UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
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NUTRITIONAL STATUS IN SYSTEMIC SCLEROSIS

Doctoral thesis abstract

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2019

Keywords: *systemic sclerosis, nutritional status, diet, malnutrition, weight loss, autoantibodies, capillaroscopy.*

The doctoral thesis includes:

- 131 pages - of which 40 pages contitute the General Part;
- 70 figures - of which 55 in the Personal Part;
- 51 tables - of which 40 in the Personal Part;
- 706 references;
- 5 annexes (EPIC-Norfolk food frequency questionnaire, a list of the published articles as well as the 3 articles *in extenso*).

In the present abstract, the contents, the numbering of the selected figures and the list of abbreviations are kept in the same form as in the doctoral thesis.

CONTENTS

LIST OF ABBREVIATIONS

95% IC	Interval de confidenţă 95%
AAN	Anticorpi antinucleari
ACR	*eng. American College of Rheumatology*
ADA	*eng. American Dietetic Association*
AGMN	Acizi graşi mononesaturaţi
AGPN	Acizi graşi polinesaturaţi
AGS	Acizi graşi saturaţi
ANOVA	*eng. ANalysis Of VAriance*
ASPEN	Societatea Americană de Nutriţie Enterală şi Parenterală
BAPEN	*eng. British Association for Parenteral and Enteral Nutrition*
BIA	*eng. Bioelectrical Impedance Analysis*
CA	Circumferinţa abdominală
CD	Celule dendritice
CPA	Celule prezentatoare de antigen
CSURI	*eng. Capillaroscopy Skin Ulcers Risk Index*
DM	Dermatomiozită
DXA	Absorbţiometrie dual cu raze X
ECRPC	*eng. European Palliative Care Research Collaborative*
ESPEN	Societatea Europeană de Nutriţie Enterală şi Parenterală
EULAR	*eng. European League Against Rheumatism*
EWGSOP	*eng. European Working Group on Sarcopenia in Older People*
FFQ	*eng. Food Frequency Questionnaire*
FR	Factor reumatoid
G	Greutate
Hg	Hemoglobină
Ht	Hematocrit
PAH	Hipertensiune arteriala pulmonară
IAF	Indice abdomino-fesier
IFN	Interferon
IL	Interleukină
IMC	Indice de masă corporală
IQR	Interval intercuartilic
IRM	Imagistică prin rezonanţă magnetică
LES	Lupus eitematos sistemic
MAG	*eng. Malnutrition Advisory Group*
MAR	Masa adipoasă relativă
MNA	*eng. Mini Nutritional Assessment*
MNA-SF	*eng. Mini Nutritional Assessment-Short Form*
MST	*eng. Malnutrition Screening Tool*
MUST	*eng.* Malnutrition Universal Screening Tool
NOURISH	*eng. Nutrition effect On Unplanned Readmissions and Survival în Hospitalized patients*
NRS-2002	*eng. Nutritional Risk Screening-2002*
OMS	Organizaţia Mondială a Sănătăţii
CRP	Proteina C reactivă
PM	Polimiozită
PPARγ	*eng. Peroxisome Proliferator-Activated Receptor gamma*

PR	Poliartrita reumatoidă
RSMI	*eng. Relative Skeletal Muscle Index*
QCT	Tomografie computerizată cantitativă
RR	Risc relativ
SCRINIO	*eng. SCReening the Nutritional status In Oncology*
SNP	*eng. Single Nucleotide Polymorphism*
SRM	Spectroscopie prin rezonanţă magnetică
SS	Sclerodermie sistemică
DcSS	Sclerodermie sistemică forma cutanat difuză
LcSS	Sclerodermie sistemică forma cutanat limitată
T	Talie
TGFβ	Transforming Growth Factor beta
Th	Limfocite T helper
TLR	*eng. Toll-Like Receptor*
TNFα	Factorul de necroză tumorală alfa
ESR	Viteza de sedimentare a hematiilor
ZPV	Zona perivasculară

INTRODUCTION

Systemic sclerosis (SS) is a rare autoimmune disease associated with a mortality risk up to 3 times higher than the general population. The severity of the disease derives mainly from its characteristic plurivisceral involvement. SS patients demonstrate an abundance of risk factors for malnutrition and cachexia including chronic inflammation, the involvement of the entire digestive tract, an unbalanced diet, and the absence of standardized recommendations for the screening and management nutritional decline. A small number of papers published to date address the problem of nutritional status abnormalities in scleroderma, although the association between malnutrition and the significant increase in mortality risk has been demonstrated.

Digestive symptoms (present in up to 90% of patients with SS) often exhibit important relationships with body composition abnormalities and diet. With respect to other types of clinical and paraclinical changes, the connection with weight loss or malnutrition risk in scleroderma patients has seldom been described in literature.

The evaluation of SS patients through a nutritional perspective is rarely addressed, offering the opportunity to highlight the relationships between nutritional status - digestive symptoms/visceral involvement - weight loss/risk of malnutrition.

The present research aimed to investigate the nutritional status of scleroderma patients, focusing on the relationships between: 1. diet and body composition/malnutrition risk; 2. diet and digestive symptoms; 3. organ damage and body composition/risk of malnutrition; 4. body composition/weight loss/malnutrition risk and certain paraclinical findings.

PERSONAL PART

Chapter 3. Nutritional status, clinical and paraclinical characteristics in systemic sclerosis: a prospective study (group A)

3.1. Motivation and objectives

Scleroderma is often associated with a decline in body composition and a high risk of malnutrition. The factors that promote weight loss in these patients include digestive involvement and disease-related complications, diet and food-related behavior currently playing an uncertain role.

The main objective of the present research was to identify and describe the risk factors (both modifiable and non-modifiable) for malnutrition, weight loss or alteration of body composition in patients with systemic sclerosis.

3.2. Materials and methods

We conducted a prospective observational study in which we included adult patients with systemic scleroderma (SS) treated in the Ist Rheumatology Clinic of the Clinical Rehabilitation Hospital Iasi. The study protocol was approved by the local Ethics

Committees on Research (University of Medicine and Pharmacy "Grigore T Popa", Clinical Rehabilitation Hospital).

The inclusion criteria were the following: age over 18 years, positive diagnosis (ACR 1980 or the ACR/EULAR 2013 classification criteria), written consent to participate. We excluded persons under the age of 18, patients who did not meet the criteria for diagnosis or classification, persons with intellectual disabilities and patients who refused to participate.

The general data collected included gender, age and clinical phenotype (diffuse cutaneous - dcSS, and limited cutaneous - lcSS respectively). The clinical evaluation involved the calculation of the modified Rodnan score (mRSS) and the presence of digital ulcers (present/absent).

The anthropometric evaluation allowed the analysis of the following parameters: height (H): measured in cm, body mass/weight (W): measured in kg, abdominal circumference (AC): measured in cm, hip circumference (HS): measured in cm.

The anthropometric indices which derived from the aforementioned parameters were as follows:

- Body mass index (BMI);
- Waist/hip ratio (WHR);
- Relative fat mass (RFM) calculated according to the formulas:

Women: RFMf = 76 - (20 X T / CA) (obesity defined as MAR ≥ 33.9);

Men: RFMm = 64 - (20 X T / CA) (obesity defined as MAR ≥ 22.8);

or

RFM = 64 - (20 X T / CA) + (12 X gender) (where 0 corresponds to the male gender and 1 to the female gender).

We investigated the patients' diets using the EPIC-Norfolk food frequency questionnaire (translated, adapted and validated through a pilot study). The questionnaire included 130 items (food, alcoholic and non-alcoholic beverages) and evaluated the patients' diets over a 12-month period (the year preceding the study). The resulting data were included in a Microsoft Excel database and further processed using FETA version 2.53.

The energy requirement (the average daily intake recommended for each patient in kcal/day) was calculated as follows:

Women: Recommended energy intake (kcal/day) = 2403 - 7 X (age - 19);

Men: Recommended energy intake (kcal/day) = 3067 - 10 X (age - 19).

We compared the intake of vitamin D with the recommended amount of 5 mcg/day for people between 19-50 years of age, 10 mcg/day for people between 51-70, and 10 mcg/day for patients over 70 years.

We collected biological samples (venous blood) to evaluate vitamin D titers (ng/ml) by ENzyme-Linked ImmunnoAssay (25OH Vitamin D Total ELISA 90 ', DIAsource ®ImmunoAssays, Nivelles, Belgium; optimal values ≥ 30 ng/ml, insufficiency 10 - 30 ng/ml; deficiency ≤ 10 ng/ml) and serum albumin by the spectrophotometric method (Albumin Bromocresol Green, BioSystems®, Barcelona, Spain; normal values: 34 mg/l for patients over 60 years, 35 mg/l for those under 60).

We estimated the risk of malnutrition using the Malnutrition Universal Screening Tool (MUST).

The statistical analysis was performed using IBM SPSS Statistics version 20 for Microsoft Windows.

3.3.Rezults

3.3.1. General characteristics of the study group

The group consisted of patients aged between 25 and 83 years. According to the LeRoy classification, 24 patients (57.1%) had the limited cutaneous form of the disease, while 18 (42.9%) had the diffuse phenotype.

3.3.2. Rodnan score and digital ulcers

The percentage of patients with Rodnan ≥ 20 in the dcSS group was significantly higher compared to lcSS (Fisher exact test, $p = 0.002$). The presence of digital ulcers was observed in 15 cases (35.7% of patients).

3.3.3. Gastrointestinal symptoms

Patients with dcSS reported the presence of digestive symptoms more frequently compared to the lcSS subgroup (Fisher's exact test, $p = 0.007$). Patients with early satiety exhibited higher mRSS values (Mann-Whitney, $p = 0.003$).

3.3.4. Anthropometric parameters

Patients with mRSS ≥ 20 had significantly lower BMI and body mass (Mann-Whitney, $p = 0.005$, $p = 0.042$). Patients with early satiety presented with significantly lower body mass compared to the rest of the group (Mann-Whitney, $p = 0.011$).

Assuming that the patients' height remained constant between the time W_{max} was recorded and the time of the study, the distribution according to BMI_{max} revealed a higher percentage of obese patients and the absence of the underweight category.

3.3.5. Weight loss and food-related behavior

Fisher's exact test revealed a significantly higher frequency of recent weight loss among people with Rodnan scores above 20 compared to the rest of the group ($p = 0.019$). In a univariate linear regression model, the Rodnan score proved to be a good predictor of weight loss, with $F(1,40) = 7.062$ ($p = 0.011$).

The participants with early satiety demonstrated a higher prevalence of unintended weight loss over 10% (Fisher exact test, $p = 0.026$).

3.3.6. EPIC-Norfolk food frequency questionnaire

Overall, the mean daily caloric intake was lower in the study group compared to the recommended values (t-student, $p = 0.024$). Pre-obese and obese patients (according to BMI) reported lower protein intake (t-student, percentage of daily caloric intake: $p = 0.046$). In addition, this subcategory demonstrated a higher consumption of sucrose (t-student, $p = 0.031$) and total sugars (t-student, $p = 0.041$).

Patients with dysphagia demonstrated a higher consumption of sweets and preserves compared to the rest of the study group (t-student, $p = 0.014$). Moreover, patients with heartburn had a higher fat intake (t-student, $p = 0.045$).

The mean alcohol consumption was significantly higher in the male population (Mann-Whitney, $p = 0.041$) and in overweight patients according to AC (t-student, p =

0.041) and RFM (t-student, p = 0.048). Also, patients with abnormal WHR reported higher alcohol intakes (Mann-Whitney, p = 0.036).

The average daily intake of vitamin D was correlated with meat and meat products (Pearson, R = 0.746, p <0.001), dairy (Pearson, R = 0.435, p = 0.004), fish (Pearson, R = 0.481, p = 0.001) and fats (Pearson, R = 0.585, p = 0.001), not with egg consumption (Pearson, R = 0.070, p = 0.662).

Patients with weight loss reported a higher intake of sodium and chlorine compared to the rest of the group (t-student, p = 0.035, respectively p = 0.041).

3.3.7. Biochemical markers

The majority of participants had vitamin D values below 30 ng/ml (40 persons, 95.2%), 27 persons demonstrating serum titres below 10 ng/ml (64.3% of the whole group, 66.7% of women). In addition, all patients with circulating values below 30 ng/ml had vitamin D titers below 20 ng/ml.

The serum levels of vitamin D were correlated with mRSS (Spearman, R = -0.338, p = 0.028). The daily intake of vitamin D (diet and/or supplements) did not demonstrate a significant influence on serum levels.

The prevalence of hypoalbuminemia in our group was 9.5%. Compared with the rest of the BMI categories, persons with excess weight (overweight and obese) presented with significantly higher albumin levels (t-student, p = 0.028). The patients who reported an unintended weight loss over 10% also demonstrated lower serum albumin values (Mann-Whitney, p = 0.011).

3.3.8. Malnutrition risk

Persons with reduced risk of malnutrition (MUST) had significantly lower Rodnan scores (t-student, p = 0.020) compared to the rest of the group. We did not identify significant discrepancies in terms of daily energy and macronutrient intake between the three risk categories (Kruskal-Wallis, p> 0.05).

Patients at low risk of malnutrition reported higher carbohydrate intakes (percentage of average daily caloric intake), results closely approaching statistical significance (p = 0.051). The lowest serum albumin levels were detected in patients at high risk of malnutrition (Kruskal-Wallis, p = 0.006). Furthermore, early satiety was often accompanied by a high malnutrition risk (60% versus 8.1%; Fisher's exact test, p = 0.015).

3.3.9. Relationships between parameters

Of the analyzed parameters, the modified Rodnan score showed the most numerous statistically significant relationships with other variables (Figure 3.31.).

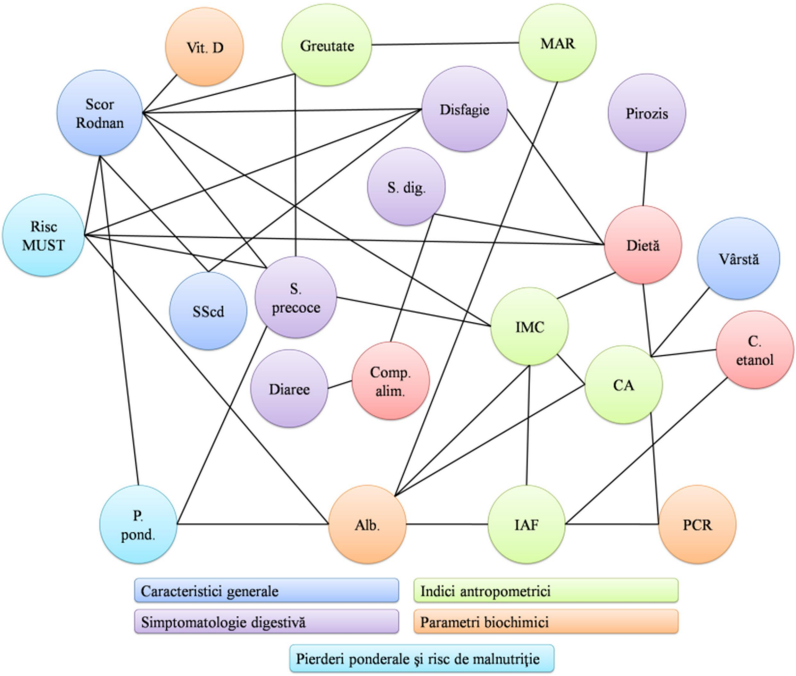


Figure 3.31. Graphical representation of the statistically significant relationships obtained in the study group: general presentation (Alb. = Albuminemia; C. etanol = alcohol consumption; CA = abdominal circumference; Food intake = dietary behavior; P. weight = weight loss; S dig. = digestive symptomatology (total); early S. = early satiety; Vit. D = vitamin D).

We identified notable associations between the Rodnan skin score and digestive symptoms (dysphagia, early satiety), weight loss and malnutrition risk.

3.4. Discussions

The significance of nutritional decline of patients with scleroderma remains a matter of debate. Scientific evidence is scarce, the recruited groups are often small (given the rarity of the disease), and the research protocols adopted as well as the results obtained differ from one study to another.

Dysphagia showed a notable relationship with the risk of malnutrition in the analyzed group. The association between dysphagia and malnutrition has been demonstrated, the two entities exhibiting a number of common characteristics such as high frequency and potentially severe consequences.

In contrast to Romanian study which used the same ELISA kit for the evaluation of vitamin D titres in patients with scleroderma, the results of our research revealed a statistically significant correlation with the Rodnan skin score. Moreover, the prevalence of vitamin D deficiency (below 10 ng/ml) was higher in our group (64.3%

vs. 9.8%). The relationship between vitamin D and cutaneous fibrosis could be explained by the impact on the signaling pathway of TGFβ (cytokine central in the pathogenesis of the disease).

According to recent data, older age does not play a decisive role in increasing the risk of malnutrition in SS. The results obtained in the present study also invalidate the relationship between age and malnutrition risk in scleroderma.

Early satiety showed a significant association with weight loss (over 10%), low body mass and high malnutrition risk. The data published so far support the link between early satiety and unintended weight loss, as well as the risk of malnutrition.

The number of meals consumed daily was reduced in patients with early satiety, this category often reporting only one meal per day. However, it has been shown that the intake of food in small amounts consumed in the form of small and frequent meals or snacks (6-10 meals/snacks per day) may bring significant benefits to patients with early satiety or anorexia.

The patients with dysphagia consumed sweets more frequently than the rest of the group. A study published in 2018 focused on dysphagia in the elderly and described the patients' preference for foods which were high in fats and sugar, especially semi-solid foods such as ice cream, panna cotta and parfait. Currently, the problem of altering the consistency of certain foods according to the severity of dysphagia is an active research topic. However, data regarding the potential benefits of such strategies applied to patients with SS-related dysphagia are currently lacking.

The prevalence of hypoalbuminemia in our group was 9.5%. BMI values below 18.5 kg/m^2 constitute an important indicator of protein-calorie malnutrition, while albuminemia may remain within normal values even in the context of marasmus. This phenomenon can be explained by the appearance of adaptive changes in cases where the patients' nutritional status is altered over long periods of time. In our study cohort, hypoalbuminemia was more frequent than underweight and demonstrated a significant relationship with MUST. One cannot assume that the underweight patients were not malnourished. Furthermore, it cannot be considered that the main mechanism of occurrence of hypoalbuminemia was malnutrition. However, the association between MUST and hypoalbuminemia as well as between the latter and weight loss suggests that nutritional status was in the process of declining in (at least part of) the study group. Moreover, it is possible that the normal values of serum albumin in the underweight may be one of the consequences of maintaining an abnormal body composition for a long period of time before the study. An argument in this regard could be the fact that the underweight category was absent from the classification of BMI_{max}.

3.5. Conclusions

• Patients with SS have an abundance of risk factors for nutritional decline.

• In addition to the specific digestive involvement and the debilitating nature of this disease, abnormal food-related behavior and an unbalanced diet may promote the appearance and/or accelerate the evolution of body composition abnormalities.

• The risk of malnutrition exhibited significant relationships with diet and digestive symptoms (early satiety and dysphagia).

• MUST demonstrated a statistically significant inverse relationship with albuminemia.

• Unintentional weight loss showed notable relationships with albuminemia and digestive symptoms (early satiety).

• The presence of heartburn was correlated with higher intakes of fatty foods.

• The number of meals consumed daily was reduced in patients with early satiety, this subgroup often declaring only one meal per day. Seeing as small and frequent meals have been shown to be beneficial for patients with early satiety or anorexia, this finding constitutes a valuable argument in favor of nutritional counseling.

• Salt consumption was significantly higher in the subgroup who experienced unintentional weight loss. Thus, the latter cannot be accounted for by water loss.

• The modified Rodnan score demonstrated many statistically significant relationships with other parameters (dysphagia, early satiety, weight loss, MUST).

• RFM is a sensitive indicator of central adiposity; in the present cohort RFM was correlated with patients' weight, age and ethanol consumption.

• Systemic inflammation showed notable relationships with central obesity.

• The main dietary sources of vitamin D were meat, dairy products, fish and fats. Similar to other studies on this topic, serum titers of vitamin D were correlated with patients' Rodnan score and not with the intake of vitamin D (diet or supplements).

• Our results highlight the need to initiate nutritional interventions for patients with SS.

Chapter 4. Body composistion, malnutrition risk and clinical and paraclinical correlates in scleroderma: a retrospective study (group B)

4.1. Motivation and objectives

The main objective of the present research was to identify the clinical, hematological, biochemical, immunological and capillaroscopic features of the SS patients who are underweight/present with unintended weight loss and/or malnutrition risk.

4.2. Materials and methods

We conducted a retrospective observational study in which we included adult patients diagnosed with SS treated in the 1st Rheumatology Clinic of the Clinical Rehabilitation Hospital. The study protocol was approved by the local Ethics Committees on Research (University of Medicine and Pharmacy "Grigore T Popa", Clinical Rehabilitation Hospital). We extracted the relevant data from the patients' files and the 1st Rheumatology Clinic electronic database.

The inclusion criteria were as follows: age over 18 years, positive diagnosis according to the ACR 1980 or the ACR/EULAR 2013 criteria, as well as the availability of clinical, biological, imaging (capillaroscopy) and immunological data. We excluded persons under the age of 18 and patients with incomplete data.

We evaluated the patients' body composition by BMI, classifying subjects according to WHO recommendations. Weight loss was presented as a percentage of the patients' initial body mass and classified according to the Medsger severity scale. The risk of malnutrition was estimated using the Malnutrition Universal Screening Tool (MUST). Subsequently, patients were classified into the 3 categories (low, moderate or high risk).

We evaluated hematological parameters, the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and various biochemical markers (AST, ALT, total cholesterol, triglycerides, glycemia, uric acid, urea, creatinine).

The autoantibodies analyzed were anti-topoisomerase I (Scl70), centromere, SSA, SSB, RNP/Sm and nucleosome antibodies (Enzyme-Linked-ImmunoAssay - ELISA). The analysis was performed in the same independent laboratory for the entire group. We compared the results obtained in SS patients with 3 other age and gender-matched groups (67 persons each) with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and healthy controls (HC).

We performed capillaroscopies using a 100N FEDMED Digital Dino-Lite Digital Microscope (AnMo Electronics Corporation, New Taipei, Taiwan) with a resolution of 1.3 Megapixel. We captured images at 200X magnification; image processing and analysis were performed using the DinoCapture 2.0 software (provided by the manufacturer of the aforementioned digital microsccope). We captured and analyzed a minimum of 4 pictures/finger (except for the thumb). We recorded morphological changes and capillary density (minimum - D_{min}, average - D_{med}, maximum - D_{max}) and stratified the patients according to the 3 capillarocopic patterns (*early, active, late*).

The statistical analysis was performed using IBM SPSS Statistics version 20 for Microsoft Windows.

4.3. Results

4.3.1. General characteristics of the study group

The study group consisted of 67 patients aged 33-80 years with a mean value of 51.51 years (DS = 15.24 years). Patients with dcSS constituted 47.8% of the group (32 persons), while 35 persons were classified as lcSS (52.2%).

Interstitial pulmonary fibrosis was associated with PAH in 16.7% of cases (compared with 2.3% with interstitial pulmonary fibrosis in the absence of PAH), with the results closely approaching statistical significance (Fisher's exact test, $p = 0.052$).

Male patients exhibited an increased risk of digital ulcers (RR = 1,939, 95% CI: 1,182-3,182), while women showed a 48.4% decrease in risk (RR = 0.516, 95% CI: 0.314-0.846).

In patients with interstitial pulmonary fibrosis, both the age of at the time of assessment (t-student, $p = 0.003$) and the age at onset (t-student, $p = 0.006$) were greater compared to the rest of the study group. In addition, patients with PAH were older both at the onset of the disease and at the time of assessment (Mann-Whitney, $p = 0.003$). Participants aged ≥60 years at onset demonstrated cardiac manifestations more frequently (50% vs. 10.5%; Fisher, $p = 0.008$), as well as pulmonary fibrosis (70% vs. 29.8%; Fisher, $p = 0.028$) and PAH (30% versus 3.5%; Fisher, $p = 0.021$).

4.3.2. Weight loss

Patients with dcSS were more likely to experience weight loss (10 patients, 31.2%, versus 4 patients with lcSS, 11.4%), the chi^2 test providing the statistical confirmation of the difference between the 2 disease phenotypes ($p = 0.046$). Subjects with digestive symptoms had a higher chance of experiencing weight loss over 5% (26.1% versus 4.8%, Fisher's exact test, $p = 0.049$). Moreover, patients with arrhythmias/conduction defects or pulmonary fibrosis also demonstrated an increased risk of weight loss (45.5% versus 14.3%; 37.5% versus 9.3%), Fisher's exact test offering the statistical confirmation in this respect ($p = 0.031$, respectively $p = 0.009$).

The presence of early satiety was accompanied by a high risk of weight loss over 5% (RR = 6,400; 95% CI: 3,622-11,309). The same symptom showed a significant association with weight loss over 10% (RR = 16,000; 95% CI: 6,195-41,324). Anorexia was accompanied by increased risk of weight loss over 5% of the initial body mass (RR = 5.357, 95% CI: 2.414-11.890).

4.3.3. Body composition and malnutrition risk

Patients with the diffuse phenotype were more likely to be underweight (Fisher exact test, $p = 0.021$) and in the high-risk MUST category compared to lcSS (Fisher's exact test, $p = 0.039$).

Patients with digestive involvement, PAH and arrhythmias/conduction defects did not demonstrate a significantly higher prevalence of high malnutrition risk ($p = 0.153$, $p = 0.566$, $p = 0.476$, respectively $p = 0.051$). However, subjects with pulmonary fibrosis were more frequently found in the moderate and high risk categories (37.5% versus 14%, chi^2 test, $p = 0.027$).

4.3.4. Hematological and biochemical parameters

The analysis of hematological and inflammatory markers revealed significant discrepancies in relation to disease phenotype, patients with dcSS demonstrating a lower lymphocyte count ($p = 0.010$) and higher CRP titers ($p = 0.048$); the rest of the parameters exhibited a similar distribution in the two subgroups.

Individuals with recent weight loss demonstrated lower mean hemoglobin (t-student, $p = 0.001$) and hematocrit levels (t-student, $p < 0.001$), and a more accelerated ESR (t-student, $p < 0.001$) compared to the rest of the study population. Furthermore, underweight patients had lower hemoglobin and hematocrit levels compared to the rest of the group (Mann-Whitney, $p = 0.030$, respectively $p = 0.015$).

Fasting hypoglycemia was more frequent in the Methotrexate subgroup (19.2% versus 2.4% in the rest of the group; Fisher's exact test, $p = 0.029$). Knowing that the elderly demonstrated a higher prevalence of hypoglycemia, we analyzed the age difference between the patients undergoing Methotrexate therapy and the rest of the study population; the t-student test showed the absence of a marked discrepancy between the two subgroups ($p = 0.406$). The subjects undergoing Azathioprine therapy had a lower erythrocite count (Mann-Whitney, $p = 0.044$). Furthermore, the only patient with neutropenia was under treatment with Azathioprine. Patients treated with Cyclosporin A had significantly higher triglyceride levels (t-student, $p = 0.044$).

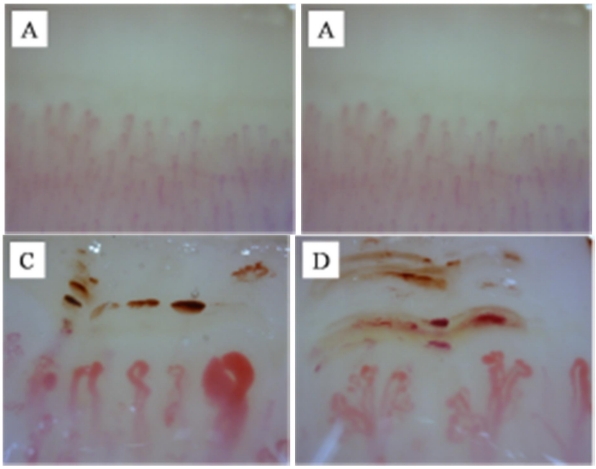
4.3.5. Immune markers

We identified higher titers of anti-nucleosome antibodies in people with PAH. Moreover, we noticed a significantly higher prevalence of PAH in the subgroups who were positive for anti-nucleosome antibodies (16.1% versus 0%, Fisher exact test, p = 0.018) or RNP/Sm (50% versus 4 , 8%, Fisher exact test, p = 0.025).

Compared with 3 age and gender-matched groups of patients with Rheumatoid Arthritis (RA, 67 persons), Systemic Lupus Erythematosus (SLE, 67 persons) and healthy controls (HC, 67 persons), the subjects with scleroderma showed significantly higher values of anti-Scl70 and centromere antibodies (p <0.001). With the exception of anti-centromere and anti-Scl70 antibodies, all other immunological markers demonstrated significantly higher titers in patients with SLE compared with SS. However, the prevalence of anti-nucleosome antibodies in SS was comparable to that of SLE (p >0.05). In addition, together with anti-Scl70 and centromere antibodies (present in SS only), the prevalence of anti-nucleosome antibodies was significantly greater among patients with scleroderma compared to RA (chi^2 test, p <0.001).

4.3.6. Capillaroscopy

We noticed notable discrepancies regarding the duration of the disease in relation to the 3 capillaryoscopic patterns, the highest values being recorded in patients with the late pattern (p = 0.002). We identified giant capillaries in 59 cases (88.1%), microhemorrhages in 52 cases (77.6%), avascular areas in 11 cases (16.4%), bushy/ramified capillaries in 24 patients (8%) (Figure 4.23.).



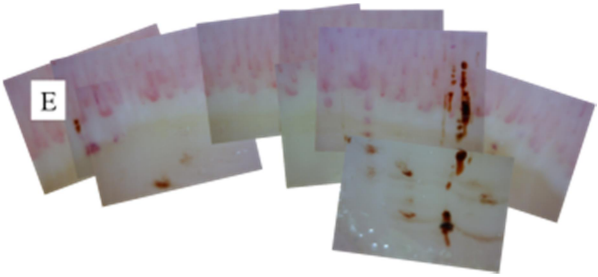


Figure 4.23. Capillaroscopy (200X): A - Normal aspect, "hairpin" aspect of the capillary loops, normal density; B - Giant capillarie, decreased density; C - Tortuous, crossed capillaries, capillary ectasia, microhemorrhages and hemosiderin deposits, giant capillary, decreased density; D - Significant decrease in density, ramified and bushy capillaries, hemorrhages, disorganized capillary network, significantly reduced density; E - Patient included in the study: complete (reconstructed) image of capillaryoscopic changes identified in the 3rd finger, right hand (right image - old hemorrhage, large hemosiderin deposits - post-traumatic).

We identified an inverse relationship between D_{min}, D_{med} and the Medsger score for general health (Spearman, R = -0.278, p = 0.023, R = 0.280, p = 0.022).

The capillary density did not differ significantly according to BMI; however, the underweight patients demonstrated the late capillaroscopic pattern more frequently (40% compared to 21% in the rest of the group). With respect to capillary density, we did not notice significant differences between the 3 MUST categories, but the late capillaroscopic pattern was more frequently described in the subgroup with high malnutrition risk (30% versus 14%).

Overall, visceral involvement was accompanied by significantly lower D_{min} and a higher prevalence of microhemorrhages (85.2% versus 46.2%; Fisher's exact test, p = 0.006). We also noticed a higher percentage of patients with capillary *drop-out* in the subgroup with organ damage (74.1% versus 38.5%; Fisher exact test, p = 0.022).

The patients who were positive for anti-centromere antibodies exhibited higher D_{med} and D_{max} (Mann-Whitney: p = 0.032, p = 0.045), not D_{min} (Mann-Whitney, p = 0.102). Furthermore, D_{min}, D_{med}, and D_{max} had significantly lower values in the anti-Scl70 positive subgroup. Nonetheless, these results did not reach statistical significance (Mann-Whitney, p> 0.05).

4.4. Discussions

Malnutrition is an independent risk factor associated with mortality in scleroderma. Cruz-Dominguez et al. used the Chang method in their analysis of SS patients' nutritional status, identifying severe malnutrition in 15% of their study population. Moreover, more than half of the patients who were deceased at he end of the study were severely malnourished. In our study group, the prevalence of high malnutrition risk (according to MUST) was almost identical to the one described by Cruz-Dominguez et al. (14.9%).

Although most studies associate older age with a higher risk of malnutrition in the general population, the elderly in our group did not show more severe changes in this area.

Patients with gastrointestinal involvement were more likely to present with weight loss over 5%. Digestive involvement was present in 90% of people at high risk of malnutrition according to MUST. The close relationship between gastrointestinal manifestations and nutritional decline has previously been described. Baron et al. recommend investigating digestive complications in parallel with the risk of malnutrition in patients with scleroderma.

Pulmonary fibrosis also demonstrated a significant association with malnutrition risk. In this regard, subjects with pulmonary fibrosis were more likely to be in the moderate/high risk categories. Although they did not use the same methods of assessing nutritional status, Jouneau et al. also identified a high prevalence of malnutrition among patients with idiopathic pulmonary fibrosis.

Early satiety was accompanied by a high risk of weight loss over 5%. The same symptom showed a notable association with weight loss over 10%, while anorexia was associated with a higher risk of weight loss over 5%. Previously published research reported similar results.

Although capillary density did not differ significantly according to BMI, underweight patients were more likely to exhibit the late capillaroscopic pattern. Moreover, people with excess weight rarely showed capillaroscopic changes specific to this pattern. Moreover, the late capillaroscopic pattern was more frequently described in the high-risk MUST subgroup. The currently available literature does not mention a direct connection between nutritional decline and capillaroscopic changes in scleroderma. Nevertheless, these anomalies (capillaroscopic changes and malnutrition risk) constitute important marks of disease progression and severity.

Herrick et al. identified a statistically significant association between capillaroscopic changes and positive anti-centromere antibodies. However, the authors analyzed the intercapillary (inter-apical) distance while the present study described density/linear mm. Furthermore, the patients who exhibited anti-centromere antibody positivity were associated with slightly higher D_{med} and D_{max} in our group. In this regard, our results (although statistically unconfirmed) resemble those obtained by Sato et al. and Bredemeier et al., capillary density being lower in the anti-Scl70 positive subgroup.

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A systematic review focused on determining the prevalence of severe disease manifestations describing *the 15 percent rule in scleroderma*. In group B, the prevalence of arrhythmias/conduction defects was close to 15% (16.4%), and interstitial pulmonary fibrosis was associated with PAH in 16.7% of cases. Moreover, high malnutrition risk was observed in 14.9% of group B and 14.28% of group A. Muangchan et al. do not mention the applicability of this rule in the case of malnutrition risk, although this variable was considered a severe manifestation at the time of selection of the relevant bibliographic resources. Seeing as the present study

was not longitudinal, we cannot confirm the link between clinical manifestations or malnutrition and mortality. However, *the 15 percent rule in scleroderma* remains a hypothesis worthy of further analysis in the context of longitudinal studies.

4.5. Conclusions

• Cardiopulmonary involvement (pulmonary fibrosis, arrhythmias/conduction defects) was associated with unintentional weight loss and/or malnutrition risk.

• Patients with visceral involvement exhibited unintended weight loss in more than a quarter of cases, the difference compared the rest of the group approaching statistical significance. The association between SS-related organ damage and weight loss may indicate a negative prognosis in these patients.

• We identified a statistically significant relationship between capillaroscopic changes and the Medsger score for general health (weight loss, hemoglobin and hematocrit).

• Although the capillary density did not differ significantly according to BMI, the underweight patients presented the late capillaroscopic pattern more frequently. Moreover, the late pattern was most commonly described in the MUST high-risk subgroup. These anomalies (capillaroscopic changes and malnutrition risk) may constitute important marks of disease progression and severity.

• While capillary density does not differ with respect to the gender of the patients, male patients are at high risk of developing digital ulcers.

• The duration of the disease contributed significantly to the progression of capillary changes.

• We identified a high prevalence of anti-nucleosome antibodies in the SS group, approaching the one observed in SLE. Furthermore, these autoantibodies demonstrated a remarkable connection with PAH.

Chapter 5. General conclusions

Despite the fact that we have formulated conclusions for both groups (A and B), we consider it appropriate to state general conclusions of the doctoral research.

• The progressive and potentially severe nature of the pluriorganic involvement in scleroderma predisposes to a high risk of nutritional decline. Digestive involvement is highly prevalent and significantly increases the risk of weight loss and malnutrition in these patients.

• Extensive skin involvement (the diffuse phenotype and a Rodnan score exceeding 20 points) is often associated with more severe clinical manifestations, underweight, weight loss and/or higher malnutrition risk.

• Nutritional decline may accompany the appearance and/or progression of visceral involvement and certain biochemical and capillaroscopic changes. Contrary to the results obtained in the general population, the elderly with scleroderma do not exhibit a higher risk of malnutrition.

• The *15 percent rule in scleroderma* may be applicable for high malnutrition risk (according to MUST, with a prevalence closely approaching 15% in the two analyzed groups).

• We obtained statistical significance for the link between capillary changes and the Medsger score for general health status (weight loss, hemoglobin and hematocrit) in group B.

• The prevalence of hypovitaminosis D remains high in scleroderma, certain authors raising the issue of its involvement in the pathogenesis of the disease. Furthermore, the intake of vitamin D (diet and supplements) does not significantly affect serum levels.

• In group A, the main dietary sources of vitamin D were meat, dairy products, fish and fats. However, the serum level of vitamin D did not show significant associations with diet/supplement intakes.

• Albeit studies reporting discrepant results regarding the correlation between the Rodnan score and serum vitamin D titers, our findings indicate a significant link between the latter and skin involvement.

• In group A, salt consumption was significantly higher in patients who suffered unintended weight loss. Therefore, the decrease in body mass cannot be accounted for by water loss.

• The male gender is a risk factor for the development of digital trophic lesions.

• Anti-nucleosome antibodies may be present in patients with scleroderma. Moreover, these autoantibodies may demonstrate significant relationships with disease-specific cardiopulmonary involvement.

Chapter 6. Original elements and perspectives

An emerging research direction is the correlation of nutritional status with various disease-related manifestations in SS. The present study has the advantage of being the first on this topic carried out in Romania.

Among the original elements characterizing the investigation of group A are the use of the EPIC-Norfolk questionnaire and the RFM in persons with scleroderma. Albeit using MUST for the estimation of malnutrition risk in scleroderma is not an absolute first, the score has not been used in patients with SS in our country.

Our results highlighted important relationships between nutritional status (body composition and diet) and digestive symptoms, suggesting that a non-pharmacological approach to SS-related gastrointstinal involvement could be attempted (with a potential reduction in the frequency of hospitalizations due to the exacerbation of digestive symptoms and/or decreased consumption of proton pump inhibitors and prokinetic agents).

In group A, patients with early satiety (a symptom which was linked to weight loss and the risk of malnutrition) were likely to exhibit a decreased meal frequency, while patients with heartburn reported a higher fat intake compared to the rest of the group. These results highlight the need to develop nutritional recommendations focused on

ensuring optimal caloric and macro/micronutrient intakes while also improving digestive symptoms in patients with scleroderma.

In group B we identified a relationship between nutritional decline and microvascular changes. Although in this respect our results did not reach statistical significance, this association has rarely been described in literature. Nevertheless, both capillaroscopic changes and weight loss/malnutrition accompany the progression of the disease.

Though exhibiting notable associations with morbidity and mortality, malnutrition remains frequently underdiagnosed in both connective tissue diseases and the general population. Patients with SS demonstrate a plethora of risk factors for malnutrition which is an important argument in favor of frequent evaluation from a nutritional perspective.

The study conducted in group A could be continued with the introduction of additional parameters (functional prognosis, risk of mortality, GSS, the frequency of occurrence of digestive symptoms in relation to diet or changes in body composition). Moreover, an important perspective is represented by the expansion of the group in order to give robustness to the evidence presented. With regard to capillaroscopy (group B), a possible future direction is to compare the results obtained with those from homologous groups of patients with other immuno-inflammatory diseases. Moreover, the re-analysis of the images may allow the investigation of other parameters (perivascular area, intercapillary distance, CSURI).

Our study is reproducible and remains open, providing important arguments in favor of the thorough investigation of nutritional status in relation to certain clinical and paraclinical features of systemic sclerosis.

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