Gastrointestinal Manifestations and Pathophysiological Mechanisms in Systemic Sclerosis

KARIN FRANCK-LARSSON
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Abstract

Systemic sclerosis (SSc) is a rare systemic, autoimmune disease characterized by vascular changes and fibrosis of the skin and internal organs.

Patients with SSc more frequently than healthy controls reported upper gastrointestinal (GI) symptoms, which was more abundant in the diffuse cutaneous form (dcSSc) of the disease than in the limited (lcSSc). One-third of a population-based cohort of 79 SSc patients reported faecal incontinence, compared to 11% in 158 healthy matched controls (p<0.001), and this symptom negatively influenced general well-being and social life. Impaired rectal sensibility, rectal bleeding, irritable bowel syndrome-like symptoms, abdominal pain, the need for manual assistance at defecation, and the use of oral laxatives were more common in patients than in controls. SSc patients reported lower scores in both physical and mental scales of the SF-36 questionnaire than controls, indicating worse health-related quality of life.

Gastric emptying was slower in patients than in controls, and a higher prevalence of delayed gastric emptying in patients with dcSSc indicated more severe GI tract involvement than in lcSSc. Electrogastrographic recordings did not correlate to gastric emptying results, indicating factors other than defective myoelectric signals contributed to disturbed gastric function.

SSc patients with faecal incontinence had lower anal squeeze pressures than patients without this symptom. Only patients with faecal incontinence had ultrasonographic abnormalities in the internal and external anal sphincters, and absence of the rectoanal inhibitory reflex. Thus, faecal incontinence in SSc patients may depend on both neurogenic and structural mechanisms. A discrete increase in fibre density observed in a majority of SSc patients might have implications from a disease mechanistic perspective.

Sera from 47% of 70 SSc patients had the capacity to induce interferon (IFN)-α, production which correlated to the presence of anti-RNP and anti-SSA autoantibodies. Increased serum levels of IFN-inducible protein were associated with vascular manifestations, and increased serum levels of IFN-α with digital ulcers. Increased serum levels of monocyte chemoattractant protein-1 or IFN-α were associated with lung fibrosis. An activated type I IFN system previously observed in several other systemic autoimmune diseases is also present in SSc and may contribute to vascular pathology and the pro-fibrotic process.

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To Kent, Hanna, Simon, and Lukas
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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<th>Description</th>
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<tbody>
<tr>
<td>ACA</td>
<td>anti-centromere antibodies</td>
</tr>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
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<td>CENP</td>
<td>centromere protein</td>
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<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>dcSSc</td>
<td>diffuse cutaneous systemic sclerosis</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DP</td>
<td>delta pressure, difference between resting pressure and squeeze pressure</td>
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<tr>
<td>EAS</td>
<td>external anal sphincter</td>
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<td>ECM</td>
<td>extra cellular matrix</td>
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<td>EGG</td>
<td>electrogastrography</td>
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<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>FD</td>
<td>fibre density</td>
</tr>
<tr>
<td>GAVE</td>
<td>gastric antral vascular ectasia</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>HPZ</td>
<td>high-pressure zone</td>
</tr>
<tr>
<td>IAS</td>
<td>internal anal sphincter</td>
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<tr>
<td>IBS</td>
<td>irritable bowel disease</td>
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<tr>
<td>IC</td>
<td>immune complex</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>IIF</td>
<td>indirect immunofluorescence</td>
</tr>
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<td>ILD</td>
<td>interstitial lung disease</td>
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<tr>
<td>IP</td>
<td>IFN inducible protein</td>
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<tr>
<td>Jo-1</td>
<td>histidyl tRNA synthetase</td>
</tr>
<tr>
<td>lcSSc</td>
<td>limited cutaneous systemic sclerosis</td>
</tr>
<tr>
<td>MCP</td>
<td>monocyte chemoattractant protein</td>
</tr>
<tr>
<td>MIP</td>
<td>macrophage inflammatory protein</td>
</tr>
<tr>
<td>MMS</td>
<td>modified Miller score</td>
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<tr>
<td>MRP</td>
<td>maximum resting pressure</td>
</tr>
<tr>
<td>MSP</td>
<td>maximum squeeze pressure</td>
</tr>
<tr>
<td>PAH</td>
<td>pulmonary arterial hypertension</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PNTML</td>
<td>pudendal nerve terminal motor latency</td>
</tr>
<tr>
<td>RAIR</td>
<td>rectoanal inhibitory reflex</td>
</tr>
<tr>
<td>RANTES</td>
<td>regulated upon activation, normal T-cells expressed and secreted</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNP</td>
<td>ribonucleoprotein</td>
</tr>
<tr>
<td>SSA</td>
<td>Sjögren’s syndrome antigen A</td>
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<tr>
<td>SSc</td>
<td>systemic sclerosis</td>
</tr>
<tr>
<td>SRC</td>
<td>scleroderma renal crisis</td>
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<tr>
<td>US</td>
<td>ultrasound</td>
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Introduction

Systemic sclerosis (SSc) is a rare autoimmune, multisystem, connective tissue disease with female preponderance. The hallmark of the disease is specific skin changes, but almost all patients have visceral involvement. Microvascular, neural, inflammatory, and fibrotic disturbances and changes contribute to morbidity and mortality of the disease.

Background

Epidemiology
There is little data on the prevalence of systemic sclerosis (SSc), and reports vary between regions: depending on race, age, and gender (1). In the Icelandic population, the incidence is estimated at 0.38/100,000 inhabitants/year and the prevalence to 71/million inhabitants (2). The prevalence of SSc was 88/million in northeast England in 2000 (95% confidence interval (CI) 68-108) (3), and 140/million among Europeans in a French multi-ethnic survey in 2001 (95% CI 122-170) (4). The prevalence and incidence have not been determined in Sweden. Onset of disease is reported in all ages, but is dependent on the period and the population studied, methodology used, and classification of disease (5-6). Peak incidence in both men and women is generally in the fifth and sixth decades (2-3, 7). In Iceland, there is a high female/ male ratio of 14.1:1 (2), but more often female preponderance between 3:1 and 8:1 is reported (3, 6-8). The ratio between limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc) in population-based European studies are 2.6:1-4.7:1 (2-4).
Etiology and Pathogenesis

SSc is a complex and heterogeneous disease and etiology appears linked to both genetic susceptibility and environmental factors (9). Progress has been made in identifying multiple candidate genes that potentially increase the risk of developing the disease, both in the number of polymorphisms in the human leukocyte antigen (HLA) region (10-12), and specific single nucleotide polymorphisms in genes involved in immune regulation, vascular function, and extra cellular matrix (ECM) (10, 13-15). Exogenous factors, including infectious agents, crystalline silica dust, vinyl chloride, organic solvents, and bleomycin are associated with disease onset (16-17). Microchimerism, in the form of persistent maternal or off-spring cells after pregnancy, are suggested as a possible factor in the development of SSc (18).

Functional and structural vasculopathy with endothelial cell injury and activation, inflammation, and fibrosis with increased deposition of ECM components contribute to the pathogenesis in a progressive sequence (19), but due to the rareness of the disease and that diagnosis is usually delayed by several years, the initial disease mechanisms are not fully elucidated. Raynaud’s phenomenon, the visible evidence of vasomotor instability in the digits, is often present for years before other disease manifestations appear, and is hypothesised to occur in internal organs (20-23). Endothelial cell dysfunction and ultrastructural microvascular changes appear to be the earliest pathological changes, followed by perivascular infiltration of inflammatory cells, intimal fibrosis with narrowing, and subsequent obliteration of the lumen in arterioles (24). Numerous inflammatory active cells have been detected in SSc skin lesions including macrophages, T-cells, B-cells, plasma cells, natural killer cells, mast cells, platelets, and eosinophils (24-26).

Multiple mediators synthesised by immune cells are involved in the development and sustenance of SSc, mainly growth factors (transforming growth factor-β, platelet-derived growth factor and connective tissue growth factor); cytokines (e.g. tumour necrosis factor, and interleukins), and chemokines (27-29). Chemokines are classified based on the first two cysteine residues (CC, CCX, CX3C or C) (30), but are often referred to in the literature with other names. They are related small molecules with chemotactic properties that are important in recruitment and regulation of leukocytes (31). However, chemokines can also exert other functions. Monocyte chemotactic protein (MCP)-1/CCL2 might directly stimulate the expression and accumulation of collagen by fibroblasts (32). IFN inducible protein (IP)-10/CXCL10 is a potent angiostatic factor with antifibrotic properties (33).
Activated myofibroblasts produce excessive amounts of ECM molecules. In skin biopsies from patients with SSc, where inflammatory cells are abundant, network deposition of type III collagen occurs, whereas, in specimens void of cells, the amount of collagen type III is reduced and there is dominance of type I (34).

In addition, an early involvement of the peripheral nervous system is proposed (35), with reduction of mainly unmyelinated sensory and autonomic innervations in sclerotic and apparently unaffected skin (36). Whether nerve dysfunction is caused by direct effects of autoimmune processes, or is secondary to ischemia from vascular changes or compression from oedematous or thickened tissues, and whether nerve dysfunction in turn causes further damage remain to be further explored.

**Autoantibodies**

A majority of patients are autoantibody positive. Antinuclear antibodies (ANA) are detected with indirect immunofluorescence (IIF), which is usually the first step in autoantibody testing in connective tissue disease, and are present in over 90% of SSc patients (37). All four major staining patterns of ANA (centromere, homogeneous, speckled, and nucleolar) on IIF can be seen in SSc. In addition, specific autoantibodies directed against a variety of structures and proteins in the nucleus and other parts of the cell, such as antibodies against centromere protein (CENP)-A to CENP-F, anti-topoisomerase I (anti-Topo-I; previously Scl-70), anti-ribonucleoprotein (RNP), anti-ribonucleic acid polymerase (RNAP) I-III, and anti-Sjögren’s Syndrome antigen A (SSA), can be detected with enzyme-linked immunosorbent assay (ELISA), immunodiffusion (ID), immunoblotting (IB), and other techniques. Autoantibodies typically associated with SSc are anti-Topo-I (reported in 20-43% of patients), anti-centromere antibodies (ACA) (reported in 19-24% patients), and anti-nucleolar antibodies (ANoA) (reported in 15-40% patients) (38). The autoantibody profile in SSc is linked to clinical findings (39-41) and to the plasma cytokine profile (42), and has prognostic value in determining risk and survival (40, 43-44).
Clinical features

Three major definitions of disease onset are used in epidemiological and clinical studies: onset of Raynaud’s phenomenon; onset of non-Raynaud’s symptom; or, date of diagnosis (45).

The first clinical symptom is usually Raynaud’s phenomenon, recognised by patients as discolouring of the digits, i.e. fingers and toes, which is caused by abnormal reactivity in the peripheral arteries and capillaries, and structural vascular changes involving the endothelium and platelets. Raynaud’s phenomenon is a common symptom and present in more than 95% of patients (46-47), and often appears after changes in temperature or in stress situations, and might lead to digital ulcers, necrosis, and, in severe cases, to spontaneous acrolysis or surgical amputation.

The hallmark of the disease is the presence of skin changes, in most patients starting distally and progressing proximally. The cutaneous changes tend to develop in a three-phase process (48): an early non-pitting oedematous phase, probably caused by both microvascular injury and inflammation; a later indurative phase with thick, shiny, and taut skin adherent to the subcutis, reflecting increasing fibrosis; and, a third phase with thin, atrophic skin tightly tethered to the underlying tissue. The process through these three stages might take from a couple of weeks to several decades in the individual patient. Skin changes may affect the hand function and contractures may occur. The scleroderma skin changes are sometimes accompanied by itching, that in some cases lead to sleep disturbances, and discoloration. The typical change in appearance associated with SSc skin changes in the face, i.e. thinning of the nose and diminishing of the mouth, are distressing to the patient (49). Skin changes in SSc can be evaluated by palpation (50).

SSc can affect almost every organ in the body and gastrointestinal manifestations are commonly encountered. Interstitial lung disease (ILD) is found in 75-80% of SSc patients and may present as shortness of breath and a non-productive cough (51-52). Conventional chest radiographs reveal manifest fibrosis, but early detection of ILD can be accomplished with more sensitive high-resolution computed tomography (HRCT) (53-54). Pulmonary function tests are useful for routine follow-up.

Cardiac involvement encompasses ischemic heart disease/cardiac infarction, pericarditis/pericardial effusion, heart failure, and arrhythmia (55-56). On autopsy, myocardial fibrosis is present in 81% of patients compared to 55% of controls (p<0.01) (51). However, cardiac involvement might pass unrecognised as only 16% of SSc patients have records of pericarditis, but on autopsy 53% have signs of pericardial
lesions (51). Musculoskeletal involvement, including myositis, and joint involvement, including arthritis, are common (39, 57). One of the most serious manifestations of the disease is scleroderma renal crisis (SRC) that might lead to total renal failure in a few days if not treated adequately (58). Another life-threatening manifestation of SSc is pulmonary arterial hypertension (PAH), which can be primary or secondary to pulmonary fibrosis. Screening for PAH with echocardiography is useful (59-60), although a definite diagnosis relies on the performance of invasive right heart catheterisation in suspected cases (61).

Patients with SSc have a higher mortality rate than the general population, with Standard Mortality Ratios (SMR) varying from 2.9-4.7 in different populations (62-63). With the introduction of new treatment strategies, especially vasoactive medication for the treatment of SRC and PAH, and with improved diagnosis and follow-up, survival has improved (45).

Despite recent advances in the understanding of SSc, there is still a lack of unequivocally effective treatment for SSc (64). Potential targets and new strategies have been identified but need confirming in clinical trials (64-65). At present, treatment is mainly for symptomatic relief, thus, morbidity problems, among them gastrointestinal (GI) symptoms, are important.

**Diagnosis and Classification**

In 1980, a group of rheumatologists, constituting a subcommittee of the American Rheumatism Association (ARA: nowadays called the American College of Rheumatology, ACR) presented a set of preliminary criteria for the classification of SSc (66). These criteria were based on a multi-centre study of early-diagnosed cases of SSc in comparison with patients with Systemic Lupus Erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), and Raynaud’s phenomenon: 797 patients were included in the study. The proposed preliminary criteria stated the finding of either the sole major criterion or two or more of the minor criteria, based on clinical consensus (Table 1).
Table 1. Major and minor criteria for the diagnosis of SSc.

<table>
<thead>
<tr>
<th>Major criterion</th>
<th>Minor criteria</th>
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<tr>
<td>Proximal scleroderma. Typical sclerodermatous skin changes: tightness, thickening, and non-pitting induration, excluding the localized forms of scleroderma, proximal to metacarpophalangeal or metatarsophalangeal joints; affecting other parts of the extremities, face, neck, or trunk (thorax or abdomen); usually bilateral, symmetrical, and almost always including sclerodactyly</td>
<td>1. Sclerodactyly: above-indicated changes limited to fingers and toes</td>
</tr>
<tr>
<td></td>
<td>2. Digital pitting scars or loss of substance from the finger pad: depressed areas at tips of digits or loss of digital pad tissue as a result of digital ischemia rather than trauma or exogenous causes</td>
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<tr>
<td></td>
<td>3. Bibasilar pulmonary fibrosis: bilateral reticular pattern of linear or lineonodular densities which are most pronounced in basilar portions of the lungs on standard chest roentgenogram; may assume appearance of diffuse mottling or “honeycomb lung”, and should not be attributable to primary lung disease.</td>
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The preliminary criteria have 97% sensitivity and 98% specificity (66). In the light of new research findings, especially disease specific autoantibodies, and the introduction of new investigation methods, including computed tomography (CT) scan and nailfold capillaroscopy, there have been attempts to revise the criteria (67). Among them, a proposed set of criteria for early SSc has been presented (68).

From a prognostic viewpoint, it has proven useful to divide the disease into subsets. Several different definitions are proposed (69), some definitions are based only on the extent of the skin changes, others definitions consider other disease features, including autoantibodies. In 1988, LeRoy et al (70) proposed a definition that differentiates between two subtypes: limited cutaneous SSc (abbreviated lcSSc or earlier lSSc), and diffuse cutaneous SSc (abbreviated dcSSc or dSSc). lcSSc is defined as skin thickening restricted to sites distal to the elbows, the knees, and above the clavicle, and dcSSc is diagnosed when skin thickening is present on the trunk, in addition to the face, neck, proximal and distal extremities (70). However, with this definition, skin changes of the
proximal extremities do not define a certain subtype. Therefore, it has been proposed the elbows, knees and clavicles should constitute the dividing line between lcSSc and dcSSc (49).

 lcSSc is generally regarded as the more benign subset of the disease with less visceral involvement, mainly from the GI tract and in some cases the lungs, and better survival, except for individuals who develop PAH. Conversely, dcSSc is characterised by a more rapid course with common pulmonary and cardiac involvement, as well as the GI tract and the joints (71).

Gastrointestinal anatomy and physiology

The gut wall consists principally of the mucosa, including the thin, smooth muscularis mucosae layer, the submucosa, the inner circular muscle layer, the outer longitudinal muscular layer, and the serosa. In the stomach, there is an additional third oblique muscle layer, and in the colon, the longitudinal muscle is organised into three bands of muscle, the taenia coli. The neural regulation of GI motility is provided by extrinsic and intrinsic innervation. Extrinsic innervation comprises somatic motor innervation to the striated muscles in the proximal oesophagus and the anal canal, and autonomic (parasympathetic and sympathetic) innervation to other parts of the GI tract. Intrinsic innervation is the enteric nervous system with two major plexuses, the submucosal plexus, and the myenteric plexus. The nervous system plays an important and integral role in the regulation of the mucosal and motor function of the gut through the release of peptide and non-peptide neurotransmitters. Neural transmitters, together with endocrine and paracrine substances, interact with the immune system in the GI tract. The GI tract holds the largest amount of lymphoid tissues in the body and is important in protection against infectious agents and in the process of evoking and maintaining immune tolerance (72).

Motility in the GI tract is regulated by electric impulses. In the stomach, there is a pacemaker area located on the greater curvature of the corpus of the stomach. The basal myoelectric activity of the stomach, the slow wave, consists of rhythmic waves of de- and repolarisation with a normal frequency of 3 cycles per minute that radiates downwards. When spike potentials are superimposed on the slow wave, mechanical activity is triggered. Thus, the normal maximal contraction frequency in the stomach is 3/ minute, and frequency and direction of the contractions are controlled by the slow wave (73).
The functions of the anal canal are to maintain faecal continence and control defecation, and this relies on several components, including innervation, the function of the internal anal sphincter (IAS) and the external anal sphincter (EAS), the anorectal angle created by the puborectalis muscle, rectal compliance, and bowel motility. The IAS and the EAS are the basis for the high-pressure zone (HPZ). IAS accounts for approximately 50-60 % of the resting pressure and EAS accounts for about 30 % and for the squeeze pressure (74). Many different mechanisms might lead to faecal incontinence, such as diarrheal states, neurological conditions, trauma, aging, and pelvic floor denervations (75). Defecation is triggered by distension of the rectum, leading to relaxation of the IAS through the rectoanal inhibitory reflex (RAIR) (76). Voluntary contraction of the EAS and the pelvic floor can normally suppress defecation. IAS generates mechanical activity with a frequency of 15-35 cycles per minute, and ultra-slow waves at 1.5-3 cycles per minute (77).

Gastrointestinal involvement in SSc

GI tract involvement is the third most frequent manifestation of SSc, surpassed only by Raynaud’s phenomenon and skin changes (37), and is reported in up to 90% of patients (78-79). An initial transient neuropathic phase was earlier proposed as preceding a myopathic phase (80), however, a four-grade scale for gastrointestinal pathology, involving vascular, neural, muscular and fibrotic changes, has been presented (81).

In autopsy oesophageal tissue (82), intimal proliferation of arterioles is present in 38% of SSc patients, compared to 5% of controls (p<0.001). The presence of antitymientic neuronal antibodies indicate an autoimmune process directed towards neurons in SSc (83), but vasculopathy with ischemia also contributes to neural damage. Almost all SSc patients have some smooth muscle atrophy, but without any difference in the presence of inflammatory cells in the myenteric plexus between patients and controls (82). However, specimens from the small and large intestines can contain eosinophils and mast cells close to intramural nerve plexa (84). Investigation of autopsy material from SSc patients (51) reveals GI muscle atrophy or fibrosis in the oesophagus (74% of patients), small intestine (48% of patients), and large intestine (39% of patients).

GI changes in SSc often cause dysmotility that can lead to a number of related states and symptoms in different parts of the GI tract. In the
neuropathic phase there may be response to prokinetic drugs, whereas, smooth muscle atrophy and fibrotic replacement in the myopathic phase render stimulatory efforts less effective (85). Early severe GI involvement is observed in a subset of patients (86).

A majority of SSc patients have decreased or absent motility in the lower parts of the oesophagus, with or without lower oesophageal sphincter dysfunction (decreased pressure or reflux), as registered by oesophageal manometry (87-88): patients might experience heartburn, dysphagia, and nocturnal aspirations (89). Secondary complications include oesophagitis, candidiasis, oesophageal ulcers, strictures, adenocarcinoma, and possibly ILD (90). Even with proton-pump inhibitor treatment, Barrett’s metaplasia, recurrence of symptoms, and progression of dysmotility might still occur (91-92).

Gastric dysmotility might cause malnutrition because of slow emptying of the bolus into the small bowel and a decrease in alimentary intake due to a sense of fullness: a full stomach might also cause reflux. In addition, gastric ulcers and gastric antral vascular ectasia (GAVE), the so-called watermelon stomach, can cause bleeding and anaemia (93-94).

In the small intestine, hypomotility can lead to bacterial overgrowth (95), reported in 43% of SSc patients in an unselected cohort (96). Involvement of the small intestine may cause bloating, postprandial fullness, distension, flatulence, diarrhoea and malabsorption, weight loss, and in severe cases, malnutrition (97): a BMI <18.5 is one of the few factors at initial presentation predicting increased mortality (98). Pneumatosis cystoides intestinalis and pseudodooobstruction are reported in SSc patients (97, 99-101). Slow colonic transit is seen in SSc (102-104) and might manifest as constipation, which has been reported in 30% of patients (87). In addition, loss of haustrations, dilatation, and wide-mouth diverticula in the colon may be present (105).

Deep rectal biopsies in a few SSc patients with recent onset and presence of intestinal symptoms indicate ultrastructural signs of neuronal damage, focal degeneration of smooth muscle cells, hypertrophy of endothelial cells with obliterated lumen, and mast cells in connection with nerve fibres and vessels (106). The prevalence of faecal incontinence in SSc is reported in varying frequency between 20-38% (107). Patient selection, methods of detection, and the nature of the symptom may influence the reports, as patients are hesitant to admit inadequate bowel control and physicians might be reluctant to ask. Several mechanisms, e.g. constipation, diarrhoea, and neural and IAS impairment can contribute to the development of faecal incontinence (108).
GI symptoms are often reported, but as GI symptoms are common in the general population, the relevance of these findings in the absence of non-SSc controls is uncertain (87): studies on GI symptoms in SSc are often retrospective or without controls (107, 109-110). Some GI symptoms (e.g. faecal incontinence) can be embarrassing for the individual and might not be detected when reviewing medical records. In addition, there is often disparity between the frequency and intensity of symptoms and objective signs of organ involvement (111). Prevalence of primary biliary cirrhosis in SSc is greater than expected in the general population (112).

Investigational methods of the GI tract in SSc

The GI tract is accessible for imaging studies, endoscopic techniques, and functional studies. Imaging studies include CT, ultrasound (US) and magnetic resonance tomography (MRT). With endoscopic techniques, it is possible to gain direct insight into the interior of the GI tract, to inspect the mucosa, and take biopsies. Functional methods are used to study motility and physiology in different parts of the GI system.

Depending on indication, several methods are used for investigating the stomach. Gastroscopy enables diagnosis of GAVE and gastric ulcers in anaemia in SSc, and scintigraphic gastric emptying motility disturbances. Electrogastrography (EGG) measures the electric control activity of the stomach through serosal, mucosal or cutaneous registrations, and is a more experimental method.

Common tests for anorectal assessments of faecal incontinence and constipation include anorectal manometry, anal sonography, defecography, pudendal nerve terminal motor latency (PNTML), anal electromyography (EMG), and saline continence test (113). For research purposes, anal vector manometry, scintigraphic defecography, and ambulatory anorectal manometry may be used (113).

In SSc, a set of clinical, laboratory, and instrumental methods are identified as suitable tools for assessing the presence of GI involvement in SSc (111). The potential tests are identified through literature reviews and selected by demonstrated validity, reliability, and feasibility: the proposed list includes both quantitative and qualitative investigations (111).
The type-I interferon system

The innate, or inborn, immune system plays an essential role in the initial response to microorganisms and helps to regulate the adaptive immune response. Immature dendritic cells (DC) capture and process antigens, and, after activation, are able to present antigens to lymphocytes for the induction of specific adaptive responses (114). Another important function of DC in the innate and adaptive immune system is to produce cytokines and chemokines that regulate the function of a number of cells in the immune system. The DC can be divided into two major subsets, the myeloid DC (mDC) and the plasmacytoid DC (pDC), which can be activated by many different agents. The pDCs have several receptors for sensing the external milieu. Of importance are Toll-like receptors (TLR)7 and TLR9 that are expressed at high levels in pDC. Activation of these receptors in pDC results in high production of type I interferons (IFN).

IFNs are cytokines that inhibit viral replication and are classified into types I, II, and III (115). The type I IFN (IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω) can be produced by a wide array of nucleated cells and bind to the type I IFN receptor (IFNAR), which is present on all nucleated cells. Binding of the type I IFN to the IFNAR induces dimerisation of IFNAR1 and IFNAR2, resulting in intracellular activation of tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1). This in turn activates multiple signalling through three main pathways: the JAK/STAT; mitogen-activated protein kinase; and, phosphoinositide 3-kinase pathways (116). The signalling results in induction of several hundreds of genes, and in gene expression profiling these IFN-inducible genes, coding for cytokines, chemokines, and other inflammatory mediators, are upregulated. The increased expression of type I IFN regulated genes is termed an IFN type I signature and is identified in a majority of patients with SLE and primary Sjögren’s syndrome (117-118), and in a subtype of patients with rheumatoid arthritis (RA) (119). The type I IFN system is a collective name for the cells and molecules involved in type I IFN production and response.

Even though the diagnostic criteria for SSc were created to differentiate SSc from other rheumatic diseases, some disease manifestations and mechanisms are shared, including the involvement of the IFN system. Gene expression profiles of peripheral blood leukocytes from patients with early SSc have increased expression of genes targeting blood leukocytes to the endothelium, but these cells also display an IFN signature (13-14), and possible similarities in increased expression of IFN-inducible genes are reported in SLE and SSc (120). Increased expression of the type I IFN regulated molecule Siglec-1 on monocytes is
found in SSc (121), and expression of IFN genes is associated with certain antibodies and some clinical manifestations (122).

Increased levels and induced production of several cytokines (TNF, interleukin (IL)-6, IL-10, and IL-18), and chemokines (IP-10, MCP-1, MIP-1α, MIP-1β, Regulated upon activation and normal T-cells expressed and secreted (RANTES), thymus and activation-regulated chemokine (TARC), and macrophage-derived cytokine (MDC)) are reported in SSc (29, 123-125) and are of importance for the recruitment and activation of inflammatory cells. MCP-1 correlates to early disease, anti-Topo-I, or anti-RNAP I-III, and to a greater frequency of organ-based complications, with a tendency for reduced MCP-1 serum levels with bosentan or prostacyclin analogue treatment in SSc (126). MCP-1 and MIP-1α are proposed as involved in the development of pulmonary fibrosis in SSc (124), and RANTES may be involved in the early stages of SSc (127). As in other systemic diseases, development of SSc is reported after treatment with type I IFN (128-129).
Aims of the thesis

The general aim of this thesis was to explore clinical, physiological and pathogenic aspects of SSc. The specific aims of the studies were:

- To analyse the type and frequency of GI symptoms in SSc patients, in comparison to healthy controls.
- To investigate possible mechanisms of disturbed GI function in the upper and lower GI tract.
- To assess any correlation between symptoms and anatomical and physiological alterations, and to relate symptoms and investigational findings to quality of life.
- To study the type I IFN system in SSc and its activation and relation to chemokines, autoantibodies, and disease manifestations.
Materials and Methods

Subjects (Papers I-IV)

The study populations consisted of patients with a diagnosis of definite SSc, according to the 1980 preliminary ACR criteria (66). The categorisation of the disease was based on whether cutaneous sclerosis affected skin only distally to the elbows, knees and clavicles (lcSSc) or also extended proximally (dcSSc) (49, 70).

**Paper I:** SSc patients (n=28) from the rheumatology clinic at the University Hospital in Uppsala, Sweden, were included in the study. The median age was 56 years (range 26-74), 24 (85%) patients were women and 4 (15%) patients were men: 13 (46%) of the patients had lcSSc and 15 (54%) had dcSSc. Disease duration from the first SSc symptom was mean 15 years (range 2-49). Fifteen healthy people, 12 women and 3 men, mean age 55 years (39-75), served as controls.

**Paper II.** SSc patients were recruited from an area with a total population of 1,109,765 inhabitants. Patients (n=98) aged 18-77 and fulfilling the 1980 preliminary ACR criteria for definite SSc were identified by retrospectively collected records from the 14 hospitals in the area for the period January 1, 1992, to December 31, 2001. The final responding patient group consisted of 79 patients (81%) with a median age of 61 (range 31–77): 63 were female (80%) and 16 male (20%). Sixty patients (76%) had lcSSc and 19 (24%) had dcSSc. Median disease duration was 11 years (range 1-48). Two healthy controls for each patient, matched for age, gender, and home municipality, were included in the study.

**Paper III:** Twenty-five patients from the rheumatology clinic at the University Hospital in Uppsala, Sweden, were included in the study. The median age was 59 years (range 27-77). Twenty-two were female (88%) and three were men (12%): Twenty patients (80%) had lcSSc and five (20%) had dcSSc. Mean disease duration from start of skin changes was seven years (median 4, range 1-34). Nineteen healthy volunteers (10 women, 9 men: mean age 62, range 52-73 yrs) served as a control group in the evaluation of the manometry examinations.

**Paper IV:** Seventy patients were included in the study. The median age was 62 years (range 19-93), 13 were men (19%) and 57 were women
(81%). Fifty-four had lcSSc (77%) and 16 (23%) had dcSSc. Mean disease duration was 9 years (range 0-34 years). Fifty-five (79%) were positive to ANA (with a centromere, homogenous, nucleolar or speckled pattern) and 43 (61%) were positive to any specific autoantibody (anti-CENP-B, anti-RNP, anti-Topo I, anti-SSA or anti-Jo-1).

**Evaluation of patient files (Papers I-IV)**

Patient files were used to ensure fulfilment of SSc criteria and to record subtype of disease, disease duration, laboratory tests, present medication, and information on gender, age, and residency. Information from patient files was used to characterise disease manifestation. GI involvement was defined as oesophageal dysmotility on cineradiography, dysphagia, heartburn, constipation, diarrhoea, or faecal incontinence. Pulmonary fibrosis was diagnosed by high-resolution CT and/or chest X-ray. Cardiac involvement was defined as present or previous cardiac infarction, heart failure, pleuritis, or arrhythmia. Presence of digital loss (radiographic evidence of acrolysis or surgical amputation due to necrotic ulcers), PAH, previous SRC, or episodes of peripheral thrombosis were recorded (40), as well as information on Raynaud’s phenomenon and historical or present digital ulcers.

**Questionnaires (Papers I-III)**

**Upper gastrointestinal symptoms (Paper I)**

A previously evaluated questionnaire (130) on the occurrence and frequency of gastric symptoms, i.e. nausea, emesis, early satiety, bloating, and upper abdominal pain, was used in the interviews of 28 SSc patients and 15 control persons. All interviews were conducted by the same investigator (Franck-Larsson). Symptoms were scored according to frequency: absence of a certain symptom (0 point), symptoms that occurred occasionally but not more than once a week (1 point), symptoms that occurred daily or several times a week (2 points), and symptoms that were always present (3 points).

**Lower gastrointestinal symptoms (Papers II and III)**

A formerly validated questionnaire (131) was used to explore aspects of bowel symptoms and faecal incontinence in 81 SSc patients and 162 age,
sex, and municipality-matched controls. The questionnaire consisted of 49 questions relating to faecal incontinence, constipation, and general symptoms: dichotomous, multiple-choice, open questions and Visual Analogue Scales were used. The Miller’s incontinence score (132) was calculated from the responses to the questionnaire, but the frequency of the episodes was modified, creating a modified Miller score (MMS): Grade I: incontinence episodes occasionally, Grade II: incontinence at least once per week, and Grade III: incontinence daily. The lowest score possible was 0 (no incontinence to flatus, liquid or solid stool) and the highest was 18 (daily incontinence to flatus, liquid and solid stool). A previously used composite score for symptoms possibly associated with Irritable bowel Syndrome (IBS) symptoms (133), not accounted for by faecal incontinence and excluding defecation frequency, was calculated from 11 questions in the questionnaire.

Health-Related Quality of Life (HR-QOL) (Papers II, and III)
The Medical Outcomes Survey (MOS) 36-item short-form health survey (SF-36) is a generic instrument for describing health multi-dimensionally and for measuring HR-QOL (134-135). The Swedish version is validated and normative data for the general Swedish population have been presented (136-138). The physical component summary (PCS) comprises the summation and calculation of scores from the four of the eight scales, or domains, addressing physical components: physical function (PF), role physical (RP), bodily pain (BP), and general health (GH). The mental component summary (MCS) comprises the remaining four scales, which measure mental aspects: vitality (VT), social function (SF), role emotional (RE), and mental health (MH). The scales are created so that higher scores always represent better health.

Evaluation of gastric function (Paper I)
Gastric emptying scintigraphy
The method used for scintigraphic analysis of gastric emptying has previously been described and evaluated in healthy subjects in a large nation-wide study (139). After an overnight fast, 28 patients and 15 controls were served a test meal labelled with macro-aggregated albumin (10 mBq $^{99m}$Tc). A gamma camera measured radioactivity: measurements were taken every 5 min during 50 min at the beginning of the
examination, and then at 10 min intervals during the remaining 70 min of the examination. Gastric radioactivity was manually delineated by drawing a region-of-interest (ROI) in each digitised image. The mean of the anterior and posterior registrations were used in a computer program to analyse the gastric processing of a solid meal. With automatic correction for physical decay of the isotope, the program calculated the lag phase, gastric emptying rate, and the half-emptying time (T1/2). Normal values were defined as mean of controls ± 2 SD.

**Electrogastrography**

The method used in this investigation is previously described (140). After an over-night fast, recordings were made with two active cutaneous electrodes and one reference electrode placed on the abdomen over the stomach of the 28 patients and 15 controls for signal trapping. A pre-prandial period of 30 minutes was recorded before intake of the test meal, and the recording continued after the start of the meal for further 100 to 140 minutes. The recordings were digitally analysed for the preprandial and postprandial periods separately and for the total time. The dominant frequency (DF) was analysed and the percentages of DF in the normal frequency range (2.4-3.6 cycles per minute, cpm), the bradygastria range (1.0-2.4 cpm), and the tachygastria range (3.6-10.0 cpm), and the dominant frequency instability coefficient (DFIC) were calculated (141) (Figure 1). The power increase was defined as the power (amplitude) of the signal in the postprandial period divided by the power in the preprandial period, and was calculated for the normal frequency and the bradygastria and tachygastria ranges.
Figure 1. Electrogastrography recording from a patient with SSc. Short periods of brady- and tachygastria were seen, but dominant frequency was within 2-4 cycles per minute for 88.8% of the time.

Evaluation of rectal physiology and anatomy (Paper III)

Clinical examination

Patients were examined through inspection and palpation of the perianal area. The anal canal was palpated and the resting and squeezing tone assessed. Finally, a rigid proctoscopy was performed.

Neurophysiologic examinations

For recording of single-fibre electromyography (EMG), a sterilised fine needle electrode (single-fibre electrode) with a recording surface of 25-μm diameter was inserted into the external anal sphincter (EAS) just lateral to the anal verge at 3- and 9-o’clock. Twenty needle positions were standard and the analysis of tracings allowed calculation of fibre density (FD) bilaterally in all patients. An average value greater than 1.7 was considered abnormal (142-143).

A St Mark’s electrode (144), with a stimulating electrode mounted at the tip of the index finger and a recording electrode mounted at the finger base, was used for measuring PNTML. The electrode had a constant
distance of 50 mm between stimulation of the nerve and registration in the EAS. The finger was inserted into the rectum and the pudendal nerve on each side was stimulated. Latency was measured bilaterally from stimulation of the pudendal nerve to the start of the muscle motor potential. Latency greater than 2.5 ms was regarded as abnormal (145).

Anorectal manometry

Anorectal manometry was performed with the patient in the left lateral position without any preceding anal manipulation or bowel preparation. A water-perfused catheter was used (VMC-8 Manometric Catheter, Synectics Medical AB, Stockholm, Sweden), with an outer diameter of 5 mm, eight side holes, and eight pressure detectors, radially oriented at 45° intervals. An inflatable rubber balloon was attached to the tip of the catheter: the perfusion rate was 0.5 ml/min. The catheter was connected to a compressor (Mui Scientific model PIP-4-8, Missauga, Ontario, Canada) and the software used was Polygram 98 for PC (Medtronic Functional Diagnostics A/S, Tonsbakken, Denmark). With the patient in the left lateral position, the catheter was fixed with the pressure detectors 6 cm above the anal verge. After stabilisation for 2 to 3 minutes, a recording was made for 30 seconds. Thereafter, the catheter was retracted by 1 cm, the pressure was allowed to stabilise, and another recording was made. The last measurement was taken with the detector 1 cm above the anal verge. The level with the highest resting pressure was recorded as the maximal resting pressure (MRP). The measurements were repeated during maximal squeeze to give the maximal squeezing pressure (MSP).

Subsequently, the catheter was placed and fixed with the transducer in the zone with the highest resting pressure. The balloon was inflated with 10 ml of air and if the initial inflation failed to induce RAIR, the balloon was further inflated by increments of 10 ml (up to 60 ml) until RAIR was elicited. Finally, the balloon was gradually inflated with 10-ml doses of body-temperature water (to a maximum volume of 250 ml); to record the first sensations of rectal filling, constant sensation, and maximal tolerated volume.

As a measure of external sphincter function, the difference between MSP and MRP (delta pressure (DP)) was calculated (MSP-MRP=DP). The value for the HPZ was calculated as the average of the measurements at 1 and 2 cm for MSP and MRP.
Endoanal ultrasound

Endoanal ultrasound was performed with the patient in the left lateral position and without any bowel preparation. A three-dimensional ultrasound image was captured with an Ultrasound scanner 2050, 12 to 16 MHz, (B-K Medical, Mileparken 34, Herlev, Denmark) and stored in a computer. The examination extended from the level of the upper border of the puborectal muscle to the anal verge. The internal and external sphincters were always identified and any defects or other structural abnormalities recorded.

In vitro methods (Papers I-IV)

Methods for detecting autoantibodies (Papers I-IV)

All patients’ sera were analysed for autoantibodies. ANA was investigated by indirect immunofluorescence on HEp-2 cells (Immuno Concepts, Sacramento, CA, USA, before Dec 14, 2004; after that date Bio-Rad, Stockholm, Sweden) with a screening dilution 1:200. ANA positive sera were further investigated for anti-double stranded deoxyribonucleic acid (dsDNA) antibodies on Crithidia luciliae (Immunoconcepts, Sacramento CA, USA) in a screening dilution of 1:10 and further titrated if positive. In Papers II and III, information on autoantibody profile was gathered from patient files from the nearest hospital if they had not visited Uppsala University Hospital.

In Papers I-III, all sera were screened for specific antibodies with the Varelisa ANA8 screen (Phadia AB, Uppsala, Sweden) and positive sera further investigated with immunodiffusion (Immuno Concept, Sacramento, CA, USA) before 14 October 2005, or the line blot Anti-ENA ProfilPlus with Ro-52 (Euroimmune, Lübeck, Germany) after that date.

In Paper IV, the InnoLia ANA Update Line Blot was used for all sera for detecting SmB, SmD, CENP-B, RNP-70kD, RNP-A, RNP-C, SSA/Ro52, SSA/Ro60, SSB/La, Topo-I, Ribosomale P, and histones (146).

Interferon-α immunoassays, induction, and inhibition (Paper IV)

Peripheral blood mononuclear cells (PBMC), pDC, and monocytes were prepared from healthy blood donor buffy coats and cultivated in 96-well
plates. A final concentration of 10% necrotic or 25% apoptotic cell material from monocytic U937 cells (147) were used in the cell cultures. The patient or control sera were used in final concentrations of 10%, 1%, 0.1%, or 0.01%, with or without necrotic or apoptotic material. Sera were defined as IFN-α inducing when IFN-α production was higher than the mean IFN-α production +2SD induced by control sera together with necrotic (>15 U/ml) or apoptotic (>10 U/ml) material. UV-inactivated herpes simplex virus type I (HSV) was used as a control IFN-α inducer. IgG from six SSc patient and two healthy control sera were purified. The necrotic cell material and the control IFN-α inducers poly I:C, pcDNA3 plasmid and HSV were treated with DNase-free RNase A or RNase-free DNase, as previously described (147). The inducers were incubated with the RNase, DNase, or only medium for 3 hours at 37°C before adding IgG or the transfection agent Lipofectin. The anti-FcγRII monoclonal antibody, prepared as described previously (148), and the IgG2b isotype control were used in the induction cultures with PBMC or PDC: chloroquine phosphate was used to inhibit endocytosis.

The IFN-α levels in the supernatants were measured by a dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), which detects most IFN-α subtypes, but not IFN-α2b (detection level ≥2 U/ml) (149). To detect IFN-α in serum, a more sensitive DELFIA was performed (detection level ≥0.5 U/ml) (150).

Chemokine analysis (Paper IV)

The levels of IP-10, MCP-1, MIP-1α, and RANTES were measured in serum samples by 4-Plex Bio-Plex Multiplex Cytokine Assay (Bio-Rad Hercules, California, USA). Increased chemokine levels were defined as mean values for the control sera + 2SD.

Statistical methods (Papers I-IV)

For continuous variables, comparisons between groups were with the Kruskal-Wallis test (Papers III-IV) and the Mann-Whitney U-test (Papers I-IV), and for dichotomous variables, the Mantel–Haenszel test (Paper II) or Fisher’s exact test (Papers II-III) were used. Correlations between different parameters were calculated by Spearman Rank Correlation test (Papers I-IV) and the unpaired t-test (Paper I). In Paper II, paired Wilcoxon signed rank test was used to compare the control group with the patient group, and odds ratios with 95% confidence intervals were
calculated. In all statistical analyses, P-values <0.05 (two-sided test) were considered statistically significant.

**Ethical considerations**

All studies were approved by the Local Ethics Committee at the University of Uppsala, Uppsala, Sweden, and performed according to the Declaration of Helsinki. All patients and controls gave their informed consent to participate in the studies.
Results and Discussion

Delayed gastric emptying in patients with diffuse versus limited systemic sclerosis, unrelated to gastrointestinal symptoms and myoelectric gastric activity (Paper I)

The presence of reported symptoms, regardless of frequency, in control subjects, and lcSSc patients, and dcSSc patients are presented in Table 2. This study confirmed that patients with SSc frequently report functional upper GI symptoms, but no clear difference was seen between SSc subtypes.

Table 1. Upper GI symptoms reported by control subjects and SSc patients.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Patients with lcSSc</th>
<th>Patients with dcSSc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=15</td>
<td>N=13</td>
<td>N=15</td>
</tr>
<tr>
<td>Nausea, no (%)</td>
<td>0</td>
<td>5 (38)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Emesis, no (%)</td>
<td>0</td>
<td>0</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Early satiety, no (%)</td>
<td>0</td>
<td>3 (23)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Bloating, no (%)</td>
<td>4 (26)</td>
<td>9 (69)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Epigastric pain, no (%)</td>
<td>2 (13)</td>
<td>6 (46)</td>
<td>7 (47)</td>
</tr>
</tbody>
</table>
Following the lag phase, SSc patients had slower gastric emptying than healthy controls (Figure 2).

*Figure 2.* Gastric emptying with a scintigraphic method in SSc patients compared to healthy controls measured as remaining gastric radioactivity at defined timepoints.

Patients with dSSc had slower gastric emptying (scintigraphic gastric half-emptying time median 103 min, range 75-447 min) than control subjects (median 70 min, range 38-94) and patients with lSSc (median 76 min, range 43-460), indicating more severe GI involvement in dcSSc. Except for an observed higher power increase in the normal and tachygastria range in dcSSc, compared to lcSSc and controls, electrogastrographic recordings did not differ among dSSc, lSSc, and controls. Patients with high frequency of symptoms did not differ from patients with low frequency of symptoms in gastric function, as measured with scintigraphic gastric emptying or EGG. Neither EGG nor a thorough review of upper gastrointestinal symptoms predicted delayed gastric emptying, but the lack of symptoms in some patients with pronounced delayed gastric emptying indicated an impaired sensory function, consistent with possible neuropathy (151).

All patients with BMI <20 had delayed gastric emptying. There could be numerous reasons for this, including delayed delivery to the small
intestine, decreased alimentary intake due to a sense of fullness, or due to coincidence with small bowel involvement. Taken into account the increased mortality risk (98), low body weight in SSc patients should lead to gastrointestinal investigations.

One of the aims of this study was to explore the correlation between findings from EGG and the scintigraphic method. As the scintigraphic method requires exposition to radioactivity, sophisticated and expensive equipment that is not widely available, and experienced and time-consuming interpretations, EGG was an attractive possibility, as it is ambulatory, non-invasive, and uses automated interpretation. However, no correlation was determined and it was concluded that the scintigraphic method is still the golden standard. As the gastric myoelectric signal is relatively weak, motion artefacts and detection of colonic signals could have influenced the recordings. Since this study was completed, the method has been refined, e.g. by applying multi-channel EGG (152-154).

Lower gastrointestinal symptoms and quality of life in patients with systemic sclerosis: a population-based study (Paper II)

With the study design, a prevalence of 124 SSc patients/1 million inhabitants in the age range 18-77 years could be calculated. This was the first prevalence estimate in Sweden, and was almost twice as high as the estimates of 71/million inhabitants from Iceland (2). However, it was possible to gain insight into the prevalence of subtype, autoantibody pattern, and clinical features of the 81 responding patients, which was 83% of the population-based cohort of SSc patients (Table 3). This increased knowledge about the disease could not be obtained from studies performed in tertiary centres.
Table 2. Description of the 81 SSc patients. Data derived from patient file and patient reports.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Frequency (%) or total (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), years</td>
<td>61 (31-77)</td>
</tr>
<tr>
<td>Gender rate, male/female (percent)</td>
<td>16 (20%)/65 (80%)</td>
</tr>
<tr>
<td>Disease duration, median (range), years</td>
<td>11 (1-48)</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
</tr>
<tr>
<td>lcSSc, number (percent)</td>
<td>60 (76%)</td>
</tr>
<tr>
<td>dcSSc, number (percent)</td>
<td>19 (24%)</td>
</tr>
<tr>
<td>ANA IF-positive, number (percent)</td>
<td>70 (89%)</td>
</tr>
<tr>
<td>Centromere pattern, number (percent)</td>
<td>18 (23%)</td>
</tr>
<tr>
<td>Homogenous pattern, number (percent)</td>
<td>17 (22%)</td>
</tr>
<tr>
<td>Nucleolar pattern, number (percent)</td>
<td>16 (20%)</td>
</tr>
<tr>
<td>Speckled pattern, number (percent)</td>
<td>37 (47%)</td>
</tr>
<tr>
<td>Anti-topoisomeras I, number (percent)</td>
<td>10 (13%)</td>
</tr>
<tr>
<td>Anti-RNP, number (percent)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Anti-SSA, number (percent)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Anti-Jo-1, number (percent)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Digital ulcers at any time, number (percent)</td>
<td>37 (47%)</td>
</tr>
<tr>
<td>Abnormal oesophageal motility, number (percent)</td>
<td>47 (59%)</td>
</tr>
<tr>
<td>Lung involvement, number (percent)</td>
<td>50 (67%)</td>
</tr>
<tr>
<td>Pulmonary hypertension, number (percent)</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>Scleroderma renal crisis, number (percent)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Two of the responding patients were excluded from further analysis as they both had a stoma. The use of age, sex, and municipally matched controls highlighted that SSc patients have increased prevalence of lower GI symptoms, and enabled conclusions from a more general viewpoint than hospital-based cohorts. Incontinence to liquid faeces was reported in 33% of the SSc patients, compared to 11% of control subjects, and incontinence to solid stools was reported in 9% of SSc patients and 2% of controls, indicating the need for awareness of this stigmatising symptom.
in SSc patients. Other symptoms associated with an impaired continence mechanism were also more frequently reported by patients, i.e. the use of protective pads, soiling of underwear, inability for anorectal discrimination, and sensing motions. The inability for anorectal discrimination suggested a neural defect, which prompted the investigations reported in Paper III. Thirty-nine percent of patients, compared to 54% of controls, reported stool frequency of once daily (p=0.028) and 40% of patients had at least two daily defecations, compared to 27% of controls (p=0.064). Manual assistance for evacuation and the use of oral medication to facilitate bowel evacuation were more common in SSc patients.

Although SSc patients by definition could not be diagnosed for IBS according to the Rome criteria (155-156), they reported symptoms possibly associated with IBS more often than controls did. The average composite score for these symptoms was 3.5 for patients and 2.0 for controls (p<0.01). A reduced feeling of well being due to bowel function was reported by 30% of patients and 13% of controls, and disturbances to social life was reported by 20% of patients and 5% of controls (p<0.01). MMS incontinence score strongly correlated with the concept that bowel problems were troublesome to general health (0.32; p<0.01) and social life (0.32; p<0.01).

SSc patients had lower scores in all eight scales and the two summaries of the SF-36 questionnaire than controls, and patients with dcSSc presented lower average scores on all SF-36 scales, but were only statistically significant for VT, SF, MH, and MCS scores. MMS did not correlate with any of the scales of SF-36, but other symptoms did, e.g. abdominal pain, bloating, the inability for sensing motions, and the need for dietary restrictions. Self-reported symptoms of Raynaud’s phenomenon and oesophageal, breathing, joint and muscle problems strongly correlated with both physical and mental scales of SF-36. This finding was in agreement with a study on SSc patient perspectives (157), and with the finding that SF-36 reflects pain and impaired mobility better than other problems, among them GI tract symptoms (158). However, some statistical analyses could be underpowered. At the time of investigation, limited data on HR-QOL in SSc was publicly available, but since then, a number of reports have been published (146, 148-164).
Physiological and structural abnormalities in patients with systemic sclerosis and faecal incontinence (Paper III)

Faecal incontinence was reported in 11/25 patients (44%): 11 patients reported incontinence to liquid stools, of which 5 (20%) also reported incontinence to solid stools. The median MMS was 3 (range 0-16).

In two patients, both with incontinence, the single-fibre EMG could not be completed. Increased FD was recorded in 19/23 (83%) of the patients: bilaterally in 14/23 (61%) and unilaterally in 5/23 (22%). There were no differences between the continent and the incontinent patients in the neurophysiologic results.

There was significant difference in MRP (p=0.015) and MSP (<0.001) in the computed HPZ, and threshold volume at which RAIR was elicited (p<0.001) between healthy controls and continent and incontinent SSc patients (Figures 1-3 in Paper III), but no difference in sensation of filling. In two incontinent SSc patients, no RAIR was identified. Incontinent patients had lower MSP at 1 cm (median 60.9 mm Hg), 2 cm (median 40.2 mm Hg) and in the computed HPZ (median 49.4 mm Hg) than continent patients (median 87.0 mm Hg at 1 cm; 61.7 mm Hg at 2 cm; and, 72.6 mm Hg in the HPZ: p<0.05).

Eight patients out of 23, four with faecal incontinence and four without faecal incontinence had negative DP (paradoxical reaction) at least at one level. In patients with increased FD, MSP was significantly lower at 2 cm (median 55.1 mm Hg) and 3 cm (median 33.2 mm Hg) than patients with normal FD (median 104.5 mm Hg at 2 cm, p=0.019 and 89.5 mm Hg at 3 cm, p=0.006).

Sonographic abnormalities were present in four patients, all individuals with faecal incontinence. One patient had a thin EAS in combination with an atrophic IAS, suggesting striated muscles could also be involved in the disease. The patients with these morphologic changes had lower MRP and MSP than patients with normal sonographic findings.

The increase in FD in this cohort was discrete and in most cases had no impact on anorectal symptoms, but was associated with decreased MSP, which indicated an impact on the development of impaired sphincter function. However, from a pathophysiologic viewpoint, the finding might be of relevance. Neural dysfunction is suggested as being the initial gastrointestinal lesion in patients with SSc (151). Vascular abnormalities, leading to ischemia and hypoxia occur early in SSc (159-160). As nerve tissue is susceptible to ischemia, this might be the cause for early nerve injury in SSc and explain increased FD in SSc patients,
although other causes e.g. injury during delivery cannot be excluded. Earlier variable, inconsistent, and sometimes conflicting results could be affected by compensatory mechanisms.

There is no longitudinal follow-up of the anorectal function in SSc patients; therefore, the sequence of events leading to faecal incontinence is unknown. No test on autonomic anal nerve function exists, except for RAIR, but the influence of the disease on autonomic nerves is reported in SSc (161). Therefore, early vascular changes and an initial active inflammation could be hypothesised as leading to nerve damage and possibly having a direct impact on the IAS and the EAS. The occurrence of anti-muscarinic-3 acetylcholine receptor suggests another mechanism for neuropathic disturbance of the GI tract in SSc (162). The lower volume required to elicit RAIR in incontinent patients indicates fibrosis in the rectal wall or muscles, leading to decrease in compliance.

In terms of HR-QOL, median SF-36 PCS was 34.1 in incontinent SSc patients and 38.1 in continent patients (NS), and median MCS was 39.3 in incontinent and 40.0 in continent patients (NS). SF-36 did not correlate to either faecal incontinence, other lower GI symptoms, or results from any of the investigations. Factors other than GI manifestations may play a more significant role in the quality of life in SSc, although the number of patients investigated might have been too small to draw conclusions in this respect.

Type I interferon system activation and association with disease manifestations in systemic sclerosis (Paper IV)

Sera from 33 (47%) of the SSc patients had the capacity to induce interferon production in normal PBMC when combined with necrotic cell material, and 23 (33%) also when combined with apoptotic material. Sera from patients with lcSSc or dcSSc did not differ in this capacity, which was in accordance with reports that both subtypes display an IFN-α signature (14), and that monocytes from patients with lcSSc or dcSSc show no difference in their expression of type I IFN regulated Siglec-1 (121). SSc can be included in the large group of systemic autoimmune diseases with an IFN-α signature that can be caused by the presence of interferogenic ICs which trigger pDCs to a continuous IFN-α production. However, in line with observations in other diseases (146-147, 163), only a proportion of patient sera had the capacity to form interferogenic ICs.
pDCs were identified as being responsible for the IFN-α production triggered by RNA-containing SSc-associated ICs, via an FcγRII- and endosome-dependent pathway. This was further supported by the association between the induction of high levels of IFN-α and autoantibodies to SSA and RNP, in a sub-analysis on sera with high IFN-α inducing capacity, (>200 U/ml of IFN-α in combination with necrotic material, n=11; and >50 U/ml IFN-α with apoptotic material, n=16). This suggested autoantibodies to RNA-binding proteins are the most important in the generation of interferogenic ICs in SSc, and indicated TLR7 activation is a common pathway by which the IFN-α signature is expressed in systemic autoimmune rheumatic diseases.

In contrast to a previous study (164), anti-Topo-I antibodies did not form strong interferogenic ICs; on the contrary only a low IFN-α-inducing capacity was determined in a proportion of anti-Topo-I sera void of other specific antibodies, this was not significantly different compared with patient sera lacking these antibodies. The reason for the discrepancy is not clear, however, anti-RNP or anti-SSA are not analysed in the study by Kim et al (164). In addition in a sub analysis of the high-producing sera, an association with a speckled pattern ANA was identified (p<0.05). ANA positive sera with a speckled pattern may contain antibodies to anti-Pm-Scl, anti-Ku, or the anti-RNA polymerases (165). Another explanation might be that the type I IFN production in some patients with SSc is not dependent on ICs but on other mechanisms.

High IFN-α-inducing capacity (>200 U/ml) in combination with necrotic material was associated with Erythrocyte Sedimentation Rate (ESR) (p=0.022) and digital loss (p=0.025), and sera from the two patients with a history of SRC induced high IFN-α production with necrotic or apoptotic material. Increased serum IFN-α levels were associated with a history of digital ulcers (p=0.029), lung fibrosis (p=0.048), and correlated to levels of ESR (p=0.001).

We observed that increased IP-10 levels were associated with cardiac involvement, signs of PAH, elevated ESR, and although only present in few patients, with digital loss and renal crises (p<0.005). However, serum levels of MCP-1 were associated with lung fibrosis (p=0.019), and correlated with ESR (p=0.045). MIP-1α levels correlated with ESR (p<0.001).

Type I IFN system activation might be more important in the early phases of SSc when immune activation and inflammation is more pronounced than in the later stages of the disease. In our study ESR served as a surrogate marker for an active disease.
Multiple testing was not corrected for, and the results should be interpreted cautiously, but the observation that most clinical associations were related to vascular manifestations is noteworthy. The results strengthened the view that type I IFN may be important in the development of vasculopathy in SSc. Previous studies in SSc indicate IFN-α mRNA is predominantly expressed in vascular and perivascular cells (14). IFN-α has antiangiogenic effects (166), and can impair endothelial cell differentiation in lupus (166). Consequently, activation of the type I IFN system in SSc may affect the microvascular system; however, clarification of the exact mechanisms by which IFN-α contributes to the vascular injury is important to clarify. In addition, there was an association between MCP-1 and IFN-α and pulmonary fibrosis, which highlighted the importance of the type I IFN system in the disease process.
Conclusions

- Upper and lower GI symptoms are more frequently reported in SSc patients than in healthy controls and have an influence on patient’s general wellbeing and social life. Faecal incontinence is reported by one-third of SSc patients in a population-based cohort.

- Gastric emptying is slower in patients than in controls, but this could not be explained by differences in electrogastrographic signalling. A higher prevalence of delayed gastric emptying in dcSSc indicates more severe GI involvement than in lcSSc.

- Abnormal findings in anal manometric recordings correlated to continence; and ultrasonographic defects of the IAS and the EAS and an absent RAIR were rare but associated with faecal incontinence.

- A discrete increase in neural FD present in almost all patients could indicate earlier nerve injury with consequent re-innervation.

- HR-QOL as measured with SF-36 is decreased in SSc patients compared to healthy controls, and similar to or lower compared to patients with other systemic diseases, but other disease manifestations may have greater influence than impairment of the GI tract.

- An activated type I IFN system previously identified in several other systemic autoimmune diseases is also present in a subgroup of SSc patients, and may contribute to both the vascular pathology and affect the pro-fibrotic process.
Systemisk skleros, som även kallas sklerodermi och förkortas SSc, är en ovanlig reumatisk systemsjukdom med kvinnlig dominans. I de flesta fall debuterar sjukdomen med Raynaud’s fenomen dvs kraftig kärlreaktivitet med färgförändringar i fingrar och tår, följt av hudförtjockningar med olika utbredning. En uppdelning har gjorts beroende på om sjukdomen bara når huden nedom armbågar och knäveck (begränsad SSc, lcSSc) eller om hudförändringarna är mer omfattande (diffus SSc, dcSSc). Även inre organ drabbas i många fall med inflammatorisk lungsjukdom, påverkan på mag-tarmkanalen, hjärtpåverkan och i sällsynta fall kraftig njurnäringsöver. Sjukdomen kan debutera i alla åldrar men oftast sker debuten i 40-50 årsåldern. I dagsläget finns ingen botande behandling, men dödligheten har dock minskats på senare år genom ökad kunskap och bättre uppföljning av sjukdomsförloppet.

Inom ramen för denna avhandling har studier gjorts med syfte att öka kunskapen om påverkan på gastrointestinalkanalen, livskvalitet och om de mekanismer som leder till att sjukdomen uppstår och fortskrider.

Patienter med SSc hade mer ofta förekommande och fler symptom från gastrointestinalkanalen än undersökta kontrollpersoner. Detta gällde både symptom från övre delen av magen som från de nedre delarna. Tarminkontinens för fast eller lös avföring rapporterades av 33% av patienterna jämfört med 11% av ålders- och könsmatchade kontrollpersoner och detta symptom hade inverkan på patienternas sociala situation och allmänna hälsa.

Vid undersökning av magsäckens funktion fann vi att patienterna hade en kraftigt förlängsammad tömning av magsäckens innehåll till tunntarmen. Däremot fanns det inte någon skillnad i signaler som uppmättes med hudregistreringar över magsäcken med elektrogastrografi. Ändtarmens funktion undersöktes med hjälp av neurofysiologiska metoder, tryckmätning och ultraljud. Patienter med tarminkontinens hade sämre knipförmåga i ändtarmen än patienter utan detta symptom och hos en del patienter med avföringsinkontinens förekom det förändringar både i muskulaturen runt ändtarmen och i tarmtömningsreflexen.
Vid en normal immunreaktion vid infektioner förekommer en aktivering av interferonsystemet. Vid vissa tillstånd, bland dem andra reumatiska systemsjukdomar, kan dock detta system vara sjukligt aktiverat och förstärka sjukdomsprocesser. Vi fann att nära hälften, 47%, av SSc patienterna producerade interferon vid stimuleringsförsök och att denna produktion var kopplad till förekomsten av vissa antikroppar samt till lungfibros och kärlmanifestationer av sjukdomen.
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