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The PREdictor of MAlnutrition in Systemic Sclerosis (PREMASS) score: the first validated combined index predictive of future weight loss in systemic sclerosis.

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ABSTRACT

Background/Purpose: Malnutrition and severe gastrointestinal dysfunction are the cause of mortality in 4-15% of systemic sclerosis (SSc) patients whereas overall gastrointestinal involvement is observed in 75-90% of cases. There is currently no disease modifying treatment for severe GI involvement and malnutrition in SSc, and their implementation in clinical trials is halted by our current inability to stratify patients with SSc for the risk of progression. Here we set out to identify a combined index predictive of significant weight loss at 12 months employing Malnutrition Universal Screening Tool (MUST) and serum adiponectin to leptin ratio (A/L) both with known value in other conditions.

Methods: This was an international, multicentre, longitudinal study employing 180 SSc patients in two independent cohorts: a study cohort (110 consecutive SSc patients) enrolled from University of Messina (60) and University of Padova (50), and a validation cohort (70) at the University of Leeds**.** Serum A/L ratio was measured by ELISA. MUST score, which includes BMI and weight loss reported by the patient in the last 3-6 months, was calculated as described: 0=no, 1=mild, >2=moderate/severe risk of malnutrition. End point of the study was weight loss ≥10% of baseline weight at 12 months.

Results: The two cohorts showed no significant differences in demographic and clinical features. Overall, median BMI decreased over time in both study and validation cohorts (23.5 vs 22.35 and 23.44 vs 22.49, respectively; $p<0.0001$). A/L ratio correlated significantly with BMI in both cohorts ($r^2=0.19$ for study cohort, $r^2=0.25$ for validation cohort; $p<0.0001$). MUST score had only low value in predicting weight loss in the study cohort (AUC 0.7; 95% CI: 0.58-0.82). Specifically, 46.5% of SSc patients with “no” or

"mild" MUST risk lost ≥10% weight at 12 months. Logistic regression analysis identified the combination of BMI and A/L as the best PREdictor of MAlnutrition in Systemic Sclerosis (PREMASS). The formula 12.18-(0.63*BMI) + (1.51*A/L) predicted the 10% weight loss at 12 months with an AUC=0.91(95% CI:0.77-0.84). A PREMASS score >0.23 showed 91.3% sensitivity (95% CI:79.79-100) and 80.46% specificity (95% CI:72.13-88.79) for ≥10% weight loss with an overall 55.26% positive predictive value (PPV) (95% CI:39.45-71.07) and 97.22% negative predictive value (NPV) (95% CI:93.43-100) and a relative risk (RR) of 19.90 (95% CI:4.93-80.37). In the validation cohort, PREMASS showed 76.47% sensitivity (95% CI:56.31-96.63) and 75.47% specificity (95% CI:63.89-87.06) with an overall 50% PPV (95% CI:30.78-69.22), 90.91% NPV (95% CI:82.41-99.4) and a RR of 5.5 (95% CI: 2-15.10).

Conclusion: Here we propose PREMASS as the first independently validated index for stratification of risk for severe weight loss in the following 12 months in SSc. Prediction of future weight loss in SSc could aid both in clinical management and stratification/enrichment in clinical trials.

INTRODUCTION

Systemic sclerosis overview

Systemic sclerosis (SSc) or scleroderma is a rare autoimmune disease characterized by microvascular injury, immune dysregulation and fibroblasts activation leading to progressive skin and multi-organ fibrosis with potentially life-threatening complications and poor quality of life [1].

The clinical course of the disease is highly variable, ranging from extremely benign that escapes diagnosis for decades, to extremely severe that results in death within three years of onset [2-3]. The main pathologic features of SSc are characterised by:

- autoimmune activation, defined by lymphocytic tissue infiltrate and antinuclear antibodies
- vascular abnormalities including decreased capillary density and vessel narrowing (neointima proliferation), resulting in poor perfusion and tissue ischemia
- tissue fibrosis, characterized by increased deposition of collagen and extracellular matrix proteins in the skin as well as internal organs [1-4].

Indeed, although the term scleroderma (scleros = hard and derma = skin) refers properly to the thickening of the skin, in Systemic Sclerosis virtually all organs can be affected including lungs, heart, gastrointestinal and renal systems [5].

The prevalence of SSc is hard to accurately estimate given the heterogeneity of clinical presentation [6]. A systematic review of the literature identified 32 epidemiological studies describing a geographically heterogeneous prevalence of the disease ranging from 7 to 489 per million [7].

Given the varied prevalence and the highly variable course of the disease, identifying patients who are at risk of severe clinical outcomes is essential.

Scleroderma is highly heterogeneous in clinical course for both type and severity of organs involved. Patients diagnosed with Scleroderma are often terribly frightened by the possible effects of the disease and unfortunately left wondering about their future given the lack of prognostic markers. The extent and rate of progression of skin fibrosis is a major prognostic factor correlating with both survival and functional limitations [8-10].

Patients with diffuse skin involvement (diffuse cutaneous SSc, ie dcSSc, defined as fibrotic involvement proximal to elbows and knees) have indeed higher risk of death for pulmonary fibrosis, cardiac involvement and gastrointestinal involvement [11-12]. For this reason, the assessment of skin involvement is often the primary outcome of clinical trials and is of utmost importance in assessing disease activity. The clinical semi-quantitative assessment of skin thickness (modified Rodnan skin score or mRSS) is currently the gold standard for skin evaluation in SSc but it is confounded by several limitations, including high inter-observer variability [13] and high level of skill required for its utilization [14]. Indeed, a meta-analysis of three independent studies determined an overall within patient inter-observer Standard Error as high as 26% [15].

Besides the increased risk of severe lung, gastrointestinal and heart fibrosis in patients with SSc, the overall leading cause of mortality and morbidity is represented by pulmonary involvement [16-17]. This can be due to interstitial lung disease (ILD or pulmonary fibrosis), more frequent in patients with dcSSC, pulmonary artery hypertension (PAH, more frequent in the patients without diffuse skin involvement - limited cutaneous or lcSSc) or the combination of the two [17-19].

Recently, Maungchan and colleagues published a systematic review identifying the prevalence of severe organ manifestations in SSc [20]. The results of this review indicated that pulmonary arterial hypertension occurs in 15% of SSc patients (95% CI 12 to 17). Similarly, pulmonary fibrosis, (defined by a forced vital capacity or FVC <70% predicted) occurs in 15% of SSc patients (CI 12 to 17). A growing effort has been directed toward understanding the complex pathogenesis of systemic sclerosis in order to better treat those complications associated with increased mortality which appears to be due mostly to cardiopulmonary causes.

Gastrointestinal involvement and malnutrition in systemic sclerosis

However, the gastrointestinal (GI) tract involvement occurs in 75-90% of SSc patients, or even 98% according to a recent study [21], and is second in frequency only to the cutaneous involvement [22]. GI motility, digestion, absorption and excretion can all be affected in SSc patients and GI symptoms, including pain, dysphagia, vomiting, diarrhea, constipation, faecal incontinence and substantial weight loss, represent one of the main complaints in clinical practice and drivers of poor quality of life [23].

Unfortunately, GI involvement is often noticed only when severe complications have already occurred and it remains difficult to predict. It is difficult to manage [24] and although severe GI involvement affects only 8% of scleroderma patients, mortality is high with only 15% of such patients surviving after 9 years [25]. Recently, nutritional status in SSc patients has been found to predict mortality [26] and SSc-related gastrointestinal causes of mortality account for 3-12% of overall SSc-related causes [19-27].

Nevertheless, GI involvement or significant weight loss are not included as outcomes in randomized clinical trials for scleroderma, and this is mainly due to our inability to measure its severity or to stratify patients.

Many factors may contribute to the development of nutritional impairment in SSc.

Depression and anxiety, altering the control of appetite, might influence nutritional intake. The prevalence of depression in SSc is variably reported to affect 19-69% of patients with SSc [28-29]. Indeed, depression is more frequent in SSc when compared to the adult populations (2.6-3.2%), and also compared to individuals with two or more common chronic physical diseases (23%) [30-31]. Similarly, up to 80% of patients with SSc, reports anxiety compared with 4.4% of the general adult population [28-29, 32].

Depression and/or anxiety may impair nutrition by decreasing appetite or by negatively impacting on crucial activities, such as food preparation and shopping [33].

The occurrence of digital ulcers, observed in up to 58% of patients [34], may also influence food intake and preparation, by causing pain, which subsequently alters functional status and the ability to eat [35]. In addition, the severity of lung involvement, fatigue and myalgia are all factors that influence global disability, further worsening the functional status of SSc patients [36-38].

Malnutrition could derive from several gastrointestinal manifestations: oropharyngeal disorders, such as microstomia or xerostomia, or a reduced mouth opening due to severe skin thickening may hinder food intake [39-40]. Indeed, a significant correlation between oral aperture size and malnutrition risk was demonstrated in a canadian study involving 586 SSc patients [41]. Furthermore, xerostomia in association with a reduced range of

motion of the hands, may reduce effective dental hygiene and mastication while promoting oral bacterial infections, dental caries and subsequent tooth loss [42-45].

Recent evidence suggests that autonomic neuropathy is an early aspect, and may even precede fibrosis [46-47] in the setting of GI dysmobility.

The most commonly reported symptoms in SSc originate from the upper GI tract [48]. In a study involving 402 patients, 94% reported upper GI symptoms, mostly resistant to specific therapy [48]. The diagnostic evaluation of the upper GI tract, performed by oesophageal manometry and endoscopy in 60 unselected patients, showed that hypotensive lower oesophageal sphincter is the most frequent alteration (95%), followed by oesophagitis (60%), aperistalsis (41%) and Barrett's oesophagus (18%) [49]. A defective peristalsis is one of the main factors leading to dysphagia and symptomatic oesophageal dysmotility could further impact nutritional impairment. In a case-control study involving 30 patients with GI symptoms and 30 healthy subjects, after the assessment of oesophageal function by barium swallow on intake (4 days), dysmotility was demonstrated in 93%, as well as an increased fat intake with dysphagia. However, this study may be limited by the small number of symptomatic patients [50].

The Canadian study on malnutrition in SSc showed no association between dysphagia or reflux and malnutrition risk, as assessed by the Malnutrition Universal Screening Tool (MUST) score [41]. Of 586 patients, 64% had reflux, 54% reported swallowing difficulty, and 43% retrosternal burning [41]. This may suggest that MUST has some limitations when employed in the assessment of weight loss in chronic disease.

Dysmotility can affect also the stomach and it occurs in both lcSSc and dcSSc, but there are not robust studies evaluating gastric involvement. In a study employing a radiolabeled

solid meal, 50% of 20 symptomatic patients fulfilled absolute criteria for delayed emptying, with 63% showing some degree of delay [50]. Gastric dysfunction appears to be due to autonomic nerve dysfunction but it could be also influenced by the extent of fibrotic alterations of the muscularis mucosa and submucosa [51-52].

Gastroparesis can certainly induce early satiety, nausea and vomiting [53]. Prokinetics are commonly used in clinical practice despite only a few small studies support their use in SSc [51-55]. The Canadian study reported that early satiety was significantly associated with malnutrition risk on bivariate analysis [41]. However, prospective studies evaluating the link between gastric dysmotility and nutritional risk in SSc are lacking. Dysmotility affects the small intestine in SSc, as reported in a prospective study showing that 88% of 17 unselected patients (35% with GI symptoms) have abnormal small bowel manometry [56]. Radiological contrast studies show that SSc patients could develop typical features of megaduodenum, small bowel dilation and/or diverticulae, and the typical hide-bound appearance (42%). Although pneumatosis intestinalis is not frequent (8%), compared to other GI alterations, its presence should be considered an important risk of perforation [57-60].

SSc may involve the colon and anorectum and a delayed colonic transit, using radionuclide, has been observed in 57% of 23 unselected patients (dcSSc and lcSSc) compared with 20 age-matched healthy controls [61]. Although it could appear that colonic motility should not impact nutrition, its presence is commonly associated with small bowel dysmotility. In addition, severe pseudo-obstruction can impair eating by inducing postprandial symptoms leading eventually to parenteral nutrition [62]. The occurrence of autoimmune GI comorbities in SSc, such as primary biliary cirrhosis (PBC),

which has an increased prevalence in SSc, especially lcSSc [63], and the resultant cholestasis, can impair the absorption of fat and induce liposoluble vitamin deficiency [64]. Another GI complication of SSc is exocrine pancreatic insufficiency and in a study involving 16 consecutive patients (dcSSc), 4 had a clinically relevant reduced exocrine function [65]. Untreated pancreatic insufficiency, unrelated to SSc, causes malabsorption and results in weight loss. However, there are no reports of associated nutritional compromise in SSc.

Non-GI manifestations can also contribute to malnutrition. Studies in other patients with chronic diseases with similar cardiopulmonary pathology demonstrate malnutrition. Studies on nutritional intake in patients with chronic heart failure showed that almost two out of three patients were not reaching an appropriate energy intakes [66-67]. Malnutrition is observed in slightly more than a third of patients with idiopathic pulmonary fibrosis referred to transplantation [68].

Prevalence of malnutrition in SSc was reported to be between 15-65%, but the assessment of weight loss was commonly performed using MUST, which includes patients anamnestic recall regarding previous 3-6 months weight loss, This may be considered an important bias since it could underestimate actual weight change [69-70]. Another study suggests that MUST can predict mortality in SSc, but the interpretation of the results is limited by the very low number of SSc patients with 10% weight loss (9 patients, 5.6%) [71].

A panel of experts provided a set recommendations to highlight the importance of malnutrition screening and the relevance of a multidisciplinary approach in SSc, supporting future research in detecting specific predictors of weight loss [72].

Several tools have been employed to detect malnutrition, such as MUST which has been developed for adult patients and it is the only validated tool for malnutrition screening in SSc [73-74]. However, the MUST score includes not only actual patients BMI, which can be easily assessed, but also unintentional weight loss over the last 3-6 months based on patient recall. Although the tool remains very intuitive and easy to use, the lack of specificity for disease groups and the absence of an objective determination of previous weight loss are important limitations of its applicability.

Metabolic functions of the adipose tissue and adipokines

Over the past decade, it has been recognized that adipose tissue has important functions, other than energy storage, glucose and lipid metabolism control. These functions are mediated by the secretion of a variety of endocrine, paracrine, and autocrine hormones, cytokines and growth factors, such as leptin, adiponectin, visfatin, interleukin 6, plasminogen activator inhibitor type 1, resistin and others influencing different local organs/tissues [75]. In fact, the alteration of the balance in adipokine release and levels has been linked with different conditions, including diabetes [76] and obesity [77], cancer [78] and metastasis development [79], cardiovascular diseases [80] and atherogenesis [81], and also inflammation [82] and fibrosis [83].

In the general population, leptin and adiponectin, the two major adipokines, play a central role in cardiovascular and metabolic homeostasis.

Adiponectin is probably the most studied adipokine. It exerts different biological activities and acts on several tissues due partly to its unique collagen-like domain that enables protein monomers to form small, medium, and large complexes with distinct functional

capabilities [84]. Interestingly, although the three-dimensional structure of adiponectin closely resembles that of TNF-α [85], these two proteins have completely opposite effects, suppress each other's production and also antagonize each other's action in their target tissues [86].

Adiponectin (defined also as Acrp30 [87], AdipoQ [88], GBP-28 [89]) is a 244-amino acid protein secreted mainly by the adipose tissue [90]. Although it was initially believed that the adipose tissue was the only site for the production of adiponectin, later different research groups provided evidence that adiponectin is expressed in other tissues including osteoblasts [91], hepatic cells [92], myocytes [93], epithelial cells [94], and placental tissue [95]. Human adiponectin is encoded by the Adipo Q gene and it spans 17 kb on chromosome locus 3q27 [96-98]. This human chromosome has a specific relevance for type 2 diabetes and metabolic syndrome since it has a region carrying a susceptibility gene for them [98-100]. In addition, it has been demonstrated that serum levels of adiponectin decrease with obesity and are positively associated with insulin sensitivity [100-101]. Thus, adiponectin has attracted a wide scientific interest in recent years, and has been extensively studied both in human and animal models, supporting its anti-diabetic, anti-inflammatory, and anti-atherogenic effects.

Skeletal muscle is one of the most relevant peripheral target tissue for adiponectin, as suggested by data showing that adiponectin improves glucose uptake and fatty acid oxidation in C2C12 myocytes [102].

Adiponectin, by binding of adiponectin to its membrane receptors, such as AdipoR1 and AdipoR2, activates downstream signalling, mainly AMPK and the p38 mitogen-activated

protein kinase (MAPK) pathways [103-104], essential for adiponectin-induced beneficial metabolic effects.

In addition, an elegant experiment showed that overexpression of adiponectin, AdipoR1, or AdipoR2 in liver improves insulin sensitivity, while deficiency of adiponectin aggravates insulin resistance. Since impaired insulin sensitivity has been linked with the accumulation of ceramide in skeletal muscle [105], it has been postulated that adiponectin could improve insulin sensitivity by decreasing ceramide concentration or muscular lipid content in obese mice [106].

Previous data have demonstrated that the activation of p38 MAPK and PPAR-α along with AMPK by adiponectin was able to increase fatty acid oxidation in C2C12 skeletal muscle cells [107]. Similarly, the inhibition of the AMPK/p38 MAPK signalling pathway in cardiomyocytes partially abrogates glucose uptake in response to hypoxic stimuli [108]. Indeed, it has recently been confirmed that adiponectin can stimulate fatty acid oxidation in muscles cells also by stimulating PPARα-related signalling via sequential activation of AMPK and p38 MAPK [107]. Indeed, it appears that multiple pathways could mediate adiponectin-mediated metabolic functions in muscles.

On the other side, numerous epidemiological studies support that coronary artery disease and hypertension, left ventricular hypertrophy [110], and a greater risk of myocardial infarction [111] are associated with reduced levels of adiponectin [109].

Thus, several experimental studies promoted the cardioprotective role of adiponectin, employing cell cultures and animal models, in vascular endothelial cells, smooth muscle cells, and cardiac myocytes and adiponectin-deficient mice [112]. The angiogenic properties of adiponectin have been demonstrated in adiponectin-deficient mice in which

adiponectin facilitates revascularization of ischemic limbs [113] and rescues from cerebral ischemia-reperfusion [114].

Additionally, adiponectin supplementation abrogates neointimal thickening in mechanically injured arteries through the inhibition on the proliferative properties of vascular smooth muscle cells [112]. On the high salt diet, adiponectin knock-out mice develop severely high blood pressure due in part to a dysregulation of eNOS AMPK-dependent [115], which is the main vasculoprotective mechanism activated by adiponectin [116-117]. In addition, studies had shown that overexpression of adiponectin protects arties from atherosclerotic plaques formation [118], whereas deficiency of adiponectin results in the higher incidence of atherosclerosis [119].

It is widely recognized that the liver exerts crucial effects on blood glucose homeostasis by keeping an appropriate balance between uptake and storage of glucose [120]. The administration of recombinant adiponectin in both wild type and diabetic mouse models lowers and brings serum glucose almost to normal levels [121-122]. In addition, high doses of adiponectin did not induce hypoglycaemia in mice, thus suggesting that the benenficial effect on glucose metabolism is possibly mediated by controlling gluconeogensis or glycogenolysis [123]. Adiponectin can increase also insulin sensitivity by upregulating PPARα and its target genes in the liver [124]. In addition, adiponectin exposure suppresses glucose output in primary rat hepatocytes [125]. Thiazolidinedione drugs can restore the glycemic status by inducing an increase in the circulating levels of adiponectin in type 2 diabetic patients, adiponectin transgenic models, and knockout mouse models [126-128].

When AMPK is activated by adiponectin, it downregulates lipogenic genes and enhances fat oxidative pathways [129]. In fact, it has been demonstrated that serum adiponectin

concentrations are inversely associated with liver fat content [130], and the link between fat accumulation and adiponectin is supported by studies reporting that mice lacking adiponectin have high fat accumulation even under normal diet [131-132].

The supplementation of adiponectin is able to induce a reduction of mitochondrial lipid peroxidation products [133], which might represent a relevant mechanism in counteracting lipid metabolism alterations.

Adipose tissue has been recently considered as a major regulator of metabolic status [134], as it acts as an endocrine organ and produces numerous bioactive factors such as adipokines, but it also stores energy in the form of lipid and controls lipid metabolism [135].

Adipocytes show high expression of adiponectin, which, through its autocrine activity, regulates their cell differentiation and adipogenesis [136]. The overexpression of adiponectin in ob/ob mice leads to morbid obesity due to decreased energy consumption, associated with improved vascular tone and increase the subcutaneous fat pad in experimental animals. Collectively, chronic overexpresson of adiponectin leads to massive increase in subcutaneous fat, and it protects against diet induced insulin resistance [137-138].

Some studies demonstrated that adiponectin receptors expression is reduced in adipose tissues of insulin-resistant animals, suggesting that adiponectin action is impaired in the insulin-resistant animals due to low adiponectin receptor expression [139-140].

In relation to the effect of adipokines on metabolic alterations, several lines of evidence support a prominent role for leptin. Leptin is widely known for providing primary metabolic signals to the brain, and in particular to the hypothalamus.

Leptin and various other metabolic signals participate in the regulation of appetite, energy homeostasis and body weight by controlling both behaviour and metabolic functions [141]. Leptin is able to induce a decrease in body weight both by suppressing appetite and by increasing energy consumption [142-144]. Leptin deficiency induces a morbid obesity in both animals and humans [145-147]. Similarly, genetic deficiency of functional leptin receptors (LEPR) also determines obesity and obesity-associated metabolic disturbances [147-149].

In addition to the brain, leptin also directly influences multiple peripheral tissues, including pancreatic islets, adipose tissue, skeletal muscle, and liver. Among the leptin receptor (LEPR) gene isoforms, only the longest one, LEPRb, contains a full-length intracellular domain, which is essential for cell signalling [150]. LEPRb mRNA is expressed in pancreatic islets, where it enhances first-phase insulin secretion, modestly improves glucose tolerance in mice and directly stimulates fatty acid oxidation in isolated islets [151-153]. In addition, leptin directly inhibits insulin expression and secretion [154]. In isolated adipocytes, leptin inhibits lipogenesis while enhancing lipolysis and fatty acid oxidation [155]. In skeletal muscles cells, leptin directly promotes fatty acid oxidation, presumably by activating AMPK pathway [156-157]. Leptin decreases lipid levels in isolated livers, and liver-specific overexpression of LEPRb prevents fat accumulation and thus hepatic steatosis in LEPRb-deficient Zucker diabetic fatty (fa/fa) rats [158-159], but the genetic deletion of LEPR in all these peripheral tissues does not ameliorate glucose metabolism and body wieght in mice [160]. It appears, then, that leptin, under normal conditions, acts mainly on the brain.

Adiponectin and leptin in systemic sclerosis and other metabolic diseases

More recently there has been a growing interest in analysing the role of adipokines in systemic sclerosis. It has been reported that in SSc, adiponectin and leptin levels are both decreased compared to healthy controls and they correlate with disease duration [161-163]. In another study, it has been reported that they inversely correlate with modified Rodnan skin score [164], a validated clinimetric method to measure skin fibrosis in SSc and a predictor of mortality.

Still, the results on the circulating levels of leptin remain controversial in SSc [165-166]

Several studies confirm that leptin and adiponectin have opposing effects on subclinical inflammation. Leptin upregulates cytokines such as TNF-α and IL-6 that are associated with insulin resistance in type 2 diabetes and is therefore considered as a proinflammatory cytokine [167]. In contrast, adiponectin downregulates the expression and release of many proinflammatory immune mediators and exerts anti-inflammatory properties. Thorand et al. [168] hypothesized that leptin and adiponectin interact with each other in the modulation of glucose metabolism, but that adiponectin is likely to have a stronger association with the risk of developing diabetes. Other groups also found an inverse relationship between leptin and adiponectin in diabetes patients [169] and in patients with obesity and coronary artery disease [170]. Although leptin or adiponectin were found to be separately associated with the risk of metabolic syndrome, type 2 diabetes and coronary artery disease, the association of type 2 diabetes risk with the adiponectin to leptin ratio was stronger than with leptin or adiponectin alone [171]. These results suggest that the use of a ratio for adiponectin and leptin may be a useful index for insulin resistance in clinical practice and a good indicator for assessing the effectiveness of antidiabetic therapy.

Indeed, an altered ratio between adiponectin and leptin has been observed in diseases characterized by weight alterations, such as anorexia nervosa, having low leptin and high adiponectin levels [172], and obese patients, having high leptin and low adiponectin levels [173].

Serum leptin levels strongly correlate with fat mass and are independently linked with increased risks for myocardial infarction and stroke [174]. In contrast, lower plasma adiponectin levels have been found to predict the development of type 2 diabetes and coronary artery disease [175].

A 25-year prospective study shows that adiponectin to leptin ratio (A/L) is a predictor of metabolic profile change in previously healthy individuals [176].

Thus, there may be possibility of building indices and finding predictive biomarkers for weight loss analyzing adipokines circulating levels.

Aim of the study

Here we aimed at identifying a combined clinical and biomarker based index to stratify patients for the risk of severe malnutrition in the next 12 months, using as end point a consequence of malnutrition (severe weight loss). Such an index could be of invaluable use in stratifying patients at risk of malnutrition for more intense intervention and/or nutritional support, and in clinical research to enrich for patients at risk of severe malnutrition in clinical trials that target GI function in this systemic disease.

MATERIALS AND METHODS

Design

International, multicentre, longitudinal, observational, parallel cohort study.

Study subjects

The study cohort comprised 110 consecutive patients with systemic sclerosis, diagnosed according to the EULAR/ACR 2013 criteria [177], and referring to the outpatient rheumatology service of the University Hospital of Messina (n=60) and Padova (n=50). An additional validation cohort of 70 SSc consecutive patients has been enrolled from the outpatient rheumatology service of the University of Leeds.

Every patient underwent clinical history and physical examination, providing sociodemographic information (age, sex, ethnicity and level of education).

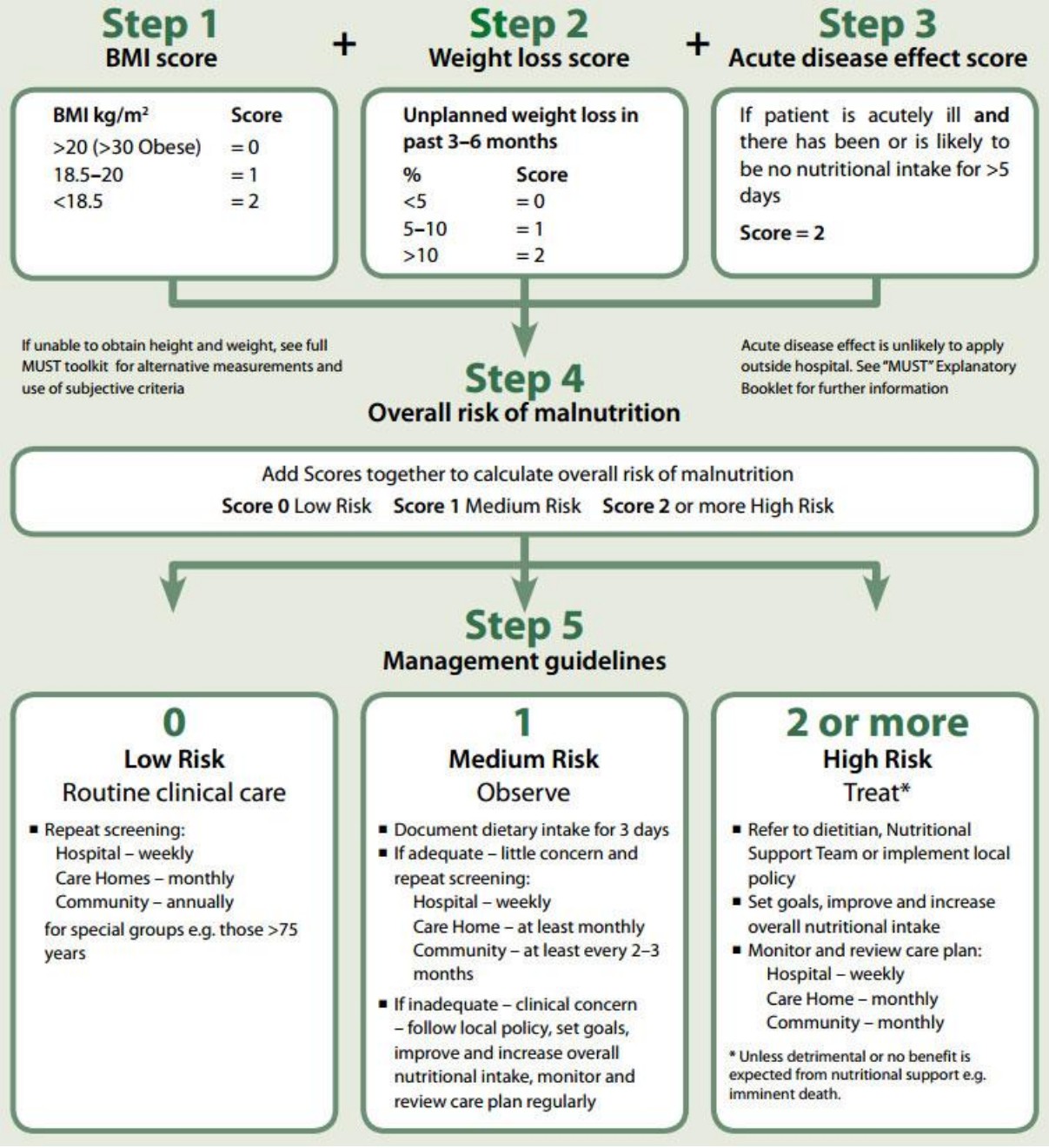
This observational study was conducted following approval by the local institutional review boards of the University of Messina (prot n°15-15) and Padova (prot 2015-32) and NHS REC Approval number (STRIKE REC) in Leeds.

Malnutrition assessment

The malnutrition universal screening tool (MUST) assigns the following scores to body mass index (BMI): > 20.0 = 0, 18.5–20.1 = 1, < 18.5 = 2. A weight loss score (unplanned weight loss in the past 3–6 months) is assigned as follows: < 5% = 0; 5%–10% = 1: >10% = 2. The MUST also adds a score of 2 if there has been or is likely to be no nutritional intake for the next 5 days or more. The scores for BMI and weight loss are summed for the total score, which is interpreted as follows: risks for malnutrition are low for MUST

score of 0, moderate for MUST score=1, and high for MUST score ≥2. Measurements of height and weight are performed at the time of the visit using standard equipment available at the sites. Weight loss was based on recall by the patient and referred to the previous year rather than the previous 3–6 months. Weight loss ≥10% was calculated after 12 months [73] (Figure 1).

Figure 1



Assessment of circulating adipokines levels

Adiponectin and leptin were measured using de-identified bar-coded serum samples, in duplicate. All the tested compounds have been quantitatively measured using an enzyme-linked immunosorbent assay kit (Abcam ltd).

Study end points and outcome measures.

Disease duration was calculated from the onset the first non-RP symptom for patients. Skin was assessed using the modified Rodnan skin score (mRSS) in order to classify patients according to limited or diffuse form of disease [178].

With regard to pulmonary function, forced vital capacity (FVC), total lung capacity (TLC) and diffusing lung capacity of carbon monoxide (DLCO) were analysed, as previously described [179]. Interstitial lung disease (ILD) was defined by DLCO and FVC less than 80% of the predicted value plus bibasilar fibrosis on High resolution CT (HRCT). The presence of basilar crackles at auscultation was also evaluated for each patient.

Pulmonary arterial hypertension was assessed by Doppler echocardiogram by measuring pulmonary artery pressure (PAP_{syst}). Patients with a PAP_{syst} greater than 40 mm Hg underwent right heart catheterisation to confirm the presence of pulmonary artery hypertension (PAH) [180-181].

Primary outcome was to assess a weight loss ≥10% after 12 months.

Statistical Analysis

Results are expressed as the mean ± standard deviation, 95% confidence intervals, median with interquartile range for quantitative variables, and relative frequencies and 95% confidence intervals for qualitative variables. The analysis was conducted on study cohort, results were then locked and validated on second cohort.

The normal distribution of each variables was assessed using the Shapiro-Wilk test. The statistical analysis was performed using the ANOVA for repeated measures for parametric variables or through the Friedman test for non-parametric variables. Contingency tables were used for organizing categorical variables and the chi-squared test to verify the hypothesis. Linear regression analysis or multiple regression analysis was used accordingly.

Comparison between percentages have been performed according to the "N-1" Chi-squared test for comparison between percentages as recommended by Campbell [182-183].

To assess predictive ability receiver operating characteristic (ROC) curves were constructed using library (pROC) [184]. The area under the receiver operating characteristic curve (AUC) was estimated and a 95% CI determined using bootstrap resamples. AUCs were compared using a bootstrap significance test with the significance of differences between bootstrap AUCs assessed using a normal approximation. Data-derived optimum cut-points were selected by optimising the sensitivity. Analysis was undertaken in the R environment for statistical computing [185].

RESULTS

Clinical features of patients, cohort determination and prevalence of organ involvement

One hundred eighty consecutive SSc patients, separated in two distinct cohorts, participated in the study. Clinical summary of the SSc patients is shown in table 1.

No statistical differences were observed between study and validation cohorts, apart from a lower frequency of ACA in the study cohort, as already reported [186].

In the study cohort, 34% of SSc patients belonged to the diffuse cutaneous subset form (vs 35% in the validation cohort). Mean age was 56.7 ± 10.9 (vs 56.1 ± 11.1 in the validation cohort) and 87% of patients were female (vs 84% in the validation cohort).

The most frequent GI disturbance was gastroesophageal reflux (GERD), being present in 75% of SSc patients in study cohort and in 74% of the validation cohort, followed by gastritis (22% in both cohorts) and hiatal hernia (19% and 25% respectively). Pulmonary fibrosis was observed in 34% and pulmonary arterial hypertension (PAH) in 8% of study cohort, while in the validation cohort 31% of the patients had pulmonary fibrosis and 7% PAH.

TABLE 1. Epidemiological and clinical features of the SSc patients

	STUDY COHORT	VALIDATION COHORT	*p*
Total group, no.	110	70	
Disease subset, D/L, no. (%)	37 (34) / 73 (66)	25 (35) / 45 (65)	0.89
Age, mean ± SD years	56.7 ± 10.9	56.1 ± 11.1	0.72
Women, no. (%)	97 (87)	59 (84)	0.57
Disease duration (onset of RP symptoms to baseline visit), mean ± SD years	15.2 ± 10.6	16.5 ± 12.7	0.45
Disease duration (onset of non-RP symptoms to baseline visit), mean ± SD years	11.7 ± 9.4	11.1 ± 8.4	0.64
mRSS for diffuse form, mean ± SD	16.2 ± 6.6	15.7 ± 7.4	0.68
mRSS for limited form, mean ± SD	4.8 ± 3.5	4.2 ± 3.9	0.57
Pulmonary fibrosis, no. (%)	38 (34)	22 (31)	0.67
TLC % predicted, mean ± SD	82 ± 20	83 ± 24	0.76
DLco % predicted, mean ± SD	69 ± 22	66 ± 15	0.31
FVC % predicted, mean ± SD	95 ± 20	99 ± 23	0.21
Pulmonary Arterial Hypertension, no. (%)	9 (8)	5 (7)	0.80
CK, mean ± SD	141 ± 51.4	130 ± 40.7	0.13
ANA+, %	100	100	1
ACA+, %	23	38	0.03
Scl70+, %	20	17	0.61
RNA III+, %	10	9	0.82
PM-Scl, %	4	5	0.75
Immunosuppressors, no. (%)	42 (38)	24 (34)	0.58
Corticosteroids, no. (%)	30 (27)	12 (17)	0.12
GERD, no. (%)	83 (75)	52 (74)	0.88
Hiatal hernia, no. (%)	21 (19)	18 (25)	0.33
Gastritis, no. (%)	24 (22)	16 (22)	1
Costipation, no. (%)	12 (11)	7 (10)	0.83
Diarrhea, no. (%)	8 (7)	4 (6)	0.79
Esophagitis, no. (%)	9 (8)	6 (8)	1
Barrett's esophagus, no. (%)	6 (5)	5 (7)	0.57
Proctite, no. (%)	4 (4)	2 (3)	0.72
PPI, no. (%)	85 (77)	50 (71)	0.36
Prokinetics, no. (%)	40 (36)	18 (25)	0.12
Antacids, no. (%)	24 (22)	17 (24)	0.75

ACA, anticentromere antibodies; ANA, antinuclear antibodies; D, diffuse cutaneous SSc; Dlco, diffusion capacity for carbon monoxide; GERD, Gastroesophageal reflux disease; FVC, forced vital capacity; L, limited cutaneous SSc; mRSS, modified Rodnan skin score; PM-Scl, anti-PM-Scl antibodies; PPI, proton pump inhibtors; RNA III, anti-RNA polymerase III antibodies; RP, Raynaud's Phenomenon; Scl70, anti-topoisomerase I antibodies; SSc, systemic sclerosis; TLC, total lung capacity;

The performance of MUST in predicting 10% weight loss after 12 months is poor

First, we wanted to assess whether the actual instrument used to assess malnutrition, MUST, could prove useful as a predictor of ≥10% weight loss after 12 months in the study cohort. 23 SSc patients among 110 enrolled in the study cohort (21%) met the end point of ≥10% after 12 months. The performance of MUST was poor (AUC 0.7; 95% CI: 0.59-0.82). Indeed, 33% of SSc patients having a none to moderate (0-1) MUST score at baseline, experienced ≥10% weight loss within 12 months (Figure 2).

Comparison of baseline outcomes between cohorts and their change after 12 months.

As outcomes of interest, BMI, MUST, adiponectin and leptin serum levels were evaluated at baseline and reassessed after 12 months. No statistical differences were observed in the outcomes of interests between study cohort and validation cohort at baseline, also when analysed according to age or gender. Table 2 shows baseline and 12 months values of each outcomes of interest for both study and validation cohort. After 12 months, BMI decreased over time ($p<0.001$) while MUST increased ($p=0.006$) in both cohorts.

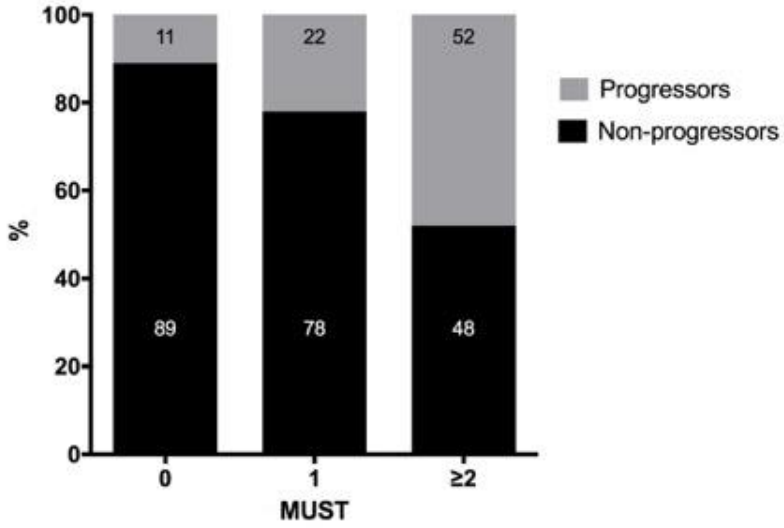
In addition, the percentage of overweight/obese patients (BMI ≥ 25 kg/m^2) decreased from 35% to 29% in the study cohort and from 30% to 26% in the validation cohort, while patients with normal weight (BMI >18.6<24.9 kg/m^2) increased from 61% to 63% in the study cohort and from 64% to 67% in the validation cohort. Similarly, the number of patients underweight (BMI <18.5 kg/m^2) increased by 4% in the study cohort and by 2% in the validation cohort after 12 months.

Figure 2.

A

	n	%
Progressors	23	21
Non-progressors	87	79

B



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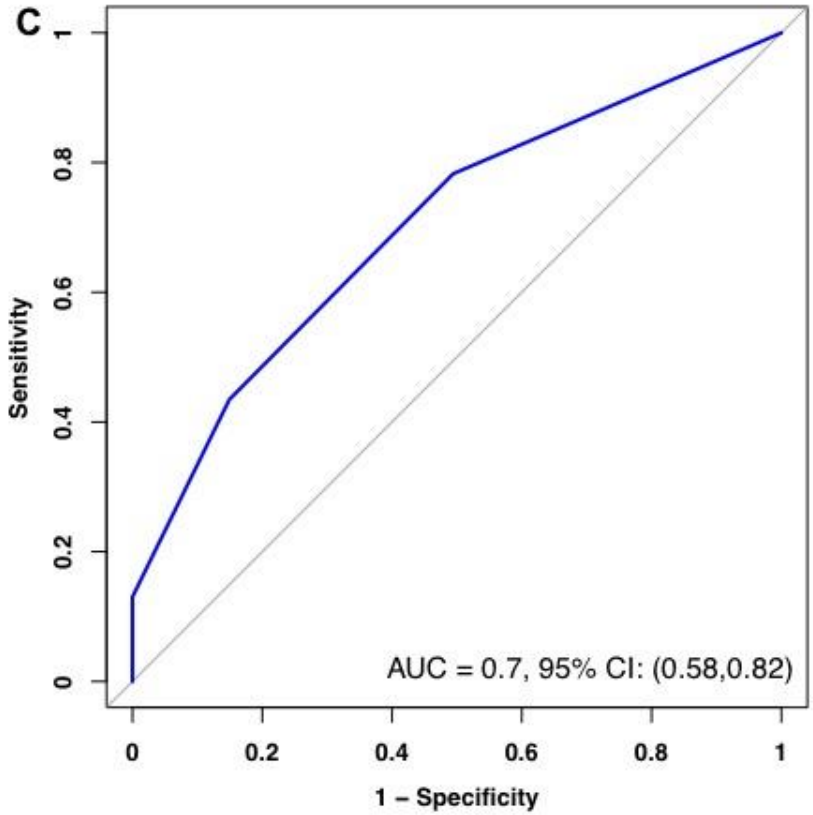


Figure 2 A-C. Performance of Malnutrition Universal Screening Tool (MUST) for the prediction of weight loss. In the study cohort, 21% of patients (n=23, panel **A**) lost ≥10% weight after 12 months (progressors) and 33% of them had a low to moderate MUST score (panel **B**). Of note, 33% of patients having a low to moderate MUST score will have ≥10% weight loss after 12 months and, on the other side, 48% of patients having a high risk of malnutrition (MUST≥2) will not have ≥10% weight loss after 12 months. Panel **C** shows the poor performance of MUST in predicting ≥10% weight loss after 12 months in the study cohort.

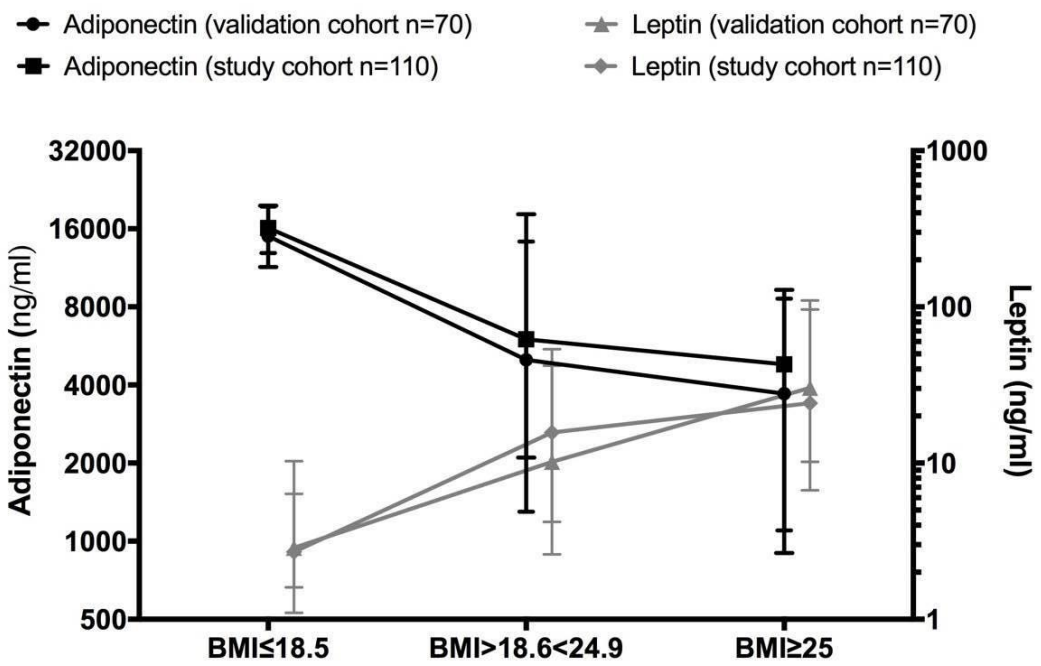
Table 2.						
	STUDY COHORT (n=110)		p	VALIDATION COHORT (n=70)		p
	BASELINE	12 months		BASELINE	12 months	
BMI, median (range)	23.5 (17.5 - 36.5)	22.3 (16.7 - 37.1)	<0.001	23.4 (17.3 - 38)	22.9 (17 - 38.1)	<0.001
BMI <18.5 kg/m^2, no. (%)	5 (4)	8 (8)	0,65	4 (5)	5 (7)	0,73
BMI >18.6<24.9 kg/m^2, no. (%)	67 (61)	69 (63)	0,78	45 (64)	47 (67)	0,71
BMI ≥ 25 kg/m^2, no. (%)	38 (35)	33 (29)	0,34	21 (30)	18 (26)	0,59
MUST, median (range)	1 (0 - 3)	1 (0 - 4)	0,006	1 (0 - 4)	1 (0 - 4)	0,006
MUST = 0	49 (44)	36 (32)	0,06	32 (46)	22 (31)	0,06
MUST = 1	40 (36)	44 (40)	0,54	25 (36)	25 (36)	1
MUST ≥ 2	23 (21)	41 (37)	0,009	13 (17)	23 (33)	0,03
BMI: body mass index; MUST: malnutrition universal screening tool;						

Association between BMI and adipokines

Next we sought to assess whether serum levels of both adiponectin and leptin were comparable between study and validation cohort. After 12 months, in both cohorts, serum levels of leptin significantly decreased ($p<0.001$) while adiponectin and consistently adiponectin to leptin ratio (A/L) increased ($p<0.001$ for both). When classified according to BMI, there was no difference between cohorts both in adiponectin (study cohort, n=5, median=16.1 vs validation cohort, n=4, median=15.1, p=0.82) and in leptin concentrations (study cohort, n=5, median=2.7 vs validation cohort, n=4, median=2.87, p=0.9) for patients underweight (BMI <18.5 kg/m^2). Also among patients with normal weight and overweight there were no differences in adiponectin and leptin serum levels between study and validation cohort. The trend of both adiponectin and leptin for each cohort is shown in Figure 3. Next we observed that adiponectin, leptin and therefore A/L significantly correlated with BMI in the entire group of patients and the correlation remained significantly also after dividing SSc patients according to BMI (Figure 4).

Figure 3.

A

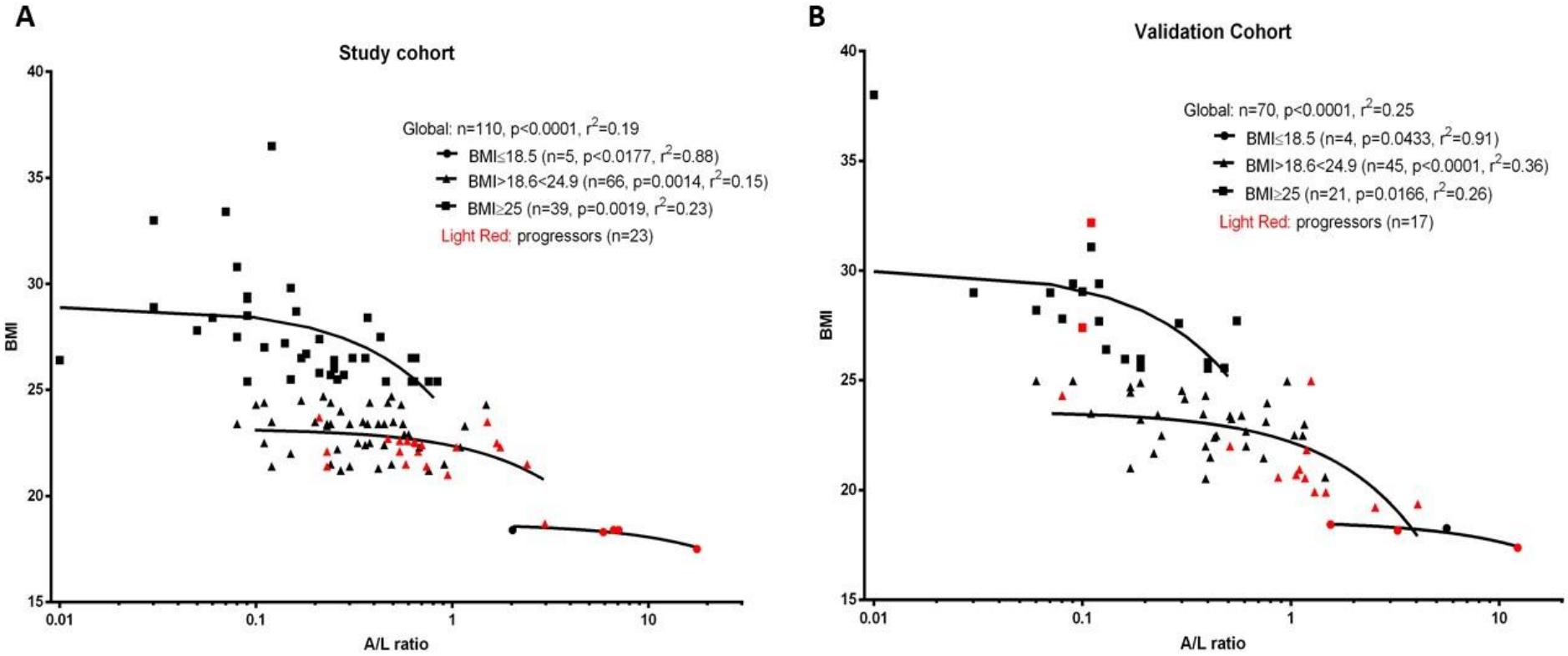


B

	study cohort (n=110)		*p*	validation cohort (n=70)		*p*
	BASELINE	12 months		BASELINE	12 months	
Adiponectin (ug/ml), median (range)	5.72 (0.6 - 19.4)	7.65 (0.05 - 22)	<0.001	5.55 (1.1 - 19.6)	6.65 (1.9 - 23)	<0.001
Leptin (ng/ml), median (range)	17.6 (1.1 - 96.4)	14.78 (0.7 - 92.3)	<0.001	15.35 (1.6 - 110)	12.5 (0.28 - 105)	<0.001
A/L, median (range)	0.36 (0.01 - 17.7)	0.56 (0.003 - 26.3)	<0.001	0.39 (0.01 - 12.25)	0.5 (0.02 - 82.14)	<0.001

Figure 3 A-B. The upper figure (A) shows that adiponectin (plotted on left ordinate as log 2) and leptin (plotted on the right ordinate as log10) have opposite trend (data shown as median ± SE) when grouped by BMI classification in underweight (BMI <18.5 kg/m2), normal weight (BMI >18.6<24.9 kg/m2) and overweight/obese (BMI ≥ 25 kg/m2) both in the study and in the validation cohort. The median with the upper and lower limit for both adiponectin, leptin and their ratio (A/L) are reported in the lower figure of the panel according to each cohort. Leptin significantly decreases while adiponectin and accordingly A/L increase after 12 months in both cohorts (B).

Figure 4.



- **Figure 4 A-C**. Linear regression analysis shows the association between BMI and A/L (A), for study cohort (**A**) and validation cohort (**B**). A significant direct correlation was observed between A/L and BMI in both cohorts. Red symbols show progressors (patients with ≥10% weight loss after 12 months).

Association between MUST scores and adipokines

Next we assessed whether MUST scores were associated with adiponectin and leptin serum concentration and in particular whether there was any difference according to the risk stratification system based on MUST. MUST scores were significantly associated with adiponectin (r^2=0.10, p<0.0001) and A/L (p<0.0001, r^2=0.21), while no association was observed with leptin alone. In addition, A/L scores were higher in SSc patients with MUST scores ≥2, corresponding to a moderate to severe risk of malnutrition, compared to those having a MUST=0 (p<0.05, data not shown).

The performance of the PREdictor of Malnutrition in Systemic Sclerosis (PREMASS) index

Considering ≥10% weight loss at 12 months our primary outcome, we built receiver operating characteristic (ROC) curves for combined indexes between BMI and A/L, MUST and A/L, and A/L alone in the study cohort. BMI+A/L had a better AUC (0.91; 95% CI: 0.86-0.97) vs MUST+A/L (0.87; 95% CI: 0.78-0.96) or A/L alone (0.85; 95% CI: 0.76-0.94). Therefore, we defined the predictor of malnutrition in systemic sclerosis (PREMASS) with the formula: 10% future weight loss ~ 12.18 - (0.63*BMI @ baseline) + (1.51 * A/L @ baseline). A PREMASS score >0.23 showed in the study cohort a 91.3% [95% CI:79.79-100] sensitivity and 80.46% specificity (95% CI: 72.13-88.79) for ≥10% weight loss with an overall 55.26 % positive predictive value (95% CI: 39.45-71.07) and 97.22% negative predictive value (95% CI: 93.43-100) and a relative risk of 19.90 (95% CI:4.93-80.37).

In the validation cohort, PREMASS showed 76.47% sensitivity (95% CI:56.31-96.63) and 75.47% specificity (95% CI:63.89-87.06) with an overall 50% PPV (95% CI:30.78-69.22), 90.91% NPV (95% CI:82.41-99.4) and a RR of 5.5 (95% CI: 2-15.10).

Clinical features of SSc patients with weight loss

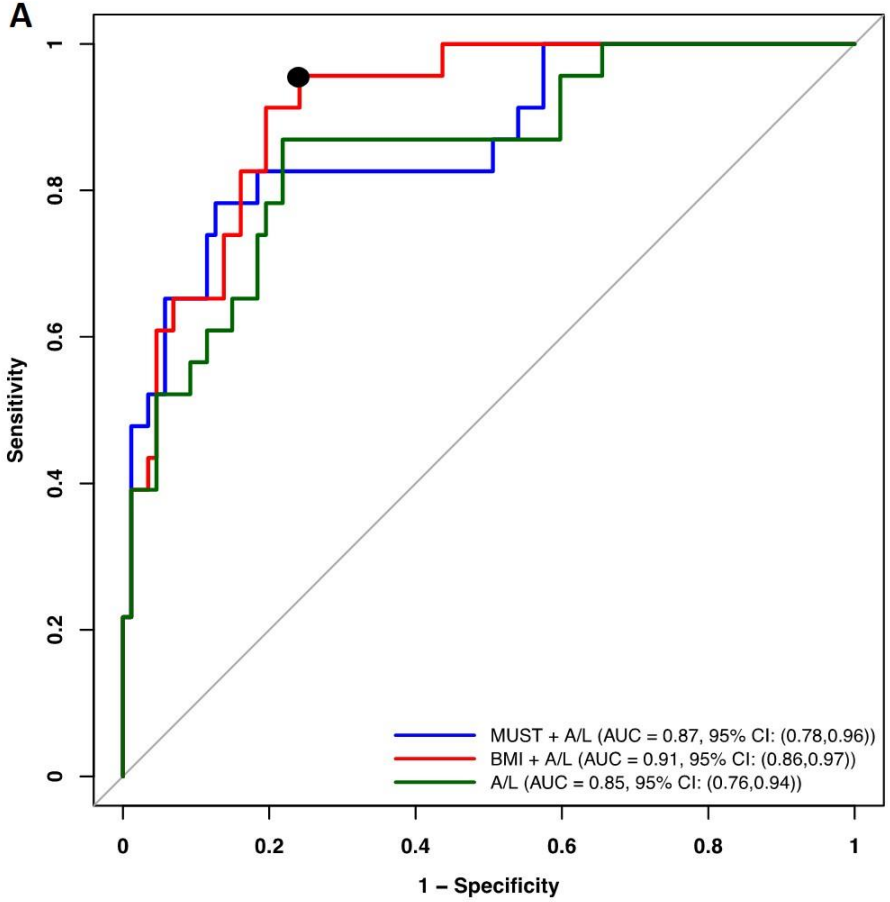
After having validated the PREMASS score, we analyzed the profile of patients with ≥10% weight loss after 12 months (23/110, 21% in the study cohort and 17/70, 24% in the validation cohort; 40/180; 22% in total) to identify other discriminants that could help identify those at higher risk of experiencing severe weight loss.

Compared to those not experiencing a clinically relevant weight loss, SSc patients with ≥10% weight loss after 12 months were older (median 63 vs 55, p=0.02), more frequently with the diffuse form (48% vs 31%, p=0.05), Scl70 positive (32% vs 17%, p=0.04), had higher modified Rodnan skin scores (median 10 vs 6, p=0.01), and HRCT evidence of pulmonary fibrosis (55% vs 30%, p=0.003), with concordant lower TLC (mean: 76.7 vs 93.7, p<0.001), and lower FVC (mean: 74.6 vs 83.5, p=0.04). No differences were observed in age, gender, disease duration, CK serum levels or presence of PAH.

In addition, SSc patients with ≥10% weight loss had a higher A/L ratio (2.34 vs 0.37, p<0.0001), and MUST scores (1.54 vs 0.57, p<0.0001), and lower BMI (21.4 vs 24.9, p<0.0001).

Figure 5.

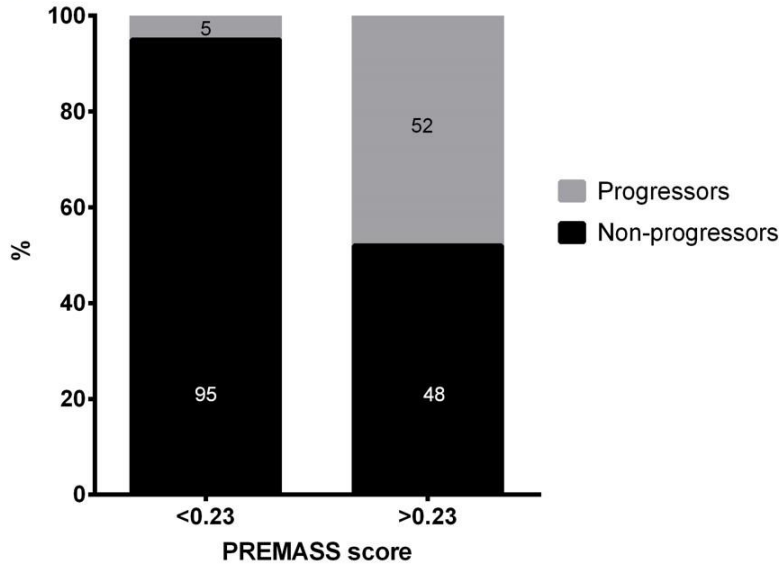
A



B

	STUDY COHORT	VALIDATION COHORT
AUC [95% CI]	0.91 [0.86-0.97]	0.82 [0.68-0.97]
Sensitivity [95% CI]	91.3 [79.79, 100]	76.47 [56.31, 96.63]
Specificity [95% CI]	80.46 [72.13, 88.79]	75.47 [63.89, 87.06]
PPV [95% CI]	55.26 [39.45, 71.07]	50 [30.78, 69.22]
NPV [95% CI]	97.22 [93.43, 100]	90.91 [82.41, 99.4]
RR [95% CI]	19.90 [4.93, 80.37]	5.50 [2.00, 15.10]

C



- **Fig. 5 A-C.** Receiver operating characteristics (ROC) curve (A) demonstrates that the best performance in predicting ≥10% weight loss after 12 months in the study cohort was represented by combined index based on BMI+A/L (red line) compared to MUST+A/L or A/L alone. The black circle corresponds to the point optimized for sensitivity and specificity. Using the formula ≥10% future weight loss= 12.18 - (0.63*BMI) + (1.51*A/L), we validated the PREdictor of MAlnutrition in Systemic Sclerosis (PREMASS) index as shown in the panel B. The number of progressors and non-progressors (C) are shown according to the cut-off (0.23) optimized for sensitivity and specificity for both cohorts.

DISCUSSION

In SSc, malnutrition is common, clinically relevant and not easily assessed by simply measuring BMI. In a recent study, employing bioelectrical impedance analysis to analyze the nutritional status in 124 consecutive patients with SSc, those having malnutrition had a higher risk for SSc-related mortality [71]. Several other lines of evidence support that severe malnutrition is an independent risk factor for mortality in SSc [69-70, 72].

In SSc, malnutrition can derive from different factors ranging from gastrointestinal manifestations, which are present in approximately 90% of patients, mood disorders, functional influences and the involvement of internal organs [187].

Our longitudinal data suggest that the percentage of SSc patients with a relevant weight loss (≥10%) after 1 year ranges between 21% and 24%, according to both study and validation cohorts. In our cohorts, we observed that patients with a clinically relevant weight loss are more frequently affected by the diffuse form and have a more severe pulmonary restrictive pattern associated with pulmonary fibrosis, as already confirmed by previous data [41]. However, we could not find any difference according to disease duration, and this could be due to the different selection criteria of the participants, being outpatients in our case and thus not exclusively very severe compared to the cohorts of other studies [41]. In addition, we found that anti-Scl70 positivity is another feature of SSc patients with weight loss, further defining a subset of patients prone to develop it.

Nonetheless, the characteristics of malnutrition remain poorly defined and the investigation of applicable predictors is needed to enable early detection and correction.

First, we started analyzed the performance of MUST in predicting ≥10% weight loss after 12 months, since it represents the most widely used instrument in SSc for the assessment

of the risk of malnutrition [41-70]. The performance of MUST is poor and, furthermore, it is not able to detect up to 33% of patients with actual relevant weight loss at 12 months, and this could be possibly attributed to the lack of validity of the subdomain of MUST that uses patients recall of weight loss.

A previous attempt to identify a biomarker of malnutrition in SSc patients was made with serum albumin, based on it is use in clinical practice for malnutrition for other diseases, but the results were disappointing [188]. Furthermore, although a recent study demonstrated that serum prealbumin was an independent predictor of mortality in SSc, no longitudinal assessment of weight loss was performed thus providing no evidence for its role in the prediction of weight loss [189]

Therefore, we focused our attention on two major adipocyte-derived hormones, adiponectin and leptin, since they play a central role in weight control [103-104, 142-144]. Although several reports have been published on the role of these two adipokines in skin and lung fibrosis and disease activity in SSc [161-166], this is to our knowledge the first study analysing their performance for the risk of weight loss in SSc in a longitudinal study. Indeed, an interesting study by Marangoni and colleagues suggests that a decline in the number of adipocytes in SSc could be considered a pathogenic mechanism contributing to the transition toward a myofibroblast profibrotic phenotype [190]. White adipose tissue exerts a critical effect on metabolism as already demonstrated in obesity; however, adipose tissue dysfunction in other pathologic conditions remains not fully defined. The loss of the subcutaneous white adipose tissue is a widely recognized feature of SSc patients and in animal models of skin fibrosis [191-192]. Thus, it could be postulated that the loss of adipose tissue in SSc could lead to dysregulated adipokynes secretion and function.

Indeed, weight loss significantly elevates plasma adiponectin levels, while the reduction of adiponectin has been associated with insulin resistance, dyslipidemia, and atherosclerosis in humans [193]. On the other hand, serum leptin concentrations massively fall in response to changes in fat mass, suggesting its important role in the events that regulate weight loss [194]. Indeed, we noted that both adiponectin and leptin are inversely associated according to BMI in SSc patients, as already observed in other metabolic diseases [168-173].

In addition, the performance of our PREMASS index (cutoff ≥0.23), based on BMI and A/L, is superior to MUST or BMI or A/L alone in predicting a ≥10% weight loss in SSc patients.

Notably, it has been reported that serum adiponectin increases in patients affected by systemic sclerosis and interstitial lung disease after the administration of cyclophosphomide, and it correlates with the improvement in ILD scores. Adiponectin levels could thus allow to identify a subset of patients prone to be resistant to cyclophosphamide therapy and at risk of exacerbations [195].

In view of our results, we could speculate that the reported increase in serum adiponectin levels after the exposure to cyclophosphamide in SSc patients could represent instead a sign of systemic, and more specifically adipose tissue, toxicity. This marker could be useful in identifying patients with a higher risk of future malnutrition after immunotherapy or possibly allow to taper the dose of immunosuppressants according to adiponectin response in order to avoid future weight loss and subsequent increase in the risk of mortality.

The performance of PREMASS index slightly decreases in the validation cohort, since the values of the AUC of the study cohort (0.91 – excellent) are higher compared to those observed in the validation cohort (0.82 – good), although they still remain acceptable and higher than the performance of MUST.

However, it is important to note that among SSc patients belonging to the validation cohort and having a PREMASS score lower than our cutoff (0.23), 3 out of 4 had a baseline BMI ≥25, being then in the overweight/obese cluster. In clinical practice, we could admit that the PREMASS index could be more useful when applied to patients in the normal weight/underweight groups as a ≥10% weight loss becomes more relevant for the risk assessment of malnutrition in this latter group.

CONCLUSIONS

A severe weight loss is frequent in SSc patients and specific clinical features define a subset of patients more susceptible to it. The PREMASS index, based on BMI and A/L, predicts ≥10% weight loss in SSc, it is easily reproducible and its use for malnutrition assessment in clinical practice might allow an appropriate identification of SSc patients not currently detected with other screening tools. Since the measurement of relevant weight loss through PREMASS index is easy to perform, it could be introduced as an outcome for future randomized clinical trial, thus including malnutrition, being a relevant risk factor for mortality, in the complex range of manifestations of SSc in the assessment of drug efficacy.

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