GASTROINTESTINAL SYMPTOMS AND MOTILITY DISORDERS IN PATIENTS WITH SYSTEMIC SCLERODERMA.

Authors: Agostino Di Ciaula¹, Michele Covelli², Massimo Berardino³, David Q-H Wang⁴, Giovanni Lapadula², Giuseppe Palasciano³, Piero Portincasa³

Affiliation: ¹ Division of Internal Medicine, Bisceglie, Bari, Italy; ² Section of Rheumatology and Internal Medicine, Department of Internal and Public Medicine (DIMIMP), University Medical School of Bari, Italy; ³ Department of Medicine, Liver Center and Gastroenterology Division, Beth Israel Deaconess Medical Center, Harvard Medical School and Harvard Digestive Diseases Center, Boston, Massachusetts, USA

Key Indexing Terms: Autonomic neuropathy; Colon; Gallbladder; Motility; Small intestine; Stomach.

Support: This work was partly supported by research grants to P.P. “Ricerca Scientifica 2003-2004”, University of Bari and to D.Q.-H.W. (DK54012) from the National Institutes of Health (US Public Health Service). P.P. was a recipient of the Short Term Mobility Grant 2005 (Harvard Medical School, Boston, MA, USA) from the Centro Nazionale delle Ricerche (CNR, Roma, Italy).

CORRESPONDING AUTHOR:
Prof. Piero Portincasa, MD, PhD
Clinica Medica “A. Murri” - Department of Internal Medicine and Public Medicine (DIMIMP)
University of Bari Medical School - Polifonico - 70124 Bari - Italy
Tel. +39-80-5478.227 - Fax +39-80.5478.232; e-mail: p.portincasa@semeiotica.uniba.it

Running footnote: Gastrointestinal function and neuropathy in scleroderma

Abbreviations: scleroderma, SSc; half-emptying time, T_{1/2}; visual analogue scale, VAS; orocecal transit time, OCTT; sweat-spot-test, SST
Abstract

Background. Studies on gastrointestinal symptoms, dysfunctions, and neurological disorders in systemic scleroderma are lacking so far.

Methods. Thirty-eight scleroderma patients (34 limited, 4 diffuse), 60 healthy controls and 68 dyspeptic controls were scored for upper and lower gastrointestinal symptoms (dyspepsia, bowel habits), gastric and gallbladder emptying to liquid meal (functional ultrasonography) and small bowel transit (H₂-breath test). Autonomic nerve function was also assessed (cardiovascular tests).

Results. Scleroderma patients had a dyspeptic score (mainly due to gastric fullness) greater than healthy controls, but lower than dyspeptic controls, who showed multiple symptoms, instead. Scleroderma patients with dyspepsia had a longer disease duration. Fasting antral area and postprandial antral dilatation were smaller in scleroderma patients than dyspeptic and healthy controls. Gastric emptying was delayed in both scleroderma patients (particularly in those with abnormal dyspeptic score) and dyspeptic controls, who also showed a larger residual area. Despite gallbladder fasting volume and postprandial emptying were comparable across the three groups, gallbladder refilling appeared delayed in dyspeptic controls and mainly dependent on delayed gastric emptying in scleroderma. Small intestinal transit was also delayed in 74% of scleroderma and 66% of dyspeptic controls. Bowel habits were similar among the three groups. Autonomic neuropathy was not associated to dyspepsia, gastric and gallbladder motility and small intestinal transit. Conclusions. Dyspepsia (mainly gastric fullness) and diffuse gastrointestinal dysmotility are frequent features in scleroderma patients with long-lasting disease, independently from autonomic neuropathy.
BACKGROUND

Systemic scleroderma (SSc) is a generalized disorder of small arteries, microvessels and connective tissue of unknown origin with the highest incidence occurring between 45 and 55 years of age [1] and with a frequency of three to eight times higher in females than in males [2]. In SSc, different types of antibodies are produced, including anti-centromere and anti-topoisomerase antibodies [3]. Major disease mechanisms are the prominent vascular changes due to endothelial-cell damage and proliferation of subendothelial connective tissue. The widespread damage of small arteries and microvessels results in increased deposition of collagen and other matrix elements, leading to thickening and fibrosis of the skin, involvement of synovia and visceral fibrosis [4]. Clinical manifestations of SSc are extremely heterogeneous and depend on the presence and degree of internal organ involvement; the two subsets of SSc include: the Diffuse Cutaneous SSc, characterized by short-term Raynaud’s, truncal and acral skin involvement and significant incidence of interstitial lung disease, oliguric renal failure, diffuse gastrointestinal disease, and myocardial involvement; and the Limited Cutaneous SSc, characterized by Raynaud’s for years and limited skin involvement but significant and late incidence of pulmonary hypertension, with or without interstitial lung disease, trigeminal neuralgia, skin calcifications, teleangectasia [4]. Following the skin, the digestive system is the second most common target of scleroderma [5], resulting in the progressive alterations of smooth muscle function along the whole gastrointestinal tract [6-16]. Gastrointestinal involvement in SSc patients has been linked to several clinical manifestations [16], but the absence of evident symptoms is also possible [8] and occurs in about one third of the patients [13]. Subjects suffering from different connective tissue disorders, including those with SSc, show a high prevalence rate of autonomic nerve dysfunction and this, in turn, is supposed to affect the gastrointestinal motility profile [17-21]. This hypothesis, however, requires further confirmation. Systematic studies focusing on gastrointestinal symptoms, dysfunctions, and neurological alterations in SSc, are lacking so far. With this in mind and considering the complex and wide multi-organ involvement in SSc, the present study is designed to better characterize the profile of gastrointestinal symptoms, motility, and autonomic nervous system functions in SSc patients, compared to both healthy and dyspeptic controls.
METHODS

Subjects

Patients with SSc were recruited from those referred to the Rheumatology Department at the University of Bari Medical School. The diagnosis of SSc fulfilled the American College of Rheumatology (ACR) criteria [4,22] and the study included 38 consecutive patients (36 females, mean age 50.9 ± 2.0 years, body mass index of 24.6 ± 0.6 Kg/m^2). According to ACR classification, 34 patients had limited cutaneous SSc and 4 had diffuse cutaneous SSc. The estimated duration of the disease was calculated from the appearance of the first symptom / sign related to SSc. A full range of symptoms was carefully investigated for the cardiovascular system (Raynaud phenomenon, dyspnea from pericardial effusion, congestive heart failure, or myocardial fibrosis, palpitations and irregular heart beats due to conduction abnormalities, and congestive heart failure), skin (diffuse pruritus, hyper- or hypopigmentation, and skin tightness and induration), gastrointestinal system (heartburn, dysphagia, hoarseness, constipation alternating with diarrhea, and dyspepsia), respiratory system (dyspnea, chest pain from pulmonary artery hypertension, and cough), musculoskeletal system (arthralgia, myalgia, loss in joint range of motion, and carpal tunnel syndrome symptoms), constitutional (fatigue and weight loss), neurological system (facial pain and hand paresthesias from peripheral entrapment neuropathies, headache, and stroke), genitourinary system (erectile dysfunction and dyspareunia), ears, nose, throat, sicca syndrome, and endocrine system (hypothyroidism). Apart from skin fibrosis, further clinical features of the patients investigated included: Raynaud’s phenomenon (100%), arterial hypertension (26%), pulmonary interstitial fibrosis (3 out 4 patients and 26% in diffuse cutaneous SSc and localized cutaneous SSc, respectively), hypertension (2 out 4 patients and 37% in diffuse cutaneous SSc and localized cutaneous SSc, respectively), and esophageal dysmotility (95%). All patients were on antireflux therapy with daily doses of proton pump inhibitors.

A group of 60 matched healthy volunteers (33 females, mean age 47 ± 2 years, body mass index 23.9 ± 0.5 Kg/m^2) served as healthy controls and 68 matched subjects with no other disease except for functional dyspepsia (45 females, mean age 49 ± 2 years, body mass index 24.9 ± 0.5 Kg/m^2) served as dyspeptic controls. Exclusion criteria for all subjects enrolled in the study were the presence of gallstones, acute or chronic pancreatitis, diabetes, pregnancy, peptic ulcer disease, history of esophageal and abdominal surgery and any other diseases or pharmacological treatment known to affect
gastrointestinal motility. Exclusion criteria also included the presence of contraindications for the entire series of cardiovascular tests, \textit{i.e.} arrhythmia, recent myocardial infarction, drug therapy that could interfere with cardiovascular activity, severe metabolic disturbance and unwillingness to cooperate. In addition, subjects were excluded if heavy smokers (\textit{i.e.} more than 10 cigarettes/day), alcohol (\textit{i.e.} more than 20g ethanol/day in both male and females, respectively) or drugs abusers. All patients were evaluated within the yearly scheduled routine work-up which included blood tests and abdominal ultrasonography. Approval from the institutional review board and written informed consent from the participating subjects were obtained.

Study design

After completing the full work-up at the Rheumatology Section, SSc patients were asked to join the study on gastrointestinal symptoms, motility, and autonomic nervous system function at the Section of Internal Medicine. All enrolled subjects underwent complete clinical examination, an interview by questionnaires for the evaluation of dyspepsia, upper gastrointestinal perception, and bowel habits, functional ultrasonography for the study of gallbladder and gastric motility, H$_2$-breath test for orocecal transit time (OCTT). SSc patients also underwent cardiovascular tests for autonomic nervous system function (see below). Tests were started at 08:00 AM. Although the maximal length of the work-up was set at 5 hours, the true length was invariably less than 3 hours.

Dyspepsia and upper gastrointestinal perception.

The presence of dyspepsia was assessed and quantified by a validated semi-quantitative scoring system measuring a subset of four symptoms (epigastric pain, burning, belching/burping, postprandial fullness), resulting in a maximal score equal to 48 [23]. In this series, the upper normal limit estimated from the mean + 2SDs of healthy controls was equal to 8 [24,25].

On the day of the motility studies, appetite, satiety, nausea, epigastric fullness and epigastric pain (or discomfort) were monitored by the visual analogue scale (VAS) [26]. The VAS consisted of a 100 mm-horizontal line indicating the current intensity of the perception between two extremes (extreme left= “no symptom at all”; right end= “maximum feeling of…”). This line was presented to the subjects who were asked to draw a vertical line through the horizontal line along its length. The distance in mm
from the extreme left of the line to the vertical line is a quantitative measure of gastrointestinal perception [27]. Scores at baseline (i.e. time 0) was the mean of 3 measurements over 3 days in the fasting state at the same time in the morning. Afterwards, VAS was scored at 15, 30, 45, 60, 90 and 120 minutes postprandially. The VAS has been previously demonstrated as a reliable method to assess subjective perception in response to experimental stimuli in the upper gastrointestinal tract [12,26-28].

Bowel habits
The weekly frequency of bowel movements was calculated from a daily diary over a time span of one month. The “Bristol” stool form scale was used to assess the quality of stools based on a semiquantitative scale, as a marker of colonic transit. The scale is deemed to be a reliable method to estimate colonic transit time [29,30].

Gastric and gallbladder emptying
Fasting and postprandial course of gastric antral area and gallbladder volume were assessed by functional ultrasonography using a 3.5MHz convex probe attached to an Ansaldo-Hitachi equipment, as previously described also by our group [27,31,32]. The test meal was a 200 mL solution with 13 g (39%) fat, 10 g (13%) protein and 35 g (48%) carbohydrates for a total of 300 kcal, 1270 kJ, 365 mOsm/l (Nutridrink®, Nutricia, Milano, Italy). The drink was taken at room temperature over one minute in the presence of the examinator. Indices of gastric emptying were: fasting antral area (mean of 3 measurements at −15, −5 and 0 minute before test meal, expressed in cm$^2$); maximal antral area at 2 minute post-meal; minimal postprandial antral area during the 2-hour; half-emptying time. Gastric emptying curves were obtained by plotting antral areas vs. time. In order to obviate inter-individual variability of antrum size [31], postprandial areas were normalized to maximal area after subtracting basal areas: \[ i.e. \quad 100 \times \frac{(A_t-a)}{(A_2-a)}, \] where $A_t$= postprandial area at any given time; $a$= basal area; $A_2$= area at 2 minute postprandially [33]. Half-emptying time was the time at which 50% decrease of antral area occurred ($T_{1/2}$, minute), and calculated by linear regression analysis from the linear descending part of the antral emptying curve. This index correlates closely with the scintigraphic $T_{1/2}$ [27]. Gastric emptying was considered abnormal if $T_{1/2}$ was greater than 35 minutes.
Gallbladder emptying was performed simultaneously to gastric motility by the same operator. Fasting and post-meal gallbladder volumes were measured by sagittal and transverse scans of the gallbladder at its largest dimension at 5-15 minute intervals during 2 hours. Gallbladder volume was measured from frozen sonograms assuming an ellipsoid shape of the organ – a reliable method when gallbladder shape is not highly irregular [34], which was the case in this study. In healthy subjects the liquid meal employed in this test (see above) induces more than 50% emptying of fasting gallbladder volume, and has been validated by our group on several occasions [24,25,32,35,36]. Indices of gallbladder motility were as follows: fasting volume (mean of 3 measurements at -15, -5 and 0 minute before test meal, expressed in mL); residual volume (minimal volume measured postprandially, expressed in mL and as percent of fasting volume); $T_{1/2}$ (time to achieve 50% decrease of fasting volume), calculated by linear regression analysis from the linear descending part of the emptying curve [35]. Gallbladder emptying was considered abnormal if half-emptying time was greater than 35 minutes (mean + 2 SD in healthy controls) [24,25].

Orocecal transit time (OCTT)

OCTT was measured by the hydrogen ($H_2$) breath test [37]. During the 5 days before the test, subjects were not allowed to take antibiotics, probiotics, prokinetics or other drugs known to affect gastrointestinal motility. To avoid prolonged intestinal $H_2$-production secondary to the presence of non-absorbable or slowly fermentable material in the colonic lumen, a special diet was given starting the day before the test and consisting of proteins and fat (meat, fish, eggs, olive oil) [24,25,38]. The meal was followed by a 12-hour fasting period. Between 08:00 and 09:00 AM the test was started. Smoking and physical exercise were prohibited one hour before and during the test [39,40]. Two breath samples were taken in the fasting state and afterwards the subject ingested 10 g of lactulose ($Duphalac$ Dry$^\text{®}$, Solvay Pharma, Torino, Italy) added directly as a powder to the liquid test meal used for simultaneous study of gallbladder and gastric emptying. End-respiratory breath samples were analyzed every 10 minutes for five hours after lactulose ingestion, using a pre-calibrated, portable hydrogen sensitive electrochemical devices (EC60-Gastrolizer, Bedfont, USA and Lactofan $H_2$ Analyzer, Fisher Analysen Instr. Leipzig, Germany, kindly donated by Italchimici SpA, Pomezia, Rome,
Italy). The concentration of hydrogen was measured in parts per million (ppm), and the accuracy of
the detector was ± 2 ppm. A rise of 10 ppm above baseline on two consecutive measurements was
considered as OCTT and expressed in minute [37]. OCTT was considered delayed if greater than 120
minutes (i.e. the mean ± 2SD obtained by a large healthy control group) [24,25]. To exclude the
presence of small intestine bacterial overgrowth, the lactulose breath test was considered normal if
there was no rise in H₂ concentration during the first 90 minutes after lactulose ingestion, with a
definitive rise never greater than 20 ppm during 180 minutes of measurement [41].

Autonomic neuropathy

The integrity of the autonomic nervous system was assessed by both the Sweat-Spot-Test (SST)
[37,42,43] and cardiorespiratory reflex tests [44], as previously described by our group [25]. The SST
was used to investigate the presence of cholinergic sympathetic fibers alterations by analyzing sweat
abnormalities on the skin of the dorsum of the foot, which was coated with iodine and a fine emulsion
of starch in arachis oil. Sweat was stimulated either by an intra-dermal injection of 0.1 mL
acetylcholine (Ach.SST), or a standardized thermal stimulus which includes pre-ganglionic fibers
(Thermal SST). A colorimetric reaction between starch and iodine was triggered by the sweat from
stimulated glands, so that each pore was seen as a little black dot after 2-5 minutes. A digital photo
was obtained and transferred to a magnifying software to measure the number and distribution of
dots appearing in a standard squared grid of 529 mm² divided into 64 squared subareas. A normal
SST was expressed by a score ≥ 12 dots/subarea and/or < 8% of abnormal subareas (each square of
the grid having less than 6 dots), according to Ryder [42] and to our group [25,43]. Only patients
with both indices (SST score and % of abnormal subareas) outside normal limits were considered to
have a positive test. A portable device (Cardionomic®, Lifescan, Italy) connected to skin electrodes
was employed to measure the “beat-to-beat” modifications of the R-R interval. Lying-to-standing and
standing-to-lying tests were used to assess sympathetic involvement. Valsalva maneuver, deep-
breathing, cough test, and postural hypotension test were used for parasympathetic involvement. The
results from each test were scored as normal, borderline or abnormal, according to age-controlled
values. The overall results for autonomic neuropathy were therefore normal (all tests normal), early
involvement (one abnormal test or two borderline abnormal), definitive involvement (two or more abnormal tests) [25,45].

Data analysis
Calculations were performed with the NCSS 2001 statistical software (Kaysville, UT, USA), and data were expressed as the mean ± standard error (SE). Continuous variables were analyzed using ANOVA followed by Fisher’s LSD test. Differences between two subgroups were evaluated by Mann-Whitney U-test or by Student’s t test, where appropriate. Linear regression analysis was performed by the method of least square. The chi-square test was used to compare categorical variables and proportions. A two-tailed probability (P) value of less than 0.05 was considered statistically significant [46,47].

RESULTS
Symptoms
The score for dyspepsia was abnormal in 61% of SSc patients, with a mean score greater than healthy controls, but lower than dyspeptic controls (Table 1). Estimated disease duration was significantly (P=0.03) longer in SSc patients with abnormal dyspepsia score (7.3±1.4 years) than in those without dyspepsia (3.4±0.6 years).

The analysis of dyspeptic symptoms by VAS throughout the motility study showed that the feeling of appetite and satiety were similar in SSc patients, healthy and dyspeptic controls. As expected, the perception of satiety and appetite (either in fasting and postprandial period) showed a strong negative correlation in both patients and controls (r = -0.80; P=0.000001). Dyspeptic controls showed more nausea than both SSc patients and healthy controls (Table 1). Of note, SSc patients had a constantly higher perception of epigastric fullness compared to healthy controls, although the highest values for this symptom were recorded in dyspeptic controls (Table 1 and Figure 1). Finally, dyspeptic controls showed higher perception of epigastric pain than both SSc patients and healthy controls (Table 1).

Despite the low number of patients with diffuse SSc enrolled in the present study, no difference was evident between patients with limited or diffuse scleroderma for either dyspepsia score (Table 2).
A. Di Ciaula et al - Gastrointestinal Function and Neuropathy in Scleroderma

Functional studies

Gastric emptying: SSc patients had a significantly smaller antral area in the fasting state and a minor postprandial dilatation of the gastric antrum than both dyspeptic and healthy controls (Table 3). During most of the postprandial period (between 10 and 120 minutes), both SSc patients and dyspeptic controls had significantly larger antral areas than healthy controls (Figure 2). Furthermore, dyspeptic controls showed the mostly impaired gastric emptying, since they also had higher postprandial areas than SSc patients between 40 and 120 minutes (Figure 2). Consequently, this resulted in a larger minimal postprandial antral area and in a delayed $T_{1/2}$ in both SSc patients and dyspeptic controls, compared to those in healthy controls (Table 3). Dyspeptic controls also showed a larger minimal postprandial antral area compared to SSc patients (Table 3).

Overall, postprandial gastric emptying was abnormal in 50% of SSc patients and in 59% of dyspeptic controls. Among SSc patients, emptying time was particularly delayed in patients with abnormal dyspeptic score, compared to those without dyspepsia ($T_{1/2}$: 49±9 minutes and 33±3 minutes, respectively, $P=0.04$). No difference was evident between patients with limited or diffuse scleroderma as far as fasting and postprandial antral areas were concerned (Table 2).

Gallbladder emptying: There was no difference in SSc patients vs dyspeptic and healthy controls for fasting volume and postprandial indices of gallbladder emptying (Table 3). However, although the gallbladder emptying profile after meal ingestion was fully comparable in SSc patients vs both dyspeptic and healthy controls, gallbladder refilling was impaired in SSc patients and dyspeptic controls, compared to healthy controls (Figure 2).

In order to assess the influence of gastric emptying on gallbladder dynamics, the postprandial gallbladder motility in subjects with normal or abnormal gastric emptying was also compared in SSc patients and dyspeptic controls. Interestingly, although the gallbladder emptying was similar in subjects with or without altered gastric emptying, an altered gallbladder refilling was only found in SSc patients with delayed gastric emptying. This was not the case, however, of dyspeptic controls, who showed similar gallbladder emptying and refilling curves accompanied with normal or impaired gastric

Dyspeptic symptoms by VAS (data not shown).
emptying (Figure 3). No difference was evident in patients with limited or diffuse scleroderma as far as fasting and postprandial gallbladder volumes were measured (Table 2).

**Orocecal transit time (OCTT):** Fasting levels of H$_2$ were invariably less than 10 ppm in all subjects and did not differ between patients and controls. In all subjects a hydrogen peak was evident. None of the subjects showed evidence of small bowel bacterial overgrowth. However, OCTT was significantly longer in both SSc patients and dyspeptic controls compared to healthy controls, with values being above the upper normal limit as detected in 74% of SSc patients and in 66% of dyspeptic controls (Table 3). No difference in OCTT was found in subjects with or without an altered gastric emptying in both SSc patients (171.2±12.2 and 173.3±13.2, respectively; P=NS) and dyspeptic controls (164.6±10.1 and 148.9±8.9, respectively; P=NS).

**Bowel habits:** There was no difference in SSc patients vs dyspeptic and healthy controls when both weekly frequency of evacuations (5.2±1.5, 5.4±1.6 and 5.9±2.0 respectively, P=NS) and bowel habits were investigated by Bristol score (3.4±0.2, 3.8±0.2 and 3.6±0.1 respectively, P=NS). No difference was observed in patients with limited or diffuse scleroderma as far as OCTT and bowel habits were examined (Table 2).

**Autonomic neuropathy**

Twenty-nine out of 38 patients and all of controls gave their informed consent to undergo tests for autonomic neuropathy. In terms of clinical features, this subgroup was fully representative of the whole SSc group of patients (n=25 with limited and n=4 with diffuse disease). Overall, tests for autonomic neuropathy were abnormal in 20 (69%) of the patients but in none of the controls. Abnormal parasympathetic function, sympathetic function or both were found in 79%, 55% and 41% of cases, respectively. According to disease classification, tests for autonomic neuropathy were abnormal in 15 out of 25 patients (60%) with limited disease and in 1 out of 4 subjects (25%) with diffuse scleroderma. No differences could be detected in patients with normal or abnormal tests for dyspeptic scores, bowel habits, gallbladder, gastric motility, and OCTT (Table 4).
DI SCUSSION

Systemic scleroderma is a rare disorder with an annual incidence of 19.3 cases per million adults in the United States. Renal and pulmonary diseases are the major causes of mortality. Survival has improved over the past few decades and is strongly dependent on the degree of organ involvement. Gastrointestinal involvement is common in both forms of limited and diffuse SSc. Indeed, in this series, esophageal dysmotility and complaints such as heartburn and various degrees of dysphagia are extensively represented and need medication with antisecretory and/or prokinetic agents. Despite gastrointestinal symptoms [8,12,13,16,26-28], motility dysfunctions [6-16] and autonomic neuropathy [17-21] have been very often associated with SSc, comprehensive studies on this topic were not available, so far. This is the first study providing an integrated overview of gastrointestinal manifestations, autonomic neuropathy and motility disorders in SSc patients and taking a group of functional dyspepsia patients as additional controls.

Several observations demonstrated that a symptomatic [16] or an asymptomatic [8] involvement of the gastrointestinal tract was the second most common manifestation of scleroderma [5] and that gastrointestinal motility dysfunction remains subclinical in about one third of SSc patients [13]. Our data confirm and expand such early reports and our results show that about one half of SSc patients have clear clinical evidence of gastrointestinal symptoms (apart from esophageal heartburn and dysphagia), although their intensity was less important than that of subjects with functional dyspepsia. Additionally, the appearance of dyspeptic symptoms in SSc is strongly related to disease duration and stage. This is probably explained by the early and progressive damage of the gastrointestinal wall (namely, smooth muscle atrophy and increased fibrosis), while symptoms appear in more advanced disease stages. This possibility is supported by histological studies showing a damage of the muscular layer at multiple levels of the gastrointestinal tract and involving the esophagus [9,48], the stomach [48], the small intestine [49] and the colon [6].

At variance with dyspeptic controls, who showed similar frequency of multiple symptoms, the main dyspeptic symptom recorded in our series of SSc patients both in the fasting state and throughout the postprandial period was the epigastric fullness. Of note, by integrating the analysis of this symptom with ultrasonographic gastric motility, we show here that a small antral area might generate epigastric fullness in SSc patients. This finding, together with a restricted distension of the gastric antrum in SSc patients
after 200 mL test meal, distinguish these patients from subjects with functional dyspepsia, who show both a basal area and a gastric distention capacity similar to those of healthy controls.

Although an histological examination of the stomach to check for the degree of fibrosis was not performed here, the hypothesis of a stiff gastric wall, with an altered smooth muscle basal tone, might be supported by previous observations demonstrating a prominent fibrosis in gastric wall of SSc patients [48]. Furthermore, similar to the suggested alterations for the duodenal wall [12], an increased sensitivity of receptors-mediated perception in SSc patients compared to healthy subjects cannot be ruled out. In SSc patients, in fact, a stiffer duodenal wall and an increased sensation of pain evoked by a controlled strain of the gut has been previously demonstrated [12].

In the postprandial period our data show the existence of an altered gastric smooth muscle function in both SSc patients and subjects with functional dyspepsia, thus confirming previous observations by ultrasonography and other diagnostic techniques [7,8,10,15,16,50-55]. Our results suggest that deranged gastric emptying is associated with the presence of symptoms, as it is particularly evident in both subjects with functional dyspepsia and SSc patients with abnormal dyspeptic score. Moreover, in this latter group, this alteration seems to be related to the duration of the disease. This last consideration is also supported by a previous ultrasonographic study, demonstrating a significant correlation between a delayed gastric emptying and the duration of scleroderma [55].

We show that OCTT is delayed in both SSc patients and dyspeptic controls, irrespective of an altered gastric emptying. In fact, OCTT was similar in subjects with or without altered gastric emptying in both SSc patients and dyspeptic controls, suggesting that this factor plays a limited role in the determination of a delayed OCTT, at least in the present series.

In SSc patients the percentage of the subjects with a delayed OCTT is doubled compared to that of another study employing the same technique [15]. Differences in patients selection and stage of the disease might probably explain such discrepancies. Of note, small intestinal bacterial overgrowth was absent in our subjects, meaning either a good therapeutic control or a preserved colonic function (see below). Previous studies demonstrated the presence of an altered colonic smooth muscle function [6] and delayed colonic transit with constipation in SSc patients [14,15,56]. However, we could not confirm these findings by using specific visual-semiquantitative scales (the Bristol Stool Form Scale is deemed to be a reliable method to estimate colonic transit time [29]). Thus, it appears that the group
of patients investigated here has a rather preserved colonic function, despite a delayed small bowel transit.

At variance from the motility data of the stomach, the present study corroborates previous observations indicating a normal gallbladder fasting volume and emptying pattern in SSc patients [55,57-59]. The fact that the gallbladder emptying is unaffected suggests that the physiological entero-hormonal and neurological pathways governing the upper gastrointestinal motility play a minor role in the genesis of functional disturbances in scleroderma. It is therefore likely that anatomically altered muscular layer of gastrointestinal organs [6,9,48,49], rather than extrinsic factors, are responsible for the smooth muscle dysfunction.

However, at variance with the gallbladder emptying, the gallbladder refilling has been shown to be altered in both SSc patients and dyspeptic controls. In SSc patients this finding appears to be related to the delayed gastric emptying rather than to a neuro-muscular dysfunction. At least in our series, however, this was not the case of dyspeptic controls, who showed an altered gallbladder refilling irrespective of gastric emptying speed.

Autonomic nerve dysfunction has been found in patients with SSc [17-21]. However, for the first time in SSc, we could add the Sweat Spot test to other standard cardiovascular tests for assessing autonomic denervation. Deranged function of the autonomic nervous system, similar to that observed in other conditions (i.e. diabetes mellitus), has been suggested for explaining the functional defects in SSc patients [17-21,50,60,61]. The integrated approach used in this study show that, although autonomic neuropathy is very frequent in SSc patients, it seems to have no role in generating gastrointestinal motility disorder, since both symptoms characteristics and motility patterns at multiple levels are similar in patients with or without abnormal parasympathetic and/or sympathetic function.

In conclusion, the present study shows that SSc patients frequently suffer from dyspepsia, mainly after a long disease duration and independently from autonomic neuropathy, and that dyspeptic symptoms are associated with gastrointestinal dysmotility. At variance with functional dyspepsia, SSc patients principally complain of epigastric fullness and, as dyspeptic subjects, exhibit multiple gastrointestinal motility defects, in which fibrosis and anatomical alterations might play a major causative role, rather than a simple neuro-muscular dysfunction.
### Table 1

Dyspepsia score and Visual Analogue Scale of five major dyspeptic symptoms in scleroderma (SSc) patients and in dyspeptic and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>SSc patients</th>
<th>Dyspeptic controls</th>
<th>Healthy controls</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dyspepsia score</strong></td>
<td>12.4±1.0^1</td>
<td>23.7±0.8^1,2</td>
<td>5.4±0.8</td>
<td>0.000001</td>
</tr>
<tr>
<td><strong>Appetite</strong></td>
<td>397.8±43.9</td>
<td>258.8±33.53</td>
<td>313.6±34.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Satiety</strong></td>
<td>784.0±40.0</td>
<td>828.5±30.4</td>
<td>772.9±31.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td>18.6±25.7</td>
<td>131.9±19.7^1,2</td>
<td>11.0±20.0</td>
<td>0.00003</td>
</tr>
<tr>
<td><strong>Epigastric fullness</strong></td>
<td>119.5±36.0^1</td>
<td>363.0±27.4^1,2</td>
<td>15.7±27.8</td>
<td>0.000001</td>
</tr>
<tr>
<td><strong>Epigastric pain</strong></td>
<td>34.0±26.0</td>
<td>136.2±19.9^1,2</td>
<td>2.0±20.2</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of Area Under Curve from 0 (fasting) to 120 minutes postprandially. Differences among groups were tested by Fisher’s LSD Multiple Comparison test.

^1P<0.05 vs controls; ^2P<0.05 vs SSc patients.
Table 2

Dyspeptic symptoms, bowel habits and gastrointestinal motility indices (gallbladder, stomach, small bowel) in patients with limited and diffuse cutaneous SSc

<table>
<thead>
<tr>
<th></th>
<th>Limited SSc</th>
<th>Diffuse SSc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>34</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Dyspepsia score</td>
<td>12.8±1.5</td>
<td>11.0±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Bristol score (bowel habits)</td>
<td>3.4±0.3</td>
<td>3.6±0.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Stomach*

<table>
<thead>
<tr>
<th></th>
<th>Limited SSc</th>
<th>Diffuse SSc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting antral area (cm²)</td>
<td>2.9±0.1</td>
<td>2.9±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal postprandial area (cm²)</td>
<td>10.7±0.3</td>
<td>10.0±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Minimal postprandial area (%)</td>
<td>8.5±2.7</td>
<td>5.1±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Minimal postprandial area (cm²)</td>
<td>3.7±0.3</td>
<td>3.3±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>42±6</td>
<td>33±7</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Gallbladder*

<table>
<thead>
<tr>
<th></th>
<th>Limited SSc</th>
<th>Diffuse SSc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting volume (mL)</td>
<td>25.0±1.7</td>
<td>23.2±3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (%)</td>
<td>26.5±2.6</td>
<td>29.1±11.5</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (mL)</td>
<td>6.5±0.8</td>
<td>6.8±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>25.2±2.8</td>
<td>26.3±10.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Small bowel*

<table>
<thead>
<tr>
<th></th>
<th>Limited SSc</th>
<th>Diffuse SSc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>31</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Orocecal transit time* (minute)</td>
<td>178.4±9.4</td>
<td>147.5±25.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. Differences among groups were tested by Mann-Whitney U-test. NS = no significant difference; * not done in 3 patients.
Table 3

Gastrointestinal motility indices in scleroderma (SSc) patients and in dyspeptic and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>SSc patients</th>
<th>Dyspeptic controls</th>
<th>Healthy controls</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stomach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting antral area (cm(^2))</td>
<td>2.9±0.1(^1)</td>
<td>3.4±0.1(^2)</td>
<td>3.5±0.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Maximal postprandial area (cm(^2))</td>
<td>10.7±0.3(^1)</td>
<td>11.8±0.2(^2)</td>
<td>12.3±0.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>Minimal postprandial area (%)</td>
<td>8.3±2.0(^1)</td>
<td>15.6±1.5(^1,2)</td>
<td>2.0±1.8</td>
<td>0.000001</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>42±4(^1)</td>
<td>47±3(^1)</td>
<td>26±3</td>
<td>0.000001</td>
</tr>
<tr>
<td>Abnormal emptying (%)</td>
<td>50(^1)</td>
<td>57(^1)</td>
<td>3</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Gallbladder</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting volume (mL)</td>
<td>25.2±1.5</td>
<td>24.8±1.0</td>
<td>22.0±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (%)</td>
<td>26.8±2.5</td>
<td>28.0±1.5</td>
<td>26.5±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (mL)</td>
<td>6.6±0.9</td>
<td>7.0±0.6</td>
<td>5.9±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>25.1±2.3</td>
<td>25.4±1.2</td>
<td>22.1±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Abnormal emptying (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Small bowel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orocecal transit time* (minute)</td>
<td>172.3±7.4(^1)</td>
<td>158.0±5.5(^1)</td>
<td>98.7±5.5</td>
<td>0.000001</td>
</tr>
<tr>
<td>Abnormal transit (%)</td>
<td>74(^1)</td>
<td>66(^1)</td>
<td>11</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. Differences among groups were tested by Fisher’s LSD Multiple Comparison test. The chi-square test was used to compare proportions. NS = no significant difference; \(^1\)P<0.05 vs controls; \(^2\)P<0.05 vs SSc. * not done in 3 SSc patients and in 3 dyspeptic patients.
Table 4
Dyspepsia score, bowel habits and gastrointestinal motility indices (gallbladder, stomach, small bowel) in scleroderma patients with normal or abnormal autonomic nerve function tests

<table>
<thead>
<tr>
<th></th>
<th>Normal ANS</th>
<th>Abnormal ANS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>9</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Male:Female</td>
<td>1:8</td>
<td>0:20</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>48.5± 4.7</td>
<td>55.1± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>23.6± 1.1</td>
<td>25.5± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Dyspepsia score</td>
<td>13.0± 3.3</td>
<td>11.6± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Bristol score (bowel habits)</td>
<td>3.7± 0.6</td>
<td>3.4± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Stomach**

<table>
<thead>
<tr>
<th></th>
<th>Normal ANS</th>
<th>Abnormal ANS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting antral area (cm²)</td>
<td>2.8± 0.1</td>
<td>2.9± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Maximal postprandial area (cm²)</td>
<td>10.5± 0.4</td>
<td>10.8± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Minimal postprandial area (%)</td>
<td>3.4± 2.1</td>
<td>5.1± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Minimal postprandial area (cm²)</td>
<td>3.1± 0.1</td>
<td>3.3± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>36.7± 4.4</td>
<td>34.3± 2.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Gallbladder**

<table>
<thead>
<tr>
<th></th>
<th>Normal ANS</th>
<th>Abnormal ANS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting volume (mL)</td>
<td>22.5± 2.3</td>
<td>25.2± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (%)</td>
<td>27.1± 5.0</td>
<td>23.7± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial residual volume (mL)</td>
<td>6.4± 1.8</td>
<td>5.7± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Half-emptying time (minute)</td>
<td>24.0± 2.8</td>
<td>23.1± 2.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Small bowel**

<table>
<thead>
<tr>
<th></th>
<th>Normal ANS</th>
<th>Abnormal ANS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>9</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Orocecal transit time* (minute)</td>
<td>190.0± 16.0</td>
<td>158.3± 11.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. Differences among groups were tested by Mann-Whitney U-test. The chi-square test was used to compare proportions. NS = no significant difference; * not done in 2 patients.
Figure Legends

Figure 1
Time-course of Visual Analogue Scale (VAS) for epigastric fullness in the fasting state (Time 0) and at regular intervals postprandially during the motility tests in SSc patients (white circles), healthy controls (black circles) and in dyspeptic controls (white squares) at each time point. Vertical lines indicate SE values. In the inset: Area Under Curve *P<0.01 vs healthy controls; °P<0.01 vs both SSc patients and healthy controls (ANOVA followed by Fisher’s LSD test).

Figure 2
Time-course of percentage antral area as index of gastric emptying (top panel) and of gallbladder volume (bottom panel) in SSc patients (white circles), healthy controls (black circles) and in dyspeptic controls (white squares) after ingestion of the standard liquid meal. Symbols indicate mean values while vertical lines indicate SE values at each time-point.

Top panel (gastric emptying): *P<0.01 SSc patients and dyspeptic controls vs healthy controls from time 10 minutes to time 120 minutes, and SSc patients vs dyspeptic controls from time 40 minutes to time 120 minutes (ANOVA followed by Fisher’s LSD test).

Bottom panel (gallbladder emptying): *P<0.05 dyspeptic vs healthy controls from time 60 minutes to time 120 minutes, and SSc patients vs healthy controls from time 90 minutes to time 120 minutes (ANOVA followed by Fisher’s LSD test).

Figure 3
Top panel: Time-course of gallbladder volume in SSc patients with normal (black circles, N=18) or abnormal (white circles, N=17) gastric emptying, after ingestion of the standard liquid meal. *P<0.05 vs patients with abnormal gastric emptying (Mann-Whitney U-test).

Bottom panel: Time-course of gallbladder volume in dyspeptic controls with normal (black squares, N=29) or abnormal (white squares, N=39) gastric emptying, after ingestion of the standard liquid meal. P=NS at each time point (Mann-Whitney U-test).
Authors’ contribution

ADC and PP conceived the study, participated in its design and coordination, contributed to acquisition of data, performed the statistical analysis and drafted the manuscript. MC, GL gave substantial contribution to design of the study, patients selection and analysis of data. MB contributed to patients selection and analysis of data. GP and DQ-HW participated in the design of the study and helped to draft the manuscript and to revise it critically. All authors read and approved the final manuscript.
Reference List


Figure 1

Epigastric Fullness, VAS (cm)

Time (min)

Healthy SSc Dyspeptic

AUC

0 100 200 300 400

° ° ° ° ° °

* * * * * *

0 1 2 3 4