Supplementary Methods

GIT Questionnaire
The UCLA SCTC-GIT 2.0 questionnaire was used as an additional measure of gastrointestinal involvement. It is comprised of 6 multi-item scales as reflux/indigestion, bloating, diarrhea, constipation, emotional well-being and social functioning plus a single-item scale for rectal incontinence. The scores of these 7 sub-groups can be classified as none to mild, moderate and severe to very severe (1).

Table S1. Description of the laboratory tests used to measure the micronutrients in the samples of patients with SSc.

Serum Marker	Normal range	Variation coefficient*	Method
Zinc	9 – 21 µmol/l	1.4% at 13.4 µmol/l 1.1% at 27.2 µmol/l	flame atomic absorption spectrometry
Selenium	0.8 – 1.1 µmol/l	4.2% at 1.2 µmol/l 4.2% at 1.71 µmol/l	graphite furnace atomic absorption spectrometry
Folic acid	> 4 µg/l	2.3% at 3.7 µg/l 2.2% at 9.8 µg/l	chemiluminiscence immunoassay
Holotranscobalamin	< 35 pmol/l: Vitamin B12 deficiency likely 35-50 pmol/l: Measurement of methyl malonic acid recommended >50 pmol/l: Vitamin B12 deficiency unlikely	unknown	chemiluminescent microparticle immunoassay
Prealbumin	200 – 400 mg/l	2.9% at 174 mg/l 2.5% at 247 mg/l	immunonephelometry

*according to http://www.uzl.usz.ch/fachwissen/uzl-analysen-auskunftssystem/Seiten/default.aspx

Supplementary Results

Table S2: Median serum values of the measured micronutrients in patients with established and very early systemic sclerosis.

Micronutrient (N=74)	Established SSc* (N=176)	Very early SSc*
Zinc (µmol/l)	10.8 (2.5)	10.8 (2.3)
Selenium (µmol/l)	1.03 (0.0)	1.07 (0.3)
Folic acid (µg/l)	6.8 (7.4)	9.1 (6.0)
Holotranscobalamin (pmol/l)	62 (39.0)	79 (57.1)
Prealbumin (mg/l)	249 (72.0)	267 (57.0)

*data shown as median (interquartile range) values in serum.

Table S3. Severity of the GIT illness in patients with established SSc, respectively very early SSc, according to the UCLA SCTC GIT 2.0 questionnaire.

Item	None-to-mild		Moderate		Severe-to-very-severe	
	SSc	veSSc	SSc	veSSc	SSc	veSSc
Reflux	89 (71.8%)	10 (100%)	26 (21.0%)	none	9 (7.3%)	none
Bloating	104 (83.9%)	8 (80%)	7 (5.6%)	1 (10%)	13 (10.5%)	1 (10%)
Diarrhea	85 (68.5%)	7 (70%)	33 (26.6%)	3 (30%)	6 (4.8%)	none
Fecal soilage	119 (96.0%)	10 (100%)	2 (1.6%)	none	3 (2.4%)	none
Constipation	87 (70.2%)	8 (80%)	26 (21.0%)	1 (10%)	11 (8.9%)	1 (10%)
Social functioning	102 (82.3%)	7 (70%)	15 (12.1%)	2 (20%)	7 (5.6%)	1 (10%)
Emotional well-being	108 (87.1%)	8 (80%)	10 (8.1%)	1 (10%)	6 (4.8%)	1 (10%)
Total GIT score	101 (81.5%)	8 (80%)	19 (15.3%)	2 (20%)	4 (3.2%)	none

Valid data for the UCLA SCTC GIT 2.0 was available for 124/176 patients with established SSc, respectively for 10/74 patients with veSSc. Data are shown as number (valid percentage). SSc: established systemic sclerosis, veSSc: very early systemic sclerosis.

Association with wound healing and/or vasculopathy

A sub-analysis looking at possible associations between levels of nutrients and markers of vasculopathy and possible wound healing was not significant (Table S4).

Table S4. Exploring the association between low micronutrients and/or prealbumin and parameters of vasculopathy.

Variable	Any low nutrient	Low Folic acid	Low Holo-transcobalamin	Low Selenium	Low Zinc	Low Prealbumin
	p	*P*	*p*	*p*	*p*	*p*
Digital ulcers (ever)	1.000	0.456	1.000	0.615	1.000	0.189
Pitting scars (ever)	0.095	0.462	1.000	0.436	1.000	0.164
SSc capillaroscopic pattern (yes/no)	0.313	0.529	0.308	1.000	0.076	1.000
Type of SSc capillaroscopic pattern (early/active/late)	0.540	0.791	0.902	0.942	0.901	0.066
PAH	0.078	1.000	0.417	0.699	0.705	0.234

Abbreviations: "*p*" represents *p* values as obtained by Chi-square test are shown. A *p* value of 0.05 or lower was considered statistically significant; the significance threshold after Bonferroni correction was 0.05/5=0.01, where 5 is the total number of hypotheses tested. Non-corrected, statistically significant *p* values, are marked with an asterisk (*). Bonferroni-corrected significant *p* values are additionally bolded.
SSc, scleroderma; PAH, pulmonary arterial hypertension.

Association with medications

We further investigated the possible influence of drug therapies in this cohort on the micronutrient or prealbumin levels, with focus on immunosupressives and proton pump inhibitors. Hereby, deficiency of prealbumin was associated with presence of methotrexate therapy, with significantly lower serum levels of prealbumin in treated patients (*p* = 0.017, Table S5). No association between micronutrients and therapy with mycophenolate mofetil, prednisone or proton pump inhibitor use was found (Table S5).

Table S5. Exploring the association between drug therapies and nutrient deficiency.

Variable	Any low nutrient	Folic acid	Holo-transcobalamin	Selenium	Zinc	Prealbumin
	p	*p*	*p*	*p*	*p*	*p*
Prednisone	0.253	0.164	0.076	0.547	0.535	0.526
Methotrexate	0.615	0.197	0.311	0.470	1.000	**0.004***
Proton pump inhibitor	0.708	0.628	0.287	0.801	0.326	0.603

Abbreviations: "*p*" represents *p* values as obtained by Chi-square test are shown. A *p* value of 0.05 or lower was considered statistically significant; the significance threshold after Bonferroni correction was 0.05/4=0.013, where 4 is the number of tests performed. Non-corrected, statistically significant *p* values, are marked with an asterisk (*). Bonferroni-corrected significant *p* values are additionally bolded.
MCP: metacarpophalangeal; HRCT: High-resolution computed tomography; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.
§Hb normal reference values according to the local laboratory: for men Hb 134-170 gG/l, for women 117-153 g/l.

The distribution of prealbumin between patients with/without therapy with methotrexate was significantly different, with lower levels in treated patients (p = 0.017, Mann-Whitney).

Supplementary references

1. KHANNA D, HAYS RD, MARANIAN P *et al.*: Reliability and validity of the University of California, Los Angeles Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument. *Arthritis Rheum* 2009; 61: 1257-63.