Impaired micronutrients and prealbumin in patients with established and very early systemic sclerosis

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Competing interests: see page S-125.

ABSTRACT

Objective. *Gastrointestinal involvement and impaired nutritional status are frequent in patients with systemic sclerosis (SSc). Hereby, we hypothesised that micronutrients and/or prealbumin could be deficitary in SSc.*

Methods. *Patients with SSc and very early SSc (veSSc) were prospectively included. Clinical assessment, data recording and quality controls followed EUSTAR standards. The UCLA SCTC-GIT 2.0 questionnaire was applied and the serum levels of zinc, selenium, prealbumin, holotranscobalamin, folic acid were measured.*

Results. *Half (52.4%) of the 176 patients with established SSc showed a deficiency in at least one of the measured nutrients. The most frequent deficit was seen in folic acid (17.9%), followed closely by selenium, prealbumin and zinc (around 15% each). Nearly a fifth (19%) of these patients had multiple deficiencies. Patients with more severe disease, including advanced skin fibrosis, positive ACR 1980 classification criteria, anaemia and elevated serum inflammation markers were more likely to be nutrient deficient. Lower BMI <20kg/m^2 was associated with several nutrient deficiencies. Prealbumin deficiency was associated with more frequent stomach symptoms and methotrexate therapy. A third of veSSc patients (27%, 44/74) presented a nutrient deficiency, mostly of zinc (10%). Surprisingly, micronutrient deficiencies were not associated with usual parameters of gastrointestinal involvement.*

Conclusion. *These novel data reveal deficiencies in micronutrients and/or prealbumin are a frequent burden in patients with SSc. Moreover, these correlate with clinical aspects of the disease. Especially patients with advanced disease appear at high risk for an impaired nutrient status, suggesting that screening of micronutrients status should be performed in these patients.*

Introduction

Systemic sclerosis (SSc) is a chronic, autoimmune, connective tissue disease characterised by inflammation, vasculopathy and tissue fibrosis, which may affect the skin and the internal organs (1).

Recent studies have reported a high prevalence of malnutrition or nutritional deficiency in SSc patients, ranging widely between 15–55% (2-4). This variability may be explained by the use of different definitions and assessment methods (2-4). An impaired nutritional status was associated with a higher risk of disease-related mortality (2, 5). Malnutrition, often multifactorial, is considered the leading gastrointestinal cause of mortality in SSc (6).

Micronutrients (minerals, vitamins) are essential dietary elements required by the organism in small quantities but essential for numerous metabolic processes, including oxidative stress, collagen synthesis and wound healing, which are also important for the pathogenesis of SSc.

There are few reports in the literature suggesting that impaired levels of micronutrients are relevant in SSc patients. A recent analysis of 82 patients diagnosed with SSc from a French cohort identified 35% of patients had a selenium deficiency, wich was associated with heart disease, 48% of patients had a zinc deficiency, 23% a folate deficiency (7). Another group previously reported significantly lower levels of selenium in 19 SSc patients in comparison to sex- and age-matched healthy controls (8).

The relation of the micronutrients with the nutritional status is unclear. Lower serum levels of selenium and ascorbic acid were reported in patients with SSc in comparison to healthy controls, irrespective of the dietary nutritional intake (9, 10).

Prealbumin, an important serum protein and macronutrient, is an established nutritional marker, which was also sug-

gested as marker of malnutrition in SSc, independent of disease activity and food intake (4). Moreover, in a large prospective study of 299 patients with SSc, with a median follow-up of 48 months, serum prealbumin was identified as a predictor of mortality (11).

Other studies focused on various vitamins (A, E, D), with heterogeneous results (12).

Although data arising from the available heterogeneous and mostly small scale studies suggest a relevant disruption of micronutrients and/or vitamins in SSc, there is to date no comprehensive and standardised assessment of the status of micro- and macronutrients in a large cohort of patients with SSc. Such a study could possibly shed light on the clinical and therapeutic relevance of these preliminary findings, if confirmed. Considering the available literature, as well as the frequent gastrointestinal involvement and impaired nutritional status, we hypothesise that micronutrients and prealbumin could be profoundly affected and of clinical relevance in SSc patients.

Material and methods

Patients and study design

This is an observational, single center, longitudinal cohort study. Consecutive patients with a diagnosis of SSc by expert opinion and meeting the ACR/EULAR 2013 classification criteria, with regular annual visits in our center between 2009 and 2014, were included. Clinical assessment, laboratory tests, data recording and quality controls were performed according to European Scleroderma Trials and Research group (EUSTAR) standards (13, 14). Data from all the recorded visits of the patients were considered for the analysis. All patients signed written informed consent. The study has been approved by the local ethical committee. Patients with lack of data for all micronutrients and prealbumin, with loss of follow up, or without written informed consent were excluded.

Malnutrition was defined according to EUSTAR as "chronic diarrhoea and/or other signs/symptoms consistent with generalised malabsorption according to the physician's opinion, confirmed by at least one of the following diagnostic tests: hydrogen breath test, D-xylose urinary test, 14C D-xylose breath test. If none of these tests were available, the physician's opinion is decisive." As markers of an impaired nutritional status, a body mass index (BMI) below 20 kg/m^2, as well as underweight (BMI below 18.5 kg/m^2) were considered.

We also analysed an additional cohort of patients with very early SSc, considering the high heterogeneity of the disease and the importance of early identification of possible complications. "Very early" SSc was defined as presence of Raynaud's phenomenon and at least one of the following: positive antinuclear antibodies, positive SSc-specific antibodies, puffy fingers, pathologic capillaroscopy, without meeting any of the classification criteria for SSc.

The UCLA SCTC-GIT 2.0 questionnaire was used as a standardised measure of patient-reported gastrointestinal involvement (see online Supplementary file) (15).

Laboratory analysis

The circulating levels of prealbumin and the following micronutrients were measured as part of the SSc assessment at each regular visit of the patients: zinc, selenium, holotranscobalamin, folic acid. These markers were chosen according to expert opinion and considering the available literature in SSc, as well as clinical relevance. Holotranscobalamin was analysed as the primary endpoint to indicate active vitamin B12 deficiency. The tests were performed as part of the routine diagnostics in the Department of Clinical Chemistry of the University Hospital Zurich, which is an ISO 17025 accredited referral centre in Switzerland. The characteristics and reference values of the standardised laboratory tests are presented in Supplementary Table S1. Deficiency in a macro-/micronutrient was defined as any value under the laboratory's normal range.

For the purpose of analysis, if multiple visits for the same patients were available, the visit with the most numerous deficiencies was selected. For patients with consistently normal nutrient levels, the latest visit was chosen.

Statistical analysis

Two main research hypotheses of the possible relevance of a deficient micronutrient or prealbumin status were investigated: i. whether gastrointestinal involvement in SSc was positively associated with nutritional deficiencies, and, ii. whether nutritional deficiencies were related to disease severity. Additionally, a secondary hypothesis of association between vasculopathy and nutritional deficiencies was analysed, as well as a possible influence of the concurrent medications. As outcomes individual deficiencies of each nutrient, and a composite parameter entitled "any deficiency", defined as levels below normal of at least one micronutrient and/or prealbumin at the respective visit, were analysed. The two-sided Fisher's exact test, the Chi-square test and the Mann-Whitney U-test were used. A p-value threshold <0.05 was considered significant. Correction for multiple testing was performed for each main hypothesis using the Bonferroni method. Additionally, ROC analysis and logistic regression were applied. The statistical analysis was performed in SPSS v. 22.0.

Results

A total of 176 patients with established SSc were included into the study. The main characteristics of the cohort are shown in Table I.

Deficiency of micronutrients and/orprealbumin: a frequent burden in patients with established systemic sclerosis

More than half (52.4%) of the patients with established SSc showed deficiency of at least one of the measured micronutrients. Multiple deficiencies were common (Table II). The median serum values are shown in Supplementary Table S2.

The most frequent deficiency in this group was found for folic acid, followed closely by selenium, prealbumin, zinc and holotranscobalamin.

Considering possible confounders, out of the 27 patients with folic acid deficiency, only one was under therapy with methotrexate and lacked folic acid substitution therapy. This patient

was the only one from the total of 18 patients treated with methotrexate with valid data who had folic acid deficiency. The majority of patients under methotrexate (16/22, 72.7%) underwent parallel substitution with folic acid. Furthermore, deficiency of prealbumin was found associated with methotrexate therapy (Supplementary file). Only 2/27 patients with folic acid deficiency, respectively 2/22 with zinc deficiency, were already under corresponding substitution therapy.

Lower BMI, but not gastrointestinal symptoms or gastrointestinal burden, is associated with nutrient deficiency

Fifteen percent of patients with established SSc had a BMI of less than 20 kg/m^2 (Table I). BMI <20 kg/m^2 was strongly associated with "any nutrient deficiency", as well as individually with low levels of prealbumin, zinc and folic acid (Table III). Further, deficiency of selenium was significantly associated with nutritional categories by BMI (normal/underweight/overweight or obese) and specifically with underweight (Table III). Only low prealbumin was associated with gastrointestinal symptoms (stomach symptoms, Table III). Overall, the patient-reported burden of gastrointestinal involvement, according to the UCLA SCTC-GIT 2.0 questionnaire, was generally mild in this cohort (according to the total GIT score: none-to-mild 81.5%, moderate 15.3%, severe in 3.2% of patients with established SSc; Suppl. Table S3). No relevant associations between the GIT categories or total score with the nutrient status were found (Table III).

Nutrient deficiency is associated with more severe disease

The presence of any deficiency in micronutrients and/or prealbumin was significantly associated with more severe SSc disease, as follows. Patients with at least one nutrient deficiency were more likely to meet the ACR 1980 classification criteria for SSc (corresponding to more extensive disease), to have skin fibrosis proximal to the MCP joints, as well as secondary joint contractures, and had a higher median mRSS (Table IV, Fig. 1). Patients with a disease du-

Table I. Main characteristics of patients with established SSc (total n=176) and very early SSc (n=74).

	Established SSc	Very early SSc
Gender (female, %)	143 (81%)	69 (93%)
Age, years	58 (49, 67)	52 (38, 61)
ACR1980 criteria for systemic sclerosis fulfilled	119/174 (68%)	None
ACR/EULAR 2013 criteria for systemic sclerosis fulfilled	167/168 (99%)	None
Disease duration since first non-Raynaud symptom, in months	71 (36, 129)	NA
Disease duration ≥3 years	119/160 (74%)	NA
Skin involvement		
Limited cutaneous	102/175 (58%)	3/74 (4%)
Diffuse cutaneous	39/175 (22%)	None
No skin involvement	35/176 (20%)	71/74 (96%)
Skin thickening of the fingers of both hands extending proximal to the MCP joints	65/136 (48%)	None
Scleredema (puffy fingers)	120/158 (76%)	3/74 (4%)
Modified Rodnan Skin Score (MRSS)	4 (2, 9)	0 (0, 0)
Joint contractures	65/173 (38%)	None
Gastrointestinal tract/ nutrition		
Oesophageal symptoms (dysphagia, reflux)	84/174 (48%)	24/74 (33%)
Stomach symptoms (early satiety, vomiting)	61/172 (36%)	NA
Intestinal symptoms (diarrhoea, bloating, constipation)	62/173 (36%)	NA
BMI (kg/m^2)	24 (21, 27)	23 (20, 26)
Underweight (BMI <18.5 kg/m^2)	8/163 (5%)	11/69 (16%)
BMI <20 kg/m^2	25/163 (15%)	23/74 (33%)
Malabsorption syndrome*	3/133 (2%)	NA
Primary biliary cholangitis (PBC)	6/176 (3%)	7/74 (10%)
Cardiopulmonal		
Dyspnea NYHA Stage I + II	142/161 (88%)	49/49 (100%)
Dyspnea NYHA Stage III + IV	19/161 (12%)	None
Pulmonary hypertension	21/171 (12%)	None
Lung fibrosis on HRCT	80/164 (49%)	1/59 (2%)
Renal crisis	4/174 (2%)	None
Vasculopathy		
Raynaud's present	166/175 (95%)	74/74 (100%)
Pitting scars on fingertips (ever)	50/134 (37%)	1/74 (1%)
Digital Ulcers (ever)	52/137 (38%)	1/74 (1%)
Scleroderma pattern on capillaroscopy	139/162 (86%)	28/72 (39%)
Laboratory parameters		
ANA positive	170/172 (99%)	72/74 (97%)
ACA positive	70/168 (42%)	37/74 (50%)
Scl-70 positive	42/171 (25%)	1/74 (1%)
RNA Polymerase III positive	17/163 (10%)	5/71 (7%)
CRP-Elevation	34/174 (20%)	7/74 (10%)
Erythrocyte sedimentation rate (mm/1h)	13 (6, 26)	6 (2, 34)
Erythrocyte sedimentation rate ≥25 mm/1h	46/168 (27%)	7/71 (10%)
Hb (g/dl)	13 (12, 14)	NA
Anaemia** n (%)	27/132 (15%)	NA
CK-elevation	24/173 (14%)	7/73 (10%)
Medication***		
Methotrexate	22/164 (13%)	3/74 (4%)
Mycophenolate mofetil	14/174 (8%)	NA
Azathioprine	3/174 (2%)	NA
Rituximab	12/175 (7%)	NA
Prednisone	32/167 (19%)	NA
Doses ≤10 mg/day	30/32 (99%)	
Doses 20 mg/day	2/32 (1%)	
Proton pump inhibitor	107/156 (69%)	NA

For nominal variables, the absolute and relative frequencies are shown. For continuous variables the median and 1st and 3rd Quartile is shown.

*Malabsorbtion was defined according to EUSTAR guidelines (see methods).

**Hb normal reference values according to the local laboratory: for men Hb 134-170 G/l, for women 117-153 G/l.

***Additionally, amongst patients with established SSc, one patient received imatinib, one patient a TNF-alpha antagonist and one patient cyclophosphamide.

NA: not available data; ACA: anti-centromere antibodies; ACR: American College of Rheumatology; ANA: antinuclear antibodies; BMI: body mass index; CK: creatine kinase; CRP: C-reactive protein; EULAR: European League against Rheumatism; Hb: haemoglobin; HRCT: high-resolution computed tomography; MCP: metacarpophalangeal; NYHA: New York Heart Association; RNA: ribonucleic acid; Scl-70: anti-topoisomerase I antibodies.

Table II. Deficiencies of nutrients in patients with established SSc and very early SSc.

Parameter	Frequency* in patients with established SSc	Frequency* in patients with very early SSc
Any deficiency	**75/143 (52.4%)**	**12/44 (27.2%)**
Deficiency of one nutrient	36/130 (27.7%)	10/44 (22.7%)
Deficiency of two nutrients	18/130 (13.8%)	2/44 (4.5%)
Deficiency of three or more nutrients	8/130 (6.2%)	0/44 (0.0%)
Single deficiencies		
Folic acid	27/151 (17.9%)	2/46 (4.3%)
Selenium	23/147 (15.6%)	4/44 (9.1%)
Prealbumin	24/144 (15.3%)	2/44 (4.5%)
Zinc	22/147 (15.0%)	5/46 (10.9%)
Holotranscobalamin	20/151 (13.2%)	1/46 (2.2%)

Measured macro-/micronutrients and their normal reference serum levels: zinc: 9-21µmol/l, selenium: 0.8-1.1µmol/l, prealbumin: 200-400mg/l, folic acid: >4 µg/l, holotranscobalamin: >35pmol/l
*data shown as number/available cases and valid percentage in brackets.

ration exceeding 3 years showed zinc deficiency more frequently (Table IV). The nutrient status did not significantly differ between SSc disease subtypes.

Furthermore, there was a strong association between any deficiency in nutrients and co-existence of elevated inflammatory markers (ESR) and anaemia (Table IV, Fig. 1). Low folic acid levels were present in only 8 out of the 27 patients with anaemia (p=0.026). Deficiencies of prealbumin and, respectively, zinc, were also strongly associated with co-existence of anaemia, as well as with higher ESR and CRP (Table IV, Fig. 1). As expected, patients with anaemia also had significantly higher ESR levels (p<0.001, median 10 *vs*. 20 mm/1h).

Micronutrients and prealbumin in patients with very early SSc

Additionally, a cohort of 74 patients with very early SSc was analysed (Table I). Interestingly, 33.3% of these patients showed signs of malnutrition (BMI <20 kg/m^2) and 15.9% were underweight (BMI <18.5 kg/m^2); 9.5% had associated PBC. Specific clinical signs of SSc, especially regarding fibrosis, were rarely present (Table I), as expected in this very early SSc cohort.

However, nearly a third of patients showed at least one deficiency of the measured nutrients, most frequently zinc (10.9%) and selenium (9.1%), Table II. Overall, the deficiencies in micronutrients were less frequent in comparison to patients with established SSc.

Patients with low zinc had significantly higher ESR levels (p=0.011, Mann-Whitney), mild gastrointestinal involvement (Table I, Suppl. Table S3), and no further association with gastrointestinal symptoms or other clinical characteristics of SSc were found.

Discussion

This comprehensive analysis of two large cohorts of patients with established and very early SSc, revealed a high frequency of an impaired nutrient status in these patients. More than half of the patients with established SSc and nearly one third of those with very early SSc showed deficiency in micronutrients and/or prealbumin.

This was especially the case for SSc patients with advanced, more severe disease, meeting the ACR1980 classification criteria, with more extensive skin fibrosis, elevated inflammation markers and anaemia.

In addition to the nutrient deficiencies, low BMI <20 kg/m^2 was relatively frequent in both cohorts (15.3% SSc *vs*. 33.3% very early SSc). This was significantly associated with "any nutrient" deficiency in patients with SSc and with low prealbumin, zinc and folic acid, respectively, further suggesting clinically meaningful nutritional impairment in these patients. Similarly, formal underweight (BMI <18.5 kg/m^2) was present in 4.9% of patients with established SSc and 15.9% of patients with very early SSc and associated with lower selenium levels. The lower BMI levels in very early SSc *vs*. SSc patients might be explained by the younger age in this cohort (Table I).

According to the UCLA SCTC-GIT 2.0 questionnaire (assessing patient-reported quality of life and gastrointestinal severity in SSc over the previous week), the overall burden of gastrointestinal symptoms was mild (Suppl. Table S3). Few patients were diagnosed with PBC (3.4% of SSc, respectively 9.4% of very early SSc patients) or malabsorption. Nonetheless, almost 50% of patients with established SSc, respectively over 30% of patients with very early SSc, reported symptoms of reflux or dysphagia. More than one third of patients with established SSc reported stomach symptoms (early satiety, vomiting) or intestinal symptoms (bloating, diarrhoea or constipation). Low prealbumin, an acknowledged marked of malabsorption, was significantly associated with presence of stomach symptoms in this cohort. However, no relevant associations between the GIT scales and any of the biomarkers were found.

These data suggest that macro-/ micronutrient measurements in serum could help identify nutritional problems even prior to an overt clinical manifestation.

Furthermore, several associations of micronutrient deficiency with inflammation and anaemia were found. Firstly, in patients with established SSc, the presence of anaemia was significantly associated with "any deficiency" in the tested nutrients, as well as with low prealbumin. ESR was significantly higher in SSc patients with "any deficiency" and with low levels of zinc or prealbumin, and a high ESR ≥25mm/1h was associated with low levels of zinc. The complex link between zinc and anaemia, as well as inflammation, is reported in the literature, (16-18) but little is known in SSc. Chronic zinc deficiency was postulated to trigger inflammation and influence the outcome of autoimmune rheumatic diseases (16, 17). Due to low number of incident deaths during follow-up, a survival analysis investigating the previously reported predictive role of prealbumin for mortality in SSc (11) was inconclusive (data not shown).

The association between therapy with methotrexate and low prealbumin in patients with established SSc could be explained by the more severe disease in

Table III. Association between low micronutrients and/or prealbumin and parameters of gastrointestinal involvement and nutritional status.

Variable	Any low nutrient	Low folic acid	Low holotrans-cobalamin	Low Selenium	Low Zinc	Low Prealbu-min
	p	*p*	*p*	*p*	*p*	*p*
BMI <18.5 kg/m^2 (underweight)	0.211	0.608	0.585	0.006*	0.546	0.172
BMI <20 kg/m^2	0.001**	0.034*	0.721	0.091	0.033*	0.027*
BMI categories (underweight, normal, overweight)	0.140	0.419	0.945	0.002**	0.385	0.307
PBC	1.000	1.000	0.580	1.000	0.162	1.000
Esophageal symptoms^{+}	1.000	0.531	0.632	1.000	0.247	0.818
Stomach symptoms^{++}	0.114	1.000	0.802	0.143	0.810	0.003**
Intestinal symptoms^{+++}	0.727	0.663	0.800	1.000	0.234	0.152
UCLA SCTC GIT 2.0 (scale)						
Reflux	0.613	0.222	0.439	0.746	0.093	0.579
Bloating	0.352	0.378	0.265	0.423	0.203	0.536
Diarrhoea	0.690	0.995	0.369	0.568	0.675	0.297
Fecal soilage	0.216	0.410	0.772	0.698	0.718	0.751
Social functioning	0.702	0.578	0.202	0.368	0.600	0.183
Emotional well-being	0.351	0.363	0.314	0.864	0.629	0.851
Constipation	0.375	0.866	0.435	0.301	0.747	0.399
Total score	0.746	0.465	0.202	0.751	0.439	0.607

"*p*" represents *p* values as obtained by Chi-square test. A *p* value of 0.05 or lower was considered statistically significant; the significance threshold after Bonferroni correction was 0.05/15=0.0033, where 15 is the total number of associations performed. Non-corrected, statistically significant *p* values, are marked with an asterisk (*). Bonferroni-corrected significant *p* values are marked with a double asterisk (**).
PBC: primary biliary cholangitis.
$^{+}$dysphagia, reflux; $^{++}$early satiety, vomiting; $^{+++}$diarrhoea, bloating, constipation.

Table IV. Association between deficient micronutrients and/or prealbumin and parameters of disease severity.

Variable	Any low nutrient	Low folic acid	Low holotrans-cobalamin	Low Selenium	Low Zinc	Low Prealbu-min
	p	*p*	*p*	*p*	*p*	*p*
ACR 1980 criteria fulfilled	0.029*	0.172	0.452	0.144	0.458	0.328
ACR/EULAR 2013 criteria fulfilled	1.000	1.000	1.000	1.000	0.144	1.000
Disease duration >3 years	0.326	0.332	0.390	1.000	0.014*	0.156
Extent of skin involvement (limited or no skin involvement *vs*. diffuse)	0.425	0.799	1.000	0.579	0.085	0.083
Skin fibrosis proximal to MCP	0.012*	0.339	0.188	0.460	0.574	0.066
Joint contractures	0.039*	0.827	0.607	0.485	0.090	0.329
Joint synovitis	0.283	0.768	1.000	0.125	0.215	0.764
Tendon friction rubs	0.747	1.000	0.361	0.372	1.000	0.621
Lung fibrosis on HRCT	0.390	0.512	0.630	0.343	1.000	0.487
CRP elevation	0.657	1.000	0.746	1.000	0.020*	0.351
ESR >25mm/1h	0.009*	0.620	0.402	0.250	**0.001****	0.008*
Anaemia§	**<0.001****	0.026*	0.310	0.201	0.007*	**0.003****

"*p*" represents *p*-values as obtained by Chi-square test. A *p*.value of 0.05 or lower was considered statistically significant; the significance threshold after Bonferroni correction was 0.05/15=0.0033, where 15 is the total number of tests performed. Non-corrected, statistically significant *p*-values, are marked with an asterisk (*). Bonferroni-corrected significant *p*-values are marked with a double asterisk (**).
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 g/l, for women 117-153 g/l.

treated patients, as well as by a possible drug side-effect through liver toxicity.
Altogether, according to our data, we postulate that the cause of the impaired nutrient status in SSc patients is likely multifactorial, consequence of the chronic disease, including changes in gut function, possibly influenced by inflammatory status or anaemia. Other possible factors like insufficient dietary intake because of the gastrointestinal symptoms and/or eating difficulties are also plausible.
Whether deficiency in serum nutrients carries a prognostic relevance is still unknown. A longitudinal analysis after the 1-years follow-up of our cohort with established SSc showed no relevant association between nutrient deficiency at baseline and further progression of skin and/or lung fibrosis at one year; nonetheless, this analysis was hindered by a low number of only 12 progressing patients (data not shown).
There are also several limitations to our study. For example, additional methods to evaluate disease-related malnutrition, like dedicated questionnaires (19, 20), or analysis of body composition (2, 20) were not applied. There are, however, to date no validated malnutrition screening tools for SSc. A normal BMI does not exclude malnutrition, and the recent Global Leadership Initiative on Malnutrition (GLIM) criteria include body composition parameters in addition to BMI in the new definition of malnutrition (21, 22). Measuring body composition parameters by modern techniques like dual energy x-ray absorptiometry (DEXA) and Bioelectrical impedance analysis (BIA) could yield a more thorough description of the burden of malnutrition in SSc (2, 20-22). Further, recent weight loss, as well as dietary habits (except from data on vitamin supplements) were not documented. We also did not perform an exhaustive analysis of the multiple possible causes of anaemia in these patients, as this was beyond the scope of this study. The measurement of the nutrients was performed within the standardised, official laboratory of the clinic using validated tests. Given the general consideration that 95% of the healthy population would fall within the reference range of the test, a certain variation can be expected even in the general population. However, the high percentage of deficiencies found in the established SSc cohort exceeds these plausible outliers. Except for zinc, the lower percentages of deficiencies in nutrients within the very early SSc cohort could, on the other side, be explained as the natural variance around

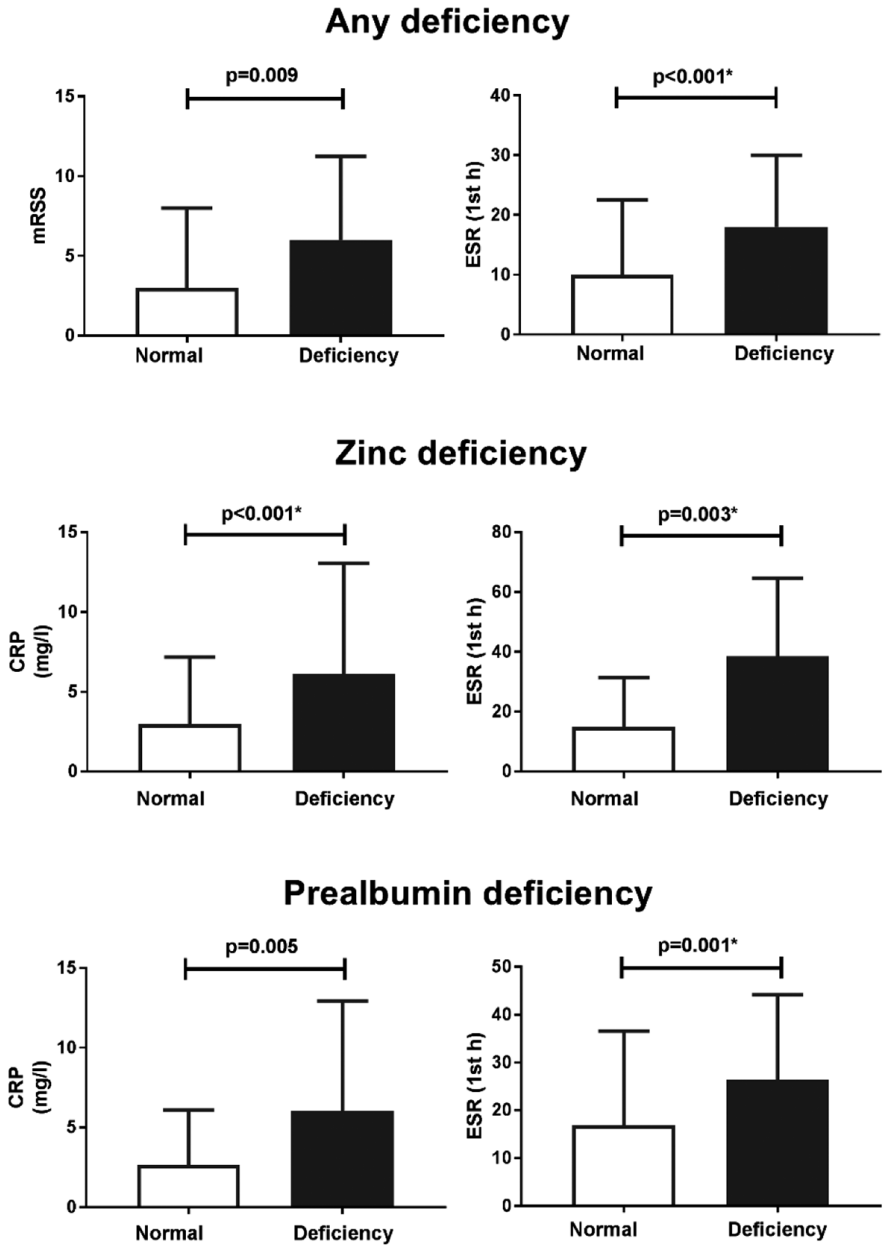

Fig. 1. Significantly differently distributed variables between patients with/without nutrient deficiency. The *p*-values obtained by Mann-Whitney test are shown. Values significant after Bonferroni correction are marked with an asterisk (*).

the reference range and should not be overinterpreted. Further, the strict definition of deficiency in active vitamin B12 (holotranscobalamin <35 pmol/l), which ensured a high specificity for identifying patients with real deficiencies, might have missed milder cases (*e.g.* where holotranscobalamin was in the "grey area" between 35–50 pmol/l), case in which the additional measurement of the methylmalonic acid and/or homocysteine would have been required. Thus, the burden of vitamin B12 deficiency might be even higher than the 13% identified in patients with established SSc.

As strengths of this analysis we would like to underline that this is the first study analysing macro-/micronutrients in two large cohorts of patients with established, and very early SSc. The diagnosis and comprehensive follow-up of the patients, with longitudinal data collection, were performed according to a systematic and standardised protocol, in line with the current EUSTAR guidelines, in a centre with longstanding expertise in SSc. The measurement of the selected key micronutrients and prealbumin was performed systematically in the same, standardised and accredited routine laboratory facility.

These data have possible clinical implications. Also based on the available literature, we believe there is evidence supporting the regular assessment of the nutritional status in the routine care of patients with SSc. In the absence of standardised guidelines, the methods may vary widely. We suggest considering the measurement of macro-/micronutrients in serum as part of this assessment. Based on our data, this should especially be considered in patients with advanced SSc (more advanced skin fibrosis, elevated inflammation markers) and with co-existent anaemia. Selection of the macro-/micronutrients to be tested remains open, but, according to our results, as well as to published data, we suggest it should (at least) include prealbumin, zinc, selenium and folic acid.

There is, so far, only inconsistent evidence based data supporting the benefit of a therapeutic substitution of the deficient macro-/micronutrients in patients with SSc in relation to the disease outcome, coming mostly from small, heterogeneous studies (12). However, recent data support standardised nutritional interventions in SSc, from dietary strategies like the low-FODMAP diet (23) to advanced nutritional support in patients with advanced SSc-related malnutrition (24). We suggest that correction of the deficiencies of macro-/micronutrients through therapeutic interventions should be considered on a case-specific basis, as it is relatively simple, cheap, well tolerated and optimises the nutritional status, which is likely to positively influence the outcome of the patient (11).

Competing interests

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B. Misselwitz reports personal fees from Gilead, personal fees and an unrestricted research grant from MSD, personal fees from Novigenix, personal fees from Vifor, personal fees from Novartis, personal fees from Takeda outside the submitted work.

O. Distler had a consultancy relationship and/or has received research funding from Abbvie, Actelion, Acceleron Pharma, Amgen, AnaMar, Baecon Discovery, Blade Therapeutics, Bayer, Boehringer Ingelheim Catenion, Competitive Drug Development International Ltd, CSL Behring, Curzion Pharmaceuticals, Ergonex, Galapagos NV, Glenmark Pharmaceuticals, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, Medscape, Mitsubishi Tanabe Pharma, MSD, Novartis, Pfizer, Roche, Target Bio Science and UCB in the area of potential treatments of scleroderma and its complications. In addition, he has a patent mir-29 for the treatment of systemic sclerosis issued (US8247389, EP2331143).

The other co-authors have declared no competing interests.

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