# Evaluation of cardiopulmonary exercise test in the prediction of disease progression in systemic sclerosis

R.A. Hemelein[1], I. Lajkó[2], K. Baráth[2], J. Varga[2], G. Ágoston[3], D. Hulló[1], M. Bocskai[1], D. Dobi[1], Z. Rózsavölgyi[2], Á. Milassin[1], A. Varga[3], A. Somfay[2], L. Kovács[1]

*[1]Department of Rheumatology and Immunology, University of Szeged, Faculty of Medicine;*
*[2]Department of Pulmonology, University of Szeged, Faculty of Medicine;*
*[3]Department of Internal Medicine, Cardiology Centre and Department of Family Medicine, University of Szeged, Faculty of Medicine, Szeged, Hungary.*

*Rita Adrienn Hemelein, Assist. Prof.*
*Imre Lajkó, Head Physician*
*Kristóf Baráth, Resident*
*János Varga*, Head Physician*
*Gergely Ágoston, Assoc. Prof.*
*Daniella Hulló, Assist. Prof.*
*Márta Bocskai, Assist. Prof.*
*Diána Dobi, Clin. Fellow*
*Zoltán Rózsavölgyi[&], Assoc. Prof.*
*Ágnes Milassin[+], Clin. Fellow*
*Albert Varga, Prof.*
*Attila Somfay, Prof.*
*László Kovács, Prof.*
**Current affiliation:*
*National Korányi Institute of Pulmonology, Budapest, Hungary.*
*[&]Current affiliation:*
*Markusovszky Hospital of Vas County, Szombathely, Hungary.*
*[+]Current affiliation:*
*University of Szeged, Faculty of Medicine, 1st Department of Internal Medicine, Szeged, Hungary.*

*Please address correspondence to:*
*László Kovács,*
*Department of Rheumatology and Immunology,*
*University of Szeged,*
*Faculty of Medicine,*
*Kálvária sgt. 57,*
*6725 Szeged, Hungary.*
*E-mail: kovacs.laszlo@med.u-szeged.hu*

*Received on July 27, 2020; accepted in revised form on January 4, 2021.*

*Clin Exp Rheumatol 2021; 39 (Suppl. 131): S94-S102.*



**Key words:** systemic sclerosis, cardiopulmonary exercise test, disease progression, desaturation

*Competing interests: none declared.*

## ABSTRACT

**Objective.** *Cardiopulmonary exercise test (CPET) is a widely used examination to predict the prognosis of many chronic pulmonary diseases, and it has also been tested in systemic sclerosis (SSc) with a focus on the development of pulmonary hypertension. CPET is a highly informative non-invasive tool that provides a more complex information than conventional lung function tests to predict the course of cardiopulmonary diseases, as it provides a general overview of the aerobic metabolism, influenced by pulmonary, cardiovascular and peripheral muscle function. The purpose of this investigation was to assess if the progression and the development of poor overall disease outcome in SSc can be predicted by this method.*

**Methods.** *Twenty-nine SSc patients were investigated prospectively with standard follow-up plus CPET for a mean of 3.7 years to match the results of conventional evaluation modalities and CPET. A composite end-point of several serious outcomes reflecting SSc-related vascular and cardiopulmonary damage was set up, and the predictive value of and correlations between the CPET parameters and resting lung function and echocardiography variables were assessed.*

**Results.** *None of the clinical parameters, resting lung function or echocardiographic test results proved to be predictive of the development of the end-point of poor prognosis in this cohort. In contrast, several CPET parameters were found to discriminate between SSc patients with or without adverse outcome. The detection of desaturation (at any CPET test) was associated with a higher risk of poor prognosis (OR: 5.265). $VO_2$ and $VE/VCO_2$ at baseline correlated with the annual decrease in FVC, anaerobic threshold with the development of digital ulcers, and $VE/VO_2$ with the increase in pulmonary arterial pressure.*

**Conclusion.** *Several CPET parameters obtained at the beginning of follow-up are informative of the appearance of various adverse end-points. CPET is a feasible examination in the care of SSc patients and provides excess information to current standard follow-up examinations.*

## Introduction

Systemic sclerosis (SSc), also termed scleroderma, is a systemic autoimmune disease with multi-organ involvement and frequently with a destructive, progressive disease course. Key pathways in the pathogenesis are vascular, fibrotic and immunological dysfunction, leading to progressive organ damage and increased mortality (1, 2). Leading causes of morbidity and mortality are interstitial lung disease (ILD), pulmonary arterial hypertension (PAH) and obliterative vasculopathy, in particular digital ulcers, gangraene and macrovascular complications. Predictors of such poor outcome are hard to definitively identify by the physician at the first encounter, but it is obviously of crucial importance in the determination of follow-up strategy and of the intensity of immunosuppressive therapy. Current guidelines prompt the treating physicians to regularly (every 6 to 12 months) perform resting lung function tests, high-resolution computed tomography (HRCT) of the lung and resting echocardiography to estimate disease progression (3-5).

Cardiopulmonary exercise test (CPET) is a widely used examination to predict the prognosis of chronic obstructive pulmonary disease (6), post-transplant lung dysfunction (7, 8), or PAH (9, 10), and it has also been examined in patients suffering from SSc in the context of circulatory dysfunction, PAH, and

exercise-triggered left ventricular diastolic disorder (11-22). As CPET provides a general overview of the aerobic metabolism, influenced by pulmonary, cardiac and vascular function (23), the purpose of this investigation was to assess if the development of poor overall disease outcome could be predicted by this non-invasive examination, and whether CPET may provide additional prognostic information to standard resting follow-up examinations. As the complexity of the disease is based upon the various individual organ involvements, we hypothesised that this synthetical examination is useful to survey the actual state of aerobic metabolism and predict the development of potential organ failures. As the predictive value of CPET has not been studied in SSc, neither to the progression of the individual organ damage nor to mortality, we have initiated a prospective study with a focus on patients of a relatively short disease duration and without advanced-stage organ damage. Our aim was to assess correlations between CPET and traditional measurements, and to compare the predictive ability of CPET to multiple adverse disease outcomes.

## Patients and methods

*Patients*

Twenty-nine patients were consecutively enrolled at the Department of Rheumatology and Immunology, University of Szeged, Hungary into this prospective study from 2010 to 2017. The diagnosis of SSc had been set up by ACR/EULAR diagnostic criteria (24). The standard exclusion criteria of CPET (NYHA stage III-IV heart failure, neurological comorbidities and unstable blood pressure) were applied, as well as severe musculoskeletal involvement, which had led to inability to participate at the examination. Each patient had at least two CPET examinations with at least one year difference. A composite end point was set up to define poor disease outcome. Our composite end point comprised: death, digital ulceration necessitating endothelin receptor antagonist therapy, pulmonary hypertension (estimated systolic pulmonary arterial pressure / PAP/ >40mmHg), change of carbon-monoxide diffusion capacity (deltaDLCO) >2%/year, or change in PAP (deltaPAP) >3mmHg/year.

*Procedures*

All patients attended spirometry to measure dynamic lung volumes (forced vital capacity, /FVC/ and forced expiratory volume in 1 s, /$FEV_1$/), body pletysmography to measure total lung capacity /TLC/, and the measurement of diffusing capacity of the lung measured by carbon monoxide (DLCO), which informs about alveolo-capillary gas exchange. Regular, yearly non-invasive examinations were performed in parallel with the CPETs. The presence, extent and activity of interstitial lung disease were assessed by HRCT, to explore (subclinical) alveolitis and progression of pulmonary fibrosis. Chest HRCT was performed at the first visit, and repeated if necessitated by worsening lung function tests or other relevant clinical findings. HRCT interpretation was based on visual analysis. The examinations, evaluated by experienced senior radiologists, determined the presence and distribution of the following CT signs suggestive of SSc involvement: Alveolitis was verified if areas of isolated ground-glass opacities were observed on high-resolution chest CT (HRCT) scans. Fibrosis was diagnosed when fine or coarser reticular pattern of pathology was noted within areas of ground-glass opacities, with or without honeycombing, traction bronchiectasis and/or bronchiolectasis. Estimated pulmonary pressure was followed by echocardiography once yearly, or at six-month intervals if necessitated by the clinical course. Systolic PAP values >40 mmHg were regarded as pulmonary hypertension (PH). We avoided the use of pulmonary arterial hypertension (PAH) in this study, since the primary focus of this study was not the specific examination in changes in PAP, but rather of a composite endpoint as mentioned above, and therefore we regarded it ethical to perform right heart catheterisation only in the event of a significant elevation of PAP (>50 mmHg), as in routine care, following national guidelines. We therefore used the term pulmonary hypertension (PH). The same cardiologist performed all the echocardiographic examinations in order to eliminate inter-observer variability. In addition to the above-listed examinations, patients were under regular gastroenterological surveillance to upper gastrointestinal involvement, including gastrointestinal reflux disease (GERD), Barrett's metaplasia or gastric antral vascular ectasia, following international guidelines (25, 26).

Resting lung function and CPET measurements were performed at the Department of Pulmonology, University of Szeged. Spirometry, body pletysmography and DLCO measurements were carried out on a Vmax229 Autobox 6200 (Sensormedics, Yorba Linda, California) equipment. Normal values were defined by NHANES (National Health and Nutrition Examination Survey) (27). Systolic PAP was estimated from the degree of tricuspid valve regurgitation by echocardiography. Other parameters of left ventricular systolic (ejection fraction), diastolic (E/A ratio) and right ventricular systolic (tricuspid annular plane systolic excursion – TAPSE) function were also recorded.

CPET was implemented on a bicycle ergometer with an electric brake (Ergoline 900) by continuously increasing (RAMP) load. A three-minute resting phase was followed by a three-minute consistent intensity warm-up phase on 20 W, next the performance was raised by 10 W/min consistently. Tidal volume ($V_T$), expired volume ($V_E$), oxygen uptake ($VO_2$), carbon dioxide production ($VCO_2$) were measured breath by breath by a mass flow meter on a metabolic load system ($V_{MAX}$ 29C Respiratory Analyzer, Sensormedics). Before every test, the system had been calibrated. Anaerobic threshold (AT) was defined by V-slope method. Heart rate, twelve-lead ECG (Cardiosoft, Sensormedics) and oxygen saturation (SatTrak, Sensormedics) were monitored during the tests. No medication was interrupted in the period of the test. Breathing reserve (BR%) was determined and if the value was 15% or below, it indicated that the test was ventilation-limited. Heart rate reserve (HRR%) was also defined, and in cases

in which the value was 10% or below, circulation-limited exercise capacity was considered. In the present study, the following parameters were analysed: peak workrate ($W_{max}$ [Watt]), heart rate (HR), aerobic capacity as measured by oxygen uptake ($VO_2$ [L/min]), specific aerobic capacity ($VO_2$/kg [mL/min/kg]), carbon dioxide output ($VCO_2$ [L/min]), minute ventilation (VE [ref%]), anaerobic threshold (AT [L/min]), oxygen saturation at rest, oxygen saturation at maximum load ($SpO_2$), and the difference of these two values (desaturation), respiratory equivalent for carbon dioxide output ($VE/VCO_2$) determined at anaerobic threshold, which inform about ventilator efficiency (ventilation-perfusion mismatch). Normal values were the following: $VO_{2max}$> 84 ref%, AT ($VO_2$ at AT / $VO_{2pred,max}$) >40%, $HR_{max}$: > 90% of the expected maximum (calculated by 220-age/years/), $VE/VCO_2$<30. Following the American Medical Association (AMA) standardised values, if $VO_{2max}$ is between 15 and 20 mL/kg/min or DLCO is between 41 and 59%, pulmonary functional damage is between 26 and 50%. If $VO_{2max}$ is <15 mL/kg/min or DLCO is <40%, the degree of pulmonary damage is more than 50% (27). In healthy individuals, $SpO_2$ and the partial pressure of oxygen in arterial blood remain stable during exercise, and saturation does not decrease more than 2%. Desaturation means $SpO_2$ decreases ≥4% or becomes less than 88% (*i.e.* it is a categorical variable). The maximum difference (change) in SpO2 during exercise (*i.e.* the degree of desaturation) is represented as $DeltaSat_{max}$ (*i.e.* it is a continuous variable).

*Statistical methods*

Continuous variables were compared between groups with t-test or analysis of variance, or, in the event of non-normal distribution of the values, with Mann-Whitney U-test. Categorical variables, including frequencies, were compared with $chi^2$ test. The predictive value of baseline categorical variables was assessed with Fisher exact test, where the odds ratio of the development of the endpoint was determined. The predictive value of continuous variables was estimated with receiver-operated curve (ROC) analysis. Correlations between certain CPET parameters with resting lung function tests or clinical variables were examined with Pearson's correlation test. For the analysis of the association between certain continuous variables and the development of the end-point, logistic regression was applied. A $p$-value of <0.05 was regarded as statistically significant throughout the study.

**Table I.** Clinical characteristics of the SSc cohort.

| | |
|---|---|
| Age (yr) | 49 (16-68) |
| Female : male (n) | 24:5 |
| DcSSc : LcSSc (n) | 15:14 |
| anti-Scl-70: anti-centromere-B antibody positivity (n) | 14:5 |
| Time since Raynaud's phenomenon (yr) | 8.2 (1-28) |
| Time since first non-Raynaud symptom (yr) | 4.8 (0-28) |
| Alveolitis (n) | 15 |
| Lung fibrosis(n) | 16 |
| Pulmonary hypertension (n) | 10 |
| Digital ulcers (n) | 12 |
| Calcinosis (n) | 2 |
| Macroangiopathy (n) | 7 |
| GERD (n) | 26 |
| Barrett oesophagus (n) | 21 |
| GAVE (n) | 5 |
| Motility disorder (n) | 11 |
| Hypercholesterolaemia (n) | 6 |
| Hypertension (systemic) (n) | 7 |
| Immunsupressive therapy (n) | 18 |
| Endothelin receptor antagonist therapy | 5 |
| Malignancy (n) | 2 |
| Overlap syndrome (n) | 4 |

Numbers indicate mean (range) or absolute numbers.
GERD: gastro-oesophageal reflux disease; GAVE: gastric antral vascular ectasia.

## Results

*Patient characteristics*

Demographic data, organ manifestations, autoantibody-positivities and some specific immunosuppressive or vasoactive medications applied in the patients are presented in Table I. Diffuse and limited cutaneous SSc subsets were represented in comparable numbers, and the disease had a relatively short duration from the onset of the first non-Raynaud symptom, with some exceptions. Immunosuppressive therapy was applied in the majority of the patients, especially in those with dcSSc, as per international guidelines, with rapid progression of skin involvement, ILD or some other inflammatory manifestations of the disease being the most frequent indications. ILD was detected with HRCT in more than half of the patients, whereas the prevalence of PH was relatively low. Scleroderma renal crisis did not occur ever in the enrolled patients.

In addition to anti-Scl-70 and anti-centromere antibodies, other autoantibody positivities have been revealed in a total of 6 patients: anti-nucleosome antibody (n=4), anti-SSA/Ro (n=3), anti-mutated citrullinated vimentin (n=2), and anti-double stranded DNA (n=2). We note that we did not examine further SSc-specific autoantibodies in this cohort. Four patients fulfilled the criteria of another autoimmune disease too: two patients had an overlapping rheumatoid arthritis (RA), one had systemic lupus erythematosus (SLE), and one had myositis. The overlapping RA or SLE were in remission at study entry, and occasional relapses manifested as polyarthritis responded well to transient low dose corticosteroid, and to immunosuppressive therapies indicated for SSc. The overlapping myositis was also controlled throughout the follow-up and did not lead to significant muscle atrophy. We did not classify any of the patients with Sjögren's syndrome overlap.

Immunosuppressive therapy was applied in the majority of the patients, especially in those with dcSSc, as per international guidelines, with rapid pro-

gression of skin involvement, ILD or some other inflammatory manifestations of the disease being the most frequent indications. A detailed presentation of the medications of the patients, including immunosuppressants, cardiovascular agents (indicated for Raynaud's phenomenon or systemic hypertension), treatment of obstructive airway symptoms and other are presented in Supplementary Table S1. Changes in these treatments were made for immunosuppressants as required by the clinical course of SSc, and in a few cases for the cardiovascular medication, but we note that none of them were specifically indicated for pulmonary hypertension or congestive heart failure.

The patients were followed-up for a median of 94 (range: 33-110) months. An average of 3.93 (range: 2-8) lung function and CPET examinations were performed per patient. During the study period, one patient died (sudden cardiac death, advanced microvascular damage, lung and myocardium fibrosis), five received endothelin-antagonist treatment necessitated by recurrent, severe digital ulcerations, in three of them did the estimated PAP increase to values over 40 mmHg, in 4 of them was the annual increase of PAP higher than 3 mmHg, and in 12 patients was the annual decline of DLCO higher than 2%. Overall, 16 patients met the pre-defined endpoint of a severe disease outcome. Comparisons between the groups who did and who did not meet the endpoint revealed no statistically significant difference in terms of age, gender, dcSSc *versus* lcSSc subset, disease duration, the presence of ILD, PH, digital ulcer or calcinosis.

*Resting lung function tests and PAP measurements*

Resting lung function test and echocardiography results at enrolment and during follow-up are summarised in Table II. Pletysmographic values were, on average, close to the normal range, but with significant variability, and markedly decreased lung volumes were also noted in a few patients. In contrast, mean DLCO value was in the moderately decreased range, again, with high variability. PAP values were in the normal or moderately elevated range. Annual changes in the DLCO and PAP values were, on average, modest, but changes in both directions occurred during follow-up. When the direction of worsening was examined separately, decreasing DLCO was observed in 19 of the 29 patients, whereas PAP increased in 10 of 14 patients in whom repeated PAP measurements could be performed. It is to be noted that PAP was estimated from the degree of tricuspid regurgitation, and, therefore, was not feasible to assess in the absence of tricuspid retrograde blood flow. None of the resting parameters, nor their changes displayed a statistically significant difference between the subgroups with or without meeting the end-point.

**Table II.** Summary of the most important resting lung function and pulmonary arterial pressure values at baseline, and the change of selected variables.

| Parameter | Mean or *median* | Range | SD or 25th-75th percentile |
|---|---|---|---|
| FVC (% of predicted) | 94.2 | 54 – 125 | 17.8 |
| FVC (L) | 3.06 | 1.97 – 5.26 | 0.72 |
| TLC(% of predicted) | 90.9 | 47 - 117 | 17.7 |
| TLC (L) | 4.75 | 3.08 – 6.14 | 0.75 |
| DLCO (% of predicted) | 56.8 | 20 – 94 | 16.9 |
| DLCO (ml/min/mmHg) | 14.51 | 4.60 – 30.10 | 5.16 |
| DeltaFVC/year (L) | *-0.02* | *-0.19 – +0.31* | *-0.08 – +0.05* |
| DeltaDLCO/year (ml/min/mmHg) | *-0.43* | *-5.92 – +2.68* | *-0.75 – +0.36* |
| PAP (mmHg) | 28.5 | 18 – 44 | 7.4 |
| deltaPAP/year (mmHg) | 1.2 | -8 - +12 | 4.5 |

FVC: forced vital capacity; DLCO: diffusion capacity of carbon monoxide; TLC: total lung capacity; PAP: pulmonary arterial pressure; L: litre. Delta values indicate changes from the first to the last measurement.
Values are presented as mean, range and SD (normal distribution) or median range and 25th and 75th percentile (non-normal distribution – in italics).

**Table III.** Cardiopulmonary exercise test results.

| Parameter | First measurement | | Last measurement | | Annual change |
|---|---|---|---|---|---|
| WR | 101.4 | 46-187 | 98.52 | 62-189 | -0.09 |
| $VO_2$ (L/min) | 1.34 | 0.5-2.4 | 1.47 | 0.84-2.81 | 0.03 |
| $VCO_2$ (L/min ) | 1.485 | 0.45-2.53 | 1.58 | 0.83-2.79 | 0.04 |
| $VO_2$/kg(ml/min/kg) | 19.64 | 9-5-36.5 | 20.2 | 10.7-33.1 | 0.43 |
| VE(L/min) | 51.42 | 17.6-85.6 | 48.3 | 28.1-83.5 | -0.74 |
| $VE/VCO_2$ | 33.24 | 26-42 | 29.43 | 21-44 | -0.85* |
| AT (L/min) | 1.00 | 0.58-1.91 | 0.92 | 0.46-1.67 | -0.02 |
| AT (% of $VO_2$max,pred) | 47.96 | 25-77 | 46.04 | 25-69 | -0.39 |
| $DeltaSat_{max}$ (Decrease in $SpO_2$) (%) | 4.5 | 1-12 | 5 | 1-12 | 0.12 |
| Desaturation (n) | 6 | | 14 | | |

WR: workrate; VO2: aerobic capacity; $VO_2$/kg: specific aerobic capacity; $VCO_2$: $CO_2$ output; VE: minute ventilation; $VE/VCO_2$: respiratory equivalent for $CO_2$output; AT: anaerobic threshold (L/min), AT% (% of predicted $VO_2$max); $SpO_2$: haemoglobin $O_2$ saturation measured by pulse-oximetry. Decrease in $SpO_2$ indicates the degree of desaturation, whereas Desaturation denotes the number of patients in whom desaturation (decrease in $SpO_2 \geq 4\%$) occurred ever during follow-up. Comparison of values at first and last visits. Numbers indicate mean (first and third column) and range (second and fourth column) or absolute number of involved patients.
*$p<0.05$ between first and last visit results.

*Cardiopulmonary exercise test results*

Mean values and ranges of the CPET results at first and last visits of the study are presented in Table III.

It can be concluded that the values at inclusion reflect moderately decreased work-rate and aerobic capacity with slightly impaired ventilation-perfusion mismatch (higher $VE/VCO_2$) compared to age-matched healthy individuals. Baseline desaturation (*i.e.* a drop in $SpO_2$ of at least 4% during the exercise test) occurred in 6 patients, and during follow-up, it was detected in further 8 patients. The degree of desaturation

(mean change in $SatO_2$ during CPET) did not change significantly during follow-up. Anaerobic threshold values were close to normal both at baseline and follow-up. Average changes in the different variables were relatively small indicating relatively stable cardiopulmonary function during exercise at the group level. Significant decrease in the $VE/VCO_2$ values during follow-up revealed improved ventilator efficiency.

In order to confirm the clinical validity of CPET in our SSc cohort, first we correlated the values of its parameters at enrolment with the resting lung function test results measured at the same time. Significant correlations ($p<0.05$) are presented in Table IV (second column). Many of the baseline CPET parameters correlated with baseline DLCO (in most instances with both absolute values expressed in ml/min/mmHg and % of predicted values), a gold-standard parameter most commonly used during the follow-up of SSc patients to screen for and monitor pulmonary parenchymal or vascular progression. As it can be seen, WR, $VO_2$, $VO_2$/kg, VE, AT and AT% all correlated positively, whereas $VE/VCO_2$ negatively with DLCO, indicating that CPET is a feasible and valid examination also in SSc patients, correlating well with the well-established measurement method of gas exchange. WR, $VO_2$, $VO_2$/kg, VE and AT also positively correlated with FVC.

Next, we assessed the correlation between baseline CPET variables and several scleroderma-specific clinical outcome variables, as our aim was to examine whether CPET performed at the diagnosis of SSc may identify disease parameters that require special focus during follow-up. Demographic data, clinical parameters included in the composite end-point, and the annual decrease in DLCO, FVC and desaturation were analysed, the latter three were selected because they are of special importance with regard to disease progression (2). These data are also presented in Table IV (third column). Lower AT% and higher $DeltaSat_{max}$ correlated with the development of digital ulcers. Higher $VE/VO_2$ values were in association with a higher annual increase in PAP. A higher decline in FVC during follow up correlated with higher $VO_2$ and lower $VE/VCO_2$ values at baseline. Furthermore, the presence of desaturation at baseline also positively correlated with the subsequent annual change (increase) in the degree of desaturation ($r=+0.57$, $p<0.05$, data not shown). Not only the baseline value of desaturation ($DeltaSat_{max}$), but also its annual change (*i.e.* increase) correlated with the annual change in DLCO ($p<0.05$, data not shown). Finally, age correlated negatively (data not shown), whereas disease duration positively with several CPET parameters.

As mentioned before, the complexity of CPET offers the opportunity to use it as a general predictor of poor prognosis is SSc. We therefore compared the two patient groups separated as to whether the patients met the serious outcome endpoint (endpoint+, n=16) or did not meet it (endpoint-, n=13). Selected parameters are presented in Table V. None of the clinical variables were different among the two groups, and although the dcSSc subset and patients with ILD were apparently represented in higher prevalence in the endpoint+ group, not even this comparison was statistically significant. PAP was also comparable in the two patient groups. Similarly, none of the resting lung function test variables either as measured at enrolment, nor the change in any of the parameters, were found to differentiate patients with poor outcome from those with a better prognosis. In contrast, desatu-

**Table IV.** Correlations of CPET parameters at enrolment with those of resting lung function tests and with standard follow-up parameters of SSc.
Results of the Pearson's correlation tests with baseline CPET results.

| CPET parameters at enrolment | Resting lung function parameter at enrolment | Standard follow-up parameters (including changes in resting lung function parameters) | Correlation (Pearson's r) ($p<0.05$) |
|---|---|---|---|
| WR | DLCO (% pred) | | +0.60 |
| | DLCO (ml/min/mmHg) | | +0.77 |
| | FVC (L) | | +0.76 |
| $VO_2$ | DLCO (% pred) | | + 0.52 |
| | DLCO (ml/min/mmHg) | | +0.76 |
| | FVC (L) | | +0.72 |
| | | DeltaFVC/year (%pred) | - 0.43 |
| | | DeltaFVC/year (L) | -0.42 |
| $VO_2$/kg | FVC (L) | | +0.33 |
| | DLCO (ml/min/mmHg) | | +0.46 |
| VE | DLCO (%pred) | | +0.45 |
| | DLCO (ml/min/mmHg) | | +0.58 |
| | FVC (L) | | +0.58 |
| AT | DLCO (%pred) | | +0.40 |
| | DLCO (ml/min/mmHg); FVC | | +0.61 |
| | | | +0.44 |
| | | duration of Raynaud's phenomenon | +0.43 |
| AT% | DLCO (%pred) | | +0.42 |
| | | duration of Raynaud's phenomenon | +0.41 |
| | | digital ulcers | -0.44 |
| $VE/VO_2$ | TLC (L) | | -0.46 |
| | | DeltaPAP/year | +0.73 |
| $VE/VCO_2$ | DLCO (%pred) | | -0.43 |
| | DLCO (ml/min/mmHg) | | -0.50 |
| $VE/VCO_2$ | | DeltaFVC/year (%pred) | +0.58 |
| | | DeltaFVC/year (L) | +0.57 |
| $DeltaSat_{max}$ | | digital ulcers | +0.53 |

For the explanation of the abbreviations, please refer to Materials and methods section, or the legends to Tables II and III. In the fourth column + Pearson's r values mean positive correlation, whereas – means inverse correlation.
$p<0.05$ for all correlations presented in the Table.

**Table V.** Comparison of selected variables between the patient subgroups who met and who did not meet the composite end-point of unfavourable outcome.

| | Endpoint + (n=16) | | Endpoint – (n=13) | |
|---|---|---|---|---|
| Age | 48.56 | (16-64) | 50.69 | (29-62) |
| dcSSc/lcSSc | 10/6 | | 5/8 | |
| ILD | 11 | (69%) | 5 | (39%) |
| Digital ulcer | 7 | (44%) | 5 | (39%) |
| Calcinosis | 2 | (6%) | 1 | (8%) |
| PAP | 28.82 | (18-44) | 28.22 | (24-35) |
| DeltaPAP/year | 1.675 | (-8 - 12) | 0.6083 | (-0.4-2) |
| DLCO | 58 | (31-94) | 55 | (20-79) |
| WR | 107.6 | (46-187) | 93.17 | (66-126) |
| $VO_2$ (L/min) | 1.45 | (0.5-2.40) | 1.21 | (0.96-1.69) |
| $VCO_2$ (L/min ) | 1.54 | (0.45-2.53) | 1.43 | (1.10-1.85) |
| VO2/kg (ml/min/kg) | 19.47 | (9.5-28.2) | 19.85 | (14.2-36.5) |
| VE (L/min) | 52.19 | (17.6-85.6) | 50.4 | (35.8-81) |
| $VE/VCO_2$ | 32.73 | (28-41) | 33.8 | (26-42) |
| AT (L/min) | 1.01 | (0.58-1.56) | 0.99 | (0.65-1.91) |
| AT (% of VO2max,pred) | 47.07 | (27-66) | 49.00 | (37-76) |
| Decrease in $SpO_2$ (%) | 5.3 | (3-12) | 11.75 | (7-20)* |
| Desaturation (n) | 10 | | 4* | |
| DeltaDesat/year ($SpO_2$%) | 1.07 | (-1 – 2.5) | 0.80 | (-1.4-6.6) |

Numbers indicate mean (range) or absolute number (percentage).
*$p<0.05$; $p=0.07$.

ration occurred less frequently in the patients who did not meet the endpoint (endpoint- patients) ($p<0.05$ with $chi^2$ test, odds ratio: 5.63 [CI: 1.08-29.38]). The mean maximal desaturation at any CPET examination (*i.e.*, the highest $SpO_2$ reduction value during exercise) was actually higher in the endpoint- patients than in the endpoint+ group, but this is counterbalanced by the fact that the number of patients who experienced desaturation was lower in the endpoint- group (4 of 13 *vs.* 10 of 14). Logistic regression of baseline CPET values did not identify any CPET parameter to indicate a significantly higher likelihood of the development of endpoint, but the annual change in desaturation displayed a near-significant risk ($p=0.07$). We also determined the specificity and sensitivity of the baseline CPET parameters to differentiate patients in whom a subsequent endpoint will evolve with ROC analysis, but none of the calculated cut-off values provided a statistically significant discriminative power.

Since the CPET values displayed a significant variation within the cohort, including a considerable number of patients with markedly increased and decreased values, resulting in near-normal mean values, we supposed that this may confound meaningful changes. In order to eliminate this problem, we have identified patients from the cohort with severely or moderately abnormal CPET results and compared them with the rest of the cohort. For this purpose, two groups were separated: 1.) patients with at least two measurement values below the 25$^{th}$ percentile of WR, $VO_2$, $VO_2$/kg, AT (L/min) or AT% at inclusion (severely abnormal – n=4), and 2.) patients with at least three results below the 50$^{th}$ percentile of the above-mentioned parameters (moderately abnormal – n=10). Comparisons of these groups with the respective remaining parts of the cohort, however, revealed only one significant difference: the degree of desaturation/year proved to be on average negative (decreasing) in the patients with severely abnormal baseline CPET values (-1.0 [-1.2-0%]), whereas it increased in the rest of the cohort (1.15 [0.76-2.0]) ($p=0.01$). Age, disease duration, and poor prognostic values, including ILD, digital ulcer, need for immunosuppressive therapy and the composite endpoint were not different in any of the comparisons.

Finally, our data indicate that resting lung function parameters reflecting the lung parenchymal involvement (TLC, FVC) on average do not display a noticeable change during follow-up in our cohort, and therefore the moderate decrease of DLCO at baseline (mean: 57%) suggests that we should focus on pulmonary vascular involvement when assessing the value of CPET. We therefore separated the patients with either an increase of >3 mmHg in PAP or >2% decrease in DLCO annually (n=12), and compared their CPET results with the remaining patients. This comparison also revealed one remarkable difference: $VCO_2$ at baseline was markedly lower in the group of patients in whom these parameters subsequently worsened more rapidly (mean: 1.29 *vs.* 1.66, $p=0.06$), indicating that $CO_2$ output might as well be a useful predictor of pulmonary vascular insufficiency.

## Discussion

An unmet need in the management of SSc patients is the accurate early identification of patients with rapid disease progression and with a high risk of irreversible organ damage and a consequent poor prognosis. The diagnostic delay is still rather long in scleroderma, and therefore early signs of lung, heart or vascular involvement are detectable in many patients at diagnosis. However, the identification of patients for example with progressive ILD – in contrast to those, in whom the initial interstitial lung changes remain relatively stable – typically requires repeated assessments of resting lung function, of the extent of involvement with HRCT, and of other clinical variables such as Rodnan skin score. Biomarkers (28-32) or 18 fluoro-desoxy-glucose-positron-emission tomography (33) have also been tested for such purposes, but information is still limited (34, 35).

In the present study, we did not wish to focus on specific organ involvements of SSc, but rather we intended to exploit the complexity of the evaluation of CPET, and hypothesised that it may thus provide prognostic information about multiple adverse disease outcomes. We have, therefore designed a composite end-point in which the individual parameters reflect the value of CPET to inform about the full spectrum of aerobic metabolism starting from oxygen uptake (deltaDLCO) through pulmonary and systemic circulation to tissue oxygenation (digital ulcers). Our end-point is somewhat arbitrary,

but composite end-points are not at all unique in SSc research. Several studies have used combinations of death, event-free survival (the lack of major cardiovascular or pulmonary or renal complications), worsening in the Scleroderma-HAQ functional index, time until major organ damage, or initiation of parenteral prostanoid therapy or long-term oxygen therapy due to worsening of PAH as components of composite end-points (36-39). In addition, our outcome measures have widely been used as single end-points in SSc studies too (40-44). We have found that the detection of desaturation at the first CPET examination, at the beginning of the follow-up indicates worse overall prognosis, i.e. the development of the composite end-point. Higher degree of desaturation at onset also predicted the development of digital ulcers, as well as a more rapid worsening of DLCO, and a faster subsequent increase in desaturation at exercise, rendering the measurement of oxygen saturation as a good indicator of the development of worsening exercise capacity and cardiovascular function. Patients with multiple and marked abnormalities in CPET (in particular, WR, $VO_2$, $VO_2$/kg and AT) at enrolment also have a higher likelihood to develop exercise-induced severe desaturation during follow-up. $VCO_2$, AT and $VE/VO_2$ also appear as useful predictors of selected outcome measures: low $VCO_2$ appeared as an indirect indicator of the development of pulmonary vascular insufficiency. Low AT at baseline correlated with the development of digital ulcers and high $VE/VO_2$ with that of pulmonary hypertension.

CPET parameters also correlated well with many of the standard resting assessment tools, indicating that this test is feasible in SSc patients too. It is important to note that CPET provides a general overview about the state of the aerobic metabolism, and is influenced by ventilation, alveolo-capillary gas-exchange, systemic and pulmonary circulation, cardiac output, microvascular capillary structure and flow and the state of intracellular oxidative processes. Although some CPET parameters can be more or less associated with specific organ dysfunction (23), it is considered more useful to predict survival or general function, as it has been shown in lung-transplant patients (7) or chronic obstructive pulmonary disease (6). This is the reason why we have constructed a composite endpoint of frequent and widely-used cardiovascular or pulmonary complications or death.

Our study indicated that CPET can provide additional information to identify SSc patients in whom the disease could run a progressive course and a poor prognosis may develop. If SSc patients display desaturation during exercise, they have a higher likelihood to experience progressive ILD, PH or peripheral vascular lesions. Importantly, standard resting lung function and echocardiographic parameters were insufficient to predict the development of such unfavourable outcome. This makes CPET a promising non-invasive examination method to estimate the progression of SSc.

Several clinical parameters, including diffuse cutaneous subset, various autoantibody-positivities, the presence of ILD or increased PAP at presentation have been proven to be helpful in identifying patients who need a closer follow-up and more aggressive therapy (1, 2, 4, 25-27). The value of decreased or decreasing DLCO or FVC and elevated or progressively increasing PAP to select patients at risk of high morbidity or mortality has also been convincingly confirmed and regular measurement of these parameters is strongly advocated during the follow-up of SSc patients (25, 26, 45). Our cohort included consecutive SSc patients with heterogeneous, but often relatively mild clinical presentation, and patients with advanced cardiopulmonary or other organ damage were excluded. Most of the patients had relatively short disease duration since the first non-Raynaud symptom. Although the follow-up time was acceptably long, these factors, together with the fact that the cohort is not as high as those in whom the above-mentioned predictive factors were identified, may explain why we did not find the standard clinical and lung function tests or echocardiography to show a significant correlation with the occurrence of the serious outcome end-point. With these limitations in mind, it is even more noteworthy that various CPET parameters, being strongly linked to oxygen utilisation, were able to discriminate between patients with or without endpoint (desaturation during exercise), or to correlate with adverse clinical outcomes, such as a more rapid decline in FVC or increase in PAP, or the development of digital ulcers ($VO_2$, $VE/VCO_2$, $VE/VO_2$, desaturation and anaerobic threshold).

There is a number of studies that have previously already addressed the value of CPET in SSc (11-22). Unlike our investigation, these studies have addressed almost exclusively two issues: First, they have searched whether CPET can help to discriminate among causes of reduced exercise capacity in SSc, and found certain CPET parameters to be associated with pulmonary vasculopathy, left ventricular dysfunction or respiratory limitation, respectively (12, 13, 15, 16), in some cases even in those patients in whom resting lung function tests and echocardiography failed to disclose such evident causes. The other area of research was to assess whether CPET may aid in the detection of PAP in SSc patients (11, 14, 18, 19). Dumitrescu *et al*. have prospectively analysed 173 SSc patients, in whom PAP was verified by right heart catheterisation, and found peak$VO_2$ and $VE/VCO_2$ to show the highest correlations with PAP, transpulmonary pressure gradient and pulmonary vascular resistance (11). Of several parameters with high sensitivity and specificity for PH detection, peak$VO_2$ showed highest diagnostic accuracy (sensitivity 87.5%, specificity 74.8% at a threshold level of 13.8 mL/min/kg). These investigations confirm that CPET is a feasible examination in SSc patients too, similarly to chronic lung disease patients. However, the above-cited publications concluded that although CPET adds certain benefits during the work-up of dyspnea or fatigue in SSc, the standard assessment techniques, such as HRCT, resting echocardiography or right heart catheterisation for the verification of the particular cardiopulmonary organ manifestations are still not replaced by CPET. Since the highest value of CPET

is that it provides a comprehensive overview about oxygen utilisation from the alveolo-capillary gas exchange to cellular metabolism, we have decided to set up endpoints that are the most relevant to patient mortality and morbidity, rather than to analyse individual organ involvements. Indeed, data about the value of CPET to assess functional outcome or survival in SSc patients is rather limited. We have found two publications about the association of between composite functional indices and CPET: Cuomo *et al*. have found that $VO_2$/kg was the only parameter that showed a significant correlation between the HAQ-DI functional index and various CPET parameters (20), whereas in another study, $VE/VCO_2$ correlated positively with both the Disease Activity Index and the Disease Severity Scale (21). One further study examined the connection between CPET and patient survival. Similarly to our results, Swigris *et al*. also demonstrated that desaturation (a drop in SpO2max below 89% or >4 percentage points) increased the risk of death during a median follow-up of 7.1 years, but it has to be noted that this study focused exclusively on SSc patients with interstitial lung disease, and it was a retrospective study (22). The recently published prospective follow-up study on the patients from the PHAROS registry, although it did not apply CPET, also confirmed that desaturation on exercise (6 minute walking test) is a predictor of mortality in SSc (46).

Our study has some weaknesses and strengths. The patient number is not as high as in many previous examinations especially in multicentre cohorts, but still it was sufficient for the identification of some statistically significant findings. We did not include right heart catheterisation in the protocol to corroborate the measurement of pulmonary arterial pressure with this most accurate method because, as we mentioned, our primary aim was not to focus on PH, and because most of the patients had relatively early disease with no significant risk factors for PH, and we decided that right heart catheterisation was not indicated and ethically established. However, we did perform right heart catheterisation in patients in whom the estimated PAP was >50 mmHg, and there was only one such patient in this cohort. A strength of the investigation is that to our knowledge, this is the first study with prospective follow-up of SSc patients and repeated CPET examinations, so we could provide data about the time-course of CPET parameters during several years of follow-up in SSc. Unlike most of the previous studies, we did not selectively analyse various organ manifestations in SSc, but concentrated on the most crucial outcomes of SSc, and we believe that such approach could help to correctly define the place of CPET in the care of SSc patients: to predict the appearance of complications that are related to oxygen-dependent processes, from tissue necrosis and PAP to patient survival.

## References


1. RUBIO-RIVAS M, ROYO C, SIMEÓN CP, CORBELLA X, FONOLLOSA V: Mortality and survival in systemic sclerosis: systematic review and meta-analysis. *Semin Arthritis Rheum* 2014; 44: 208-19.
2. POKEERBUX MR, GIOVANNELLI J, DAUCHET L *et al*.: Survival and prognosis factors in systemic sclerosis: data of a French multicenter cohort, systematic review, and meta-analysis of the literature. *Arthritis Res Ther* 2019; 21: 86.
3. WELLS AU: High-resolution computed tomography and scleroderma lung disease. *Rheumatology* 2008; 47: v59-v61.
4. MATHAI SC, HUMMERS LK, CHAMPION HC *et al*.: Survival in pulmonary hypertension associated with the scleroderma spectrum of diseases: impact of interstitial lung disease. *Arthritis Rheum* 2009; 60: 569-77.
5. COGHLAN JG, DENTON CP, GRÜNIG E *et al*.: Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann Rheum Dis* 2014; 73: 1340-9.
6. STRINGER W, MARCINIUK D: The role of cardiopulmonary exercise testing (CPET) in pulmonary rehabilitation (PR) of chronic obstructive pulmonary disease (COPD) patients. *COPD* 2018; 15: 621-31.
7. LAYTON AM, ARMSTRONG HF, KIM HP, MEZA KS, D'OVIDIO F, ARCASOY SM: Cardiopulmonary exercise factors predict survival in patients with advanced interstitial lung disease referred for lung transplantation. *Respir Med* 2017; 126: 59-67.
8. DUDLEY KA, EL-CHEMALY S: Cardiopulmonary exercise testing in lung transplantation: A review 2012. *Pulm Med* 2012: 237852
9. PTASZYŃSKA-KOPCZYŃSKA K, KRENTOWSKA A, SAWICKA E *et al*.: The strengths and weaknesses of non-invasive parameters obtained by echocardiography and cardiopulmonary exercise testing in comparison with the hemodynamic assessment by the right heart catheterization in patients with pulmonary hypertension. *Adv Med Sci* 2017; 62: 39-44.
10. ZHAO QH, WANG L, PUDASAINI B *et al*.: Cardiopulmonary exercise testing improves diagnostic specificity in patients with echocardiography-suspected pulmonary hypertension. *Clin Cardiol* 2017; 40: 95-101.
11. DUMITRESCU D, NAGEL C, KOVACS G *et al*.: Cardiopulmonary exercise testing for detecting pulmonary arterial hypertension in systemic sclerosis. *Heart* 2017; 103: 774-82.
12. BOUTOU AK, PITSIOU GG, SIAKKA P *et al*.: Phenotyping exercise limitation in systemic sclerosis: the use of cardiopulmonary exercise testing. *Respiration* 2016; 91: 115-23.
13. MARTIS N, QUEYREL-MORANNE V, LAUNAY D *et al*.: Limited Exercise Capacity in Patients with Systemic Sclerosis: Identifying Contributing Factors with Cardiopulmonary Exercise Testing. *J Rheumatol* 2018; 45: 95-102.
14. NINABER M, HAMERSMA W, SCHUERWEGH A, KOVACS G, OLSCHEWSKI H, STOLK J: Oxygen pulse slope analysis during exercise testing identifies patients with systemic sclerosis at a possible risk for developing pulmonary vasculopathy. *Eur Respir J* 2013; 42: P3969.
15. TZILAS V, BOUROS D: Cardiopulmonary exercise testing in systemic sclerosis: 'Ars longa, vita brevis'. *Respiration* 2016; 91: 202-3.
16. WALKEY AJ, IEONG M, ALIKHAN M, FARBER HW: Cardiopulmonary exercise testing with right-heart catheterization in patients with systemic sclerosis. *J Rheumatol* 2010; 37: 1871-7.
17. SCHWAIBLMAIR M, BEHR J, FRUHMANN G: Cardiorespiratory responses to incremental exercise in patients with systemic sclerosis. *Chest* 1996; 110: 1520-5.
18. DUMITRESCU D, OUDIZ RJ, KARPOUZAS G *et al*.: Developing pulmonary vasculopathy in systemic sclerosis, detected with noninvasive cardiopulmonary exercise testing. *PLoS One* 2010; 5: e14293.
19. KOVACS G, MAIER R, ABERER E *et al*.: Assessment of pulmonary arterial pressure during exercise in collagen vascular disease: echocardiography vs right-sided heart catheterization. *Chest* 2010; 138: 270-8.
20. CUOMO G, SANTORIELLO C, POLVERINO F, RUOCCO L, VALENTINI G, POLVERINO M: Impaired exercise performance in systemic sclerosis and its clinical correlations. *Scand J Rheumatol* 2010; 39: 330-5.
21. ROSATO E, ROMANIELLO A, MAGRÌ D *et al*.: Exercise tolerance in systemic sclerosis patients without pulmonary impairment: correlation with clinical variables. *Clin Exp Rheumatol* 2014; 32 (Suppl. 86): S103-8.
22. SWIGRIS JJ, ZHOU X, WAMBOLDT FS *et al*.: Exercise peripheral oxygen saturation (SpO2) accurately reflects arterial oxygen saturation (SaO2) and predicts mortality in systemic sclerosis. *Thorax* 2009; 64: 626-30.
23. ALBOUAINI K, EGRED M, ALAHMAR A, WRIGHT DJ: Cardiopulmonary exercise testing and its application. *Postgrad Med J* 2007; 83: 675-82.

24. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Ann Rheum Dis* 2013; 72: 1747-55.
25. SMITH V, SCIRÈ CA, TALARICO R *et al*.: Systemic sclerosis: state of the art on clinical practice guidelines. *RMD Open* 2019; 4: e000782.
26. KOWAL-BIELECKA O, FRANSEN J, AVOUAC J *et al*.: Update of EULAR recommendations for the treatment of systemic sclerosis. *Ann Rheum Dis* 2017; 76: 1327-39.
27. HANKINSON JL, ODENCRANTZ JR, FEDAN KB: Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159: 179-87.
28. DE LAURETIS A, SESTINI P, PANTELIDIS P *et al*.: Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. *J Rheumatol* 2013; 40: 435-46.
29. CARULLI MT, HANDLER C, COGHLAN JG, BLACK CM, DENTON CP: Can CCL2 serum levels be used in risk stratification or to monitor treatment response in systemic sclerosis? *Ann Rheum Dis* 2008; 67: 105-9.
30. HOFFMANN-VOLD AM, TENNØE AH, GAREN T *et al*.: High level of chemokine CCL18 is associated with pulmonary function deterioration, lung fibrosis progression, and reduced survival in systemic sclerosis. *Chest* 2016; 150: 299-306.
31. KUMÁNOVICS G, GÖRBE E, MINIER T, SIMON D, BERKI T, CZIRJÁK L: Follow-up of serum KL-6 lung fibrosis biomarker levels in 173 patients with systemic sclerosis. *Clin Exp Rheumatol* 2014; 32 (Suppl. 86): S138-44.
32. VOLKMANN ER, TASHKIN DP, ROTH MD *et al*.: Changes in plasma CXCL4 levels are associated with improvements in lung function in patients receiving immunosuppressive therapy for systemic sclerosis-related interstitial lung disease. *Arthritis Res Ther* 2016; 18: 305.
33. BELLANDO-RANDONE S, TARTARELLI L, CAVIGLI E *et al*.: 18F-fluorodeoxyglucose positron-emission tomography/CT and lung involvement in systemic sclerosis. *Ann Rheum Dis* 2019; 78: 577-8.
34. ORLANDI M, LEPRI G, DAMIANI A *et al*.: One year in review 2020: systemic sclerosis. *Clin Exp Rheumatol* 2020; 38 (Suppl. 125): S3-17.
35. MERKEL PA, CLEMENTS PJ, REVEILLE JD, SUAREZ-ALMAZOR ME, VALENTINI G, FURST D: Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6. *J Rheumatol* 2003; 30: 1630-47.
36. SULLIVAN KM, GOLDMUNTZ EA, KEYES-ELSTEIN L *et al*.: SCOT Study Investigators. Myeloablative autologous stem-cell transplantation for severe scleroderma. *N Engl J Med* 2018; 378: 35-47.
37. VAN LAAR JM, FARGE D, SONT JK *et al*.: EBMT/EULAR Scleroderma Study Group. Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. *JAMA* 2014; 311: 2490-8.
38. WANGKAEW S, THONGWITOKOMARN H, PRASERTWITTAYAKIJ N, EUATHRONGCHIT J: Rapid skin thickness progression rate is associated with high incidence rate of cardiopulmonary complications in patients with early diffuse cutaneous systemic sclerosis: inception cohort study. *Clin Exp Rheumatol* 2020; 38 (Suppl. 125): S98-105.
39. GAINE S, CHIN K, COGHLAN G *et al*.: Selexipag for the treatment of connective tissue disease-associated pulmonary arterial hypertension. *Eur Respir J* 2017; 50: 1602493.
40. AYANO M, TSUKAMOTO H, MITOMA H *et al*.: CD34-selected versus unmanipulated autologous haematopoietic stem cell transplantation in the treatment of severe systemic sclerosis: a post hoc analysis of a phase I/II clinical trial conducted in Japan. *Arthritis Res Ther* 2019; 21: 30-8.
41. SAUNDERS P, TSIPOURI V, KEIR GJ *et al*.: Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial. *Trials* 2017; 18: 275.
42. BRATIS K, LINDHOLM A, HESSELSTRAND R *et al*.: CMR feature tracking in cardiac asymptomatic systemic sclerosis: Clinical implications. *PLoS One* 2019; 14: e0221021.
43. ROSATO E, LEODORI G, GIGANTE A, DI PAOLO M, PAONE G, PALANGE P: Reduced ventilatory effciency during exercise predicts major vascular complications and mortality for interstitial lung disease in systemic sclerosis. *Clin Exp Rheumatol* 2020; 38 (Suppl. 125): S85-S91.
44. ELHAI M, BOUBAYA M, DISTLER O *et al*.: Outcomes of patients with systemic sclerosis treated with rituximab in contemporary practice: a prospective cohort study. *Ann Rheum Dis* 2019; 78: 979-87.
45. COTTIN V, BROWN KK: Interstitial lung disease associated with systemic sclerosis (SSc-ILD). *Respir Res* 2019; 20: 13-19.
46. HSU VM, CHUNG L, HUMMERS LK *et al*.: Risk factors for mortality and cardiopulmonary hospitalization in systemic sclerosis patients at risk for pulmonary hypertension, in the PHAROS Registry. *J Rheumatol* 2019; 46: 176-83.