serum profile of the population. In Norway, only individuals without infections, immunodeficiencies, cardiovascular and other chronic diseases are allowed to donate blood. However, controls were not matched to patients with respect to age, and it is therefore challenging to determine whether differing values are attributed to disease or age.

7.0 Ethical considerations:

There are especially two ethical pitfalls related to medical research. It is of vital importance to 1) not harm or cause unnecessary burden on included research subjects, and 2) perform analyses and report results as unbiased and honestly as possible.

Patients included in the study are registered in NOSVAR. When included in NOSVAR, patients consent that collected data may be used for research purposes. This study only involves information already collected from earlier studies or clinical work. Clinical examinations, echocardiographies, pulmonary function tests and HRCTs were performed in order to reveal organ manifestations according to clinical recommendations, irrespective of research value. Contrary, serum markers for analyzation by ELISA or Luminex were collected exclusively for research purposes. However, as the frequency of severe complications from blood sampling is very low, and as sample volumes collected were considered too small to possess any danger in non-anemic patients, we believe the value of results from sampling considerably outweighs the risks.

We have put time and effort in performing different analyses for confirmation of result findings. In advance of analyses, we have not developed hypotheses on echocardiographic or seral markers suspected of relation to outcomes, and we therefore consider our conclusions unbiased. With respect to article three, associations to echocardiographic markers were only evaluated in patients with echocardiographies at a maximum of three years apart from serum collection. Although this has reduced the number of study individuals significantly, we found this necessary in order to keep a minimum of temporal relation between echocardiography and serum sampling.

In paper I we suggest that patients with DD bear a high risk of mortality and should be followed closely. One may discuss whether patients presenting DD on the evaluated echocardiographies should be informed and monitored more closely. However, 1) results should be validated in other cohorts before recommendations are revised, 2) DD from different etiologies will likely

require heterogenic treatment regimes, and 3) to date, there is as mentioned inadequate treatment options for primary diastolic dysfunction. Our results have therefore not had a direct impact on the research participants of these studies.

It is of notice that no commercial industry has been involved in this Ph. D. thesis.

8.0 Main conclusions:

1. Left and right ventricular dysfunction are frequent complications of systemic sclerosis, already at first echocardiographic examination.
2. RV systolic function in SSc deteriorates through the disease course, while LV systolic function remains stable.
3. LV diastolic function and RV systolic function are strong independent predictors of mortality, while LV systolic function failed to predict mortality independently.
4. LV diastolic function and precapillary PH are associated conditions, of which DD presents the strongest mortality predictive ability.
5. Angiopoietin 2, osteopontin and tumor necrosis factor-related apoptosis-inducing ligand are serum markers strongly related to LV and RV dysfunction among SSc patients. These three serum markers further prove to be strong independent predictors of SSc-mortality in multivariable regression analyses.

9.0 Clinical implications and future perspective:

9.1. Clinical implications

We suggest that DD should be evaluated on all echocardiographies from SSc patients and considered a negative prognostic sign when evident. As a high number of DD patients in our cohort presented with concomitant precapillary PH, it is vital that the presence of DD on echocardiography raises the clinician's suspicion of such a devastating complication.

In the absence of precapillary PH, we consider it important that DD patients are referred to cardiologic consultations for evaluation of HFpEF. Although HFpEF treatment has proven difficult, this is an internationally prioritized research subject.

TAPSE was a strong independent predictor of mortality in SSc patients, also when adjusted for LV diastolic function. TAPSE should merit a place in standard echocardiographies in SSc.

We believe the physician must pay attention to the regulation of diabetes mellitus, hypertension and obesity in SSc as these are additional risk factors for the development of DD.

Our results on serum markers and the markers' relation to cardiac dysfunction need to be validated in other cohorts. Although one may hypothesize both diagnostic and therapeutic traits of these markers, it is at this stage too early to advocate their clinical implications.

9.2. Future perspective

Development of novel therapies directed toward optimization and/or improvement of diastolic function are highly called for. In studies on DD/HFpEF, it is however challenging to avoid including patients with components of other dyspnea-driving morbidities. Before initiating large, costly randomized controlled studies, it is therefore necessary to carefully select patients of interest.

In order to differentiate PAH and PH from occult left heart, future studies are necessary to categorize normal and abnormal physiologic response to fluid loading. This must be performed

on large samples of the normal population. Individuals of differing age, sex and ethnicities should be included. Such individuals should be without significant risk of altered cardiac function, and one should recruit a sample free from cardiovascular disease, diabetes mellitus, hypercholesterolemia and hypertension. Ideally, participants should be non-smokers and medication naïve. Realistically, studies from different centers are needed in order to give a reliable overview of the subject, not biased by methodology of a single center.

Succeedingly, cross-sectional RHC studies including fluid loading should be performed at large PH centers to evaluate the etiology of PH in SSc patients. These studies may include both new onset- and earlier diagnosed SSc-PH patients. As an addition, it would be interesting to investigate whether patients re-categorized from PAH to PH from left heart disease represent the subset of PH patients shown to respond inadequately to pulmonary vasodilators.

Given the detrimental burden of DD on mortality in SSc, a targeted approach to reducing risk factors of DD/HFpEF may be of value. Whether optimization of hypertension, diabetes and overweight may reduce the burden of DD in SSc is of interest.

The significance of ANGPT2, OPN and TRAIL on cardiac outcomes must be validated in other cohorts. We did not perform RHC simultaneously with serum sampling. It is therefore difficult to evaluate the relation between reported cardiac dysfunction and PH. It is not evident whether altered serum levels of the serum markers reflect PH, primary cardiac SSc or heart disease due to non-SSc etiologies. If our results are validated, studies investigating the therapeutic potential of the markers, or their regulation, are of interest.

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