Improvement of microangiopathy after haematopoietic stem cell transplantation in systemic sclerosis

M. Boonstra[1], J. Ciaffi[2], D.C. Noordhoek[1], S.I.E. Liem[1], L.J.J. Beaart-van de Voorde[1], S.T.A. van Bijnen[3], T.W.J. Huizinga[1], M.C. Vonk[3], J.K. de Vries-Bouwstra[1]

[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;
[2]Medicine and Rheumatology Unit, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy;
[3]Department of Rheumatology, Radboudumc, Nijmegen, The Netherlands.

Abstract

Objective

Haematopoietic stem cell transplantation (HSCT) is a treatment option for patients with severe systemic sclerosis (SSc), but the efficacy of the procedure in remodelling the nailfold microvascular array is largely unknown. Therefore, this study aimed to evaluate the effect of HSCT on microangiopathy assessed through nailfold capillaroscopy (NC) and to compare the results with findings in patients receiving conventional immunosuppression.

Methods

We included SSc patients with severe SSc and whose pre- and post-treatment NC images were available. Findings in patients treated with HSCT were compared with patients not treated with HSCT. Images were scored by two independent observers blinded for clinical data and treatment history. Capillary pattern was determined and semiquantitative scores from 0 (no changes) to 3 (>66% alterations per millimetre) were used to quantify the degree of specific microvascular characteristics. Changes in severity of microangiopathy between baseline and post-treatment were compared between groups.

Results

Images of 18 HSCT patients and 21 controls were scored. From baseline to follow-up, 33% of HSCT patients showed improvement from scleroderma pattern to normal NC, compared to 6% of controls (p=0.15). Pre- to post-treatment differences in semiquantitative scores showed significant improvement in HSCT patients compared to controls regarding capillary loss (-0.5 vs. 0.0, p<0.05) and disorganisation (-0.8 vs. 0.0, p<0.05).

Conclusion

The degree of microangiopathy improved significantly in severe SSc patients treated with HSCT compared with patients receiving conventional immunosuppressive therapy.

Key words

systemic sclerosis, nailfold capillaroscopy, microangiopathy, haematopoietic stem cell transplantation, improvement

*Maaike Boonstra, MD**
*Jacopo Ciaffi, MD**
D. Christine Noordhoek, MD
Sophie I.E. Liem, MD
Liesbeth J.J. Beaart-van de Voorde, MSc
Sandra T.A. van Bijnen, MD, PhD
Tom W.J. Huizinga, MD, PhD
Madelon C. Vonk, MD, PhD
Jeska K. de Vries-Bouwstra, MD, PhD

**These authors contributed equally.*

Please address correspondence to:
Jacopo Ciaffi,
Medicina e Reumatologia,
IRCCS Istituto Ortopedico Rizzoli,
via Pupilli 1,
40136 Bologna, Italy.
E-mail: jacopo.ciaffi91@gmail.com

Rececived on July 13, 2022; accepted in revised form on October 10, 2022.

Competing interests: none declared.

Introduction

Systemic sclerosis (SSc) is a rare disorder characterised by microvascular damage, autoimmunity and fibrosis. The pathogenesis is unclear, with a possible role of genetic, hormonal, environmental and occupational factors (1-3), but the available evidence suggests that microvascular damage and dysfunction represent the earliest morphological and functional markers of the disease (4). The degree of vasculopathy can be evaluated through nailfold capillaroscopy (NC). This method allows to detect the typical abnormalities observed in SSc and, since these alterations are dynamic, sequential NC may show worsening of microangiopathic patterns during the disease (5). Several studies have hypothesised an association between severity of microangiopathy and disease activity or organ involvement in SSc (6, 7). However, a small proportion of patients may experience improvement of capillary damage, with two early reports describing rapid and substantial amelioration of capillary morphology after haematopoietic stem cell transplantation (HSCT) in, respectively, 1 and 6 cases (8, 9). These encouraging results have been corroborated in a recent study by Santana-Gonçalves *et al.* (10) who observed a significant increase in the capillary density and a reduction in the number of giant capillaries after HSCT in 27 SSc patients. However, controls were not included. Based on the limited number of patients thus far, the lack of adequate control groups and the different methods applied to evaluate the degree of microangiopathy, it is difficult to draw conclusions on the efficacy of HSCT on microangiopathy in SSc. Therefore, we decided to evaluate NC images after HSCT in SSc patients and to compare the observed changes with a control group of SSc patients treated with conventional immunosuppressive therapy.

Methods

Patients

The study population was composed of adult patients enrolled in the Leiden Combined Care in SSc (CCISS) cohort and in the Nijmegen SSc inception cohort at Radboud University Medical Center. Both centres participated in the ASTIS trial and have applied ASTIS criteria for transplantation after completion of this trial (11). For the current study, all patients aged >18 years, with a diagnosis of diffuse cutaneous SSc meeting the ACR 1980 preliminary classification criteria (12), were selected if clinical ASTIS inclusion criteria were fulfilled at any time during follow-up. The only exception was that patients older than 65 years, which were excluded from the ASTIS trial, could be included in our study.

The criteria that had to be fulfilled were: A) disease duration ≤4 years and modified Rodnan Skin Score (mRSS) ≥15 with diffusing capacity of the lungs for carbon monoxide (DLCO) and/or forced vital capacity (FVC) ≤80% and radiologic evidence of interstitial lung disease (ILD); or B) disease duration ≤2 years, mRSS ≥20, erythrocyte sedimentation rate (ESR) >25 mm/1st hour and/or haemoglobin <11.0 g/dl. Patients fulfilling the abovementioned criteria were included in the study if longitudinal capillaroscopic assessment had been performed. Exclusion criteria were: A) age <18 years, B) unavailability of pre-treatment or post-treatment NC images.

To evaluate specific changes in NC abnormalities over time attributable to HSCT, patients treated with HSCT were classified as cases and their NC images were compared with those of the remaining patients fulfilling the criteria of severe SSc who had not received HSCT, included as frame of reference and defined as control group. Controls could be treated with any therapeutic regimen, except HSCT. For both cohorts, ethical approval was obtained from local ethics committees and participants had provided written informed consent.

Nailfold capillaroscopy scoring

For all patients, baseline and follow-up NC images, acquired with standardised methodology (13), were retrospectively evaluated. The last available images collected before the timepoint of fulfilling inclusion criteria were selected as baseline. For post-treatment evaluation, the last available images were selected

for scoring, provided that these were obtained at least 3 months after the procedure in the HSCT group or after the introduction of immunosuppressive treatment in the control group.

Two observers (MB and CN) independently scored all images blinded for patients' characteristics and treatment strategy. Baseline and follow-up NCs were directly compared. Images of the second-to-fifth fingers of both hands were collected. NCs were classified as "non-scleroderma pattern", or as "early", "active", or "late" "scleroderma pattern" (14). Capillary density was recorded based on the image with the worst pattern. Abnormalities such as capillary density reduction, dilatations, giants, microhaemorrhages and ramifications were assessed using semiquantitative scales from 0 to 3 (0: no abnormalities/reduction; 1: <33% abnormalities/reduction; 2: 33–66% abnormalities/reduction; 3: >66% abnormalities/reduction) (15-17).

Finally, a 0–100 visual analogue scale (VAS) score was expressed to summarise the overall degree of microangiopathy, with higher scores representing worse vasculopathy.

For each parameter, mean score of the two observers was used. Interobserver agreement (intraclass correlation coefficient [ICC]) was high for capillary density (ICC: 0.84), giant capillaries (ICC: 0.90), microhaemorrhages (ICC: 0.71), capillary loss (ICC: 0.83), disorganisation (ICC: 0.73), qualitative pattern (ICC: 0.78) and VAS (ICC: 0.91), moderate for capillary dilatations (ICC: 0.68) and bad for neo-angiogenesis (ICC: 0.08). Therefore, we excluded neo-angiogenesis from the analysis.

Statistical analysis

Baseline characteristics were compared using 2-sample *t*-test, Mann-Whitney U-test and chi-square test when appropriate. Degree of microangiopathy was compared between patients and controls at baseline and post-treatment. Multivariable linear regression including sex, age and NC parameters was performed to determine whether NC changes differed between groups. Adjustment for multiple testing was performed with the Holm-Bonferroni method.

Table I. Baseline characteristics of systemic sclerosis patients treated with haematopoietic stem cell transplantation (HSCT) and controls.

	HSCT patients (n=18)	Control patients (n=21)	*p*-value
Demographic and clinical characteristics			
Female, n (%): male, n (%)	7 (39):11 (61)	12 (57):9 (43)	0.26
Age [years], mean ± SD	45±10	56±15	<0.01
Current smokers, n (%)	4 (22)	3 (14)	0.52
Disease characteristics			
Disease duration [years], median (range)	1.0 (0.0-7.3)	1.3 (0.5-3.5)	0.43
Digital ulcers, n (%)	2 (11)	4 (19)	0.49
ILD on HRCT, n (%)	15 (83)	15 (71)	0.38
Severe ILD, n (%)	7 (39)	2 (10)	0.04
FVC, mean [% of predicted] ± SD	89±24	90±26	0.94
DLCO, mean [% of predicted] ± SD	55±15	65±11	0.04
PAH, n (%)	0	0	
mRSS, mean ± SD	23±9	19±9	0.23
Severe skin involvement, n (%)	4 (22)	3 (14)	0.52
Gastrointestinal involvement, n (%)	11 (61)	10 (48)	0.40
Cardiac involvement, n (%)	3 (17)	3 (14)	0.84
Renal crisis, n (%)	0	2 (10)	0.19
ANA, n (%)	17 (94)	21 (100)	0.27
Anti-centromere, n (%)	0	0	
Anti-topoisomerase I, n (%)	13 (72)	11 (52)	0.20
Anti-RNA polymerase III, n (%)	1 (6)	3 (14)	0.37
Vasoactive therapies			
Calcium channel blockers, n (%)	11 (61)	10 (48)	0.40
Endothelin receptor antagonists, n (%)	1 (6)	3 (14)	0.37
Prostacyclin analogues, n (%)	1 (6)	0	0.27
Phosphodiesterase 5 inhibitors, n (%)	0	0	
Comorbidities			
Hypertension, n (%)	1 (6)	0	0.27
Diabetes, n (%)	0	0	
Thyroid disease, n (%)	0	1 (5)	0.35
AMI or CAD, n (%)	2 (11)	2 (10)	0.87
COPD, n (%)	0	1 (5)	0.35
Nailfold capillaroscopy			
Scleroderma pattern early, n (%)	0	3 (14)	0.10
Scleroderma pattern active, n (%)	5 (28)	4 (19)	0.48
Scleroderma pattern late, n (%)	10 (56)	11 (52)	0.74
Capillary density [a] *, median (range)	4.4 (2.5-7.0)	4.6 (2.8-6.0)	0.81
VAS [b] **, median (range)	72 (30-88)	80 (10-94)	0.94
Categorical score*** for capillary loss [c], median (range)	2.0 (1.5-3.0)	2.5 (0.5-3.0)	0.75
Categorical score*** for haemorrhages [d], median (range)	1.0 (0.0-1.5)	1.0 (0.0-1.5)	0.42
Categorical score*** for dilatations [e], median (range)	2.3 (1.0-3.0)	2.0 (1.0-3.0)	0.67
Categorical score*** for giants [f], median (range)	1.3 (0.0-2.5)	1.5 (0.0-3.0)	0.50
Categorical score*** for disorganisation [g], median (range)	2.3 (1.5-3.0)	2.0 (0.5-3.0)	0.53

Disease duration was defined as time since the development of the first sign of skin thickening. Scores could not be determined in [a] 3 patients; [b] 5 patients; [c,d] 7 patients; [e] 12 patients; [f] 11 patients; [g] 10 patients.
* number of capillaries per millimetre based on the image with the worst pattern.
** VAS score for overall severity of microangiopathy.
*** 0 = no changes to 3 = >66% alterations per millimetre.
AMI: acute myocardial infarction; ANA: anti-nuclear antibodies; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; DLCO: diffusion capacity of the lungs for carbon monoxide; FVC: forced vital capacity; HSCT: haematopoietic stem cell transplantation; ILD: interstitial lung disease; mRSS: modified Rodnan Skin Score; PAH: pulmonary arterial hypertension; VAS: visual analogue scale.

Results

Patients' characteristics and baseline capillaroscopy

Eighteen HSCT and 21 control patients were included (Table I). Of the individuals in the control group, 13 received cyclophosphamide, 6 mycophenolate mofetil (MMF) and 2 methotrexate. At time of treatment, HSCT patients were younger than controls (45 *vs*. 56 years, $p<0.01$), but had similar disease duration (1.0 *vs*. 1.3 years, $p=0.43$) and

similar mRSS (23±9 *vs*. 19±9, *p*=0.23). In both groups, 15 patients (83% of the HSCT group and 71% of the control group) had ILD on high-resolution computed tomography (HRCT) of the thorax (*p*=0.38). None of the patients had pulmonary arterial hypertension (PAH). According to the Medsger Disease Severity scale ≥3 (18), severe skin involvement was defined as a mRSS ≥30 and severe lung involvement as DLCO or FVC <50% of predicted. In the HSCT group, 7 (39%) patients had severe ILD compared to 2 (10%) in the control group (*p*=0.04). No difference in the proportion of individuals with severe skin involvement was observed (*p*=0.52). Regarding concomitant treatment with vasoactive agents, calcium channel blockers were administered to 11 (61%) HSCT patients and 10 (48%) controls (*p*=0.40), while 1 patient (6%) in the HSCT group and 3 (14%) in the control group were treated with endothelin receptor antagonists (*p*=0.37) and 1 with prostacyclin analogues in the HSCT group (*p*=0.27).

Baseline images were collected at a median of 12 days (range 0-144) before treatment in the HSCT group and 20 days (range 0–314) in controls (*p*=0.07). Baseline capillaroscopic assessment of HSCT and control patients did not show significant differences in any parameter. In the HSCT group, 56% of patients had a late scleroderma pattern and 28% had an active scleroderma pattern, compared to, respectively, 52% and 14% in the control group (*p*=0.48 for the active pattern and *p*=0.74 for the late pattern).

Change in microangiopathy

Post-treatment images were collected at a median of 2.3 years (range 0.4–5.3) after the procedure in the HSCT and 1.8 years (range 0.3-5.6) after the introduction of immunosuppressive treatment in the control group (*p*=0.43). At the time of follow-up NC, 2 patients in the HSCT group had started immunosuppressive treatment with MMF. Of the patients in the control group, 10 (48%) had changed their therapy starting MMF maintenance after pulse cyclophosphamide and 1 (5%) patient was changed from methotrexate to MMF.

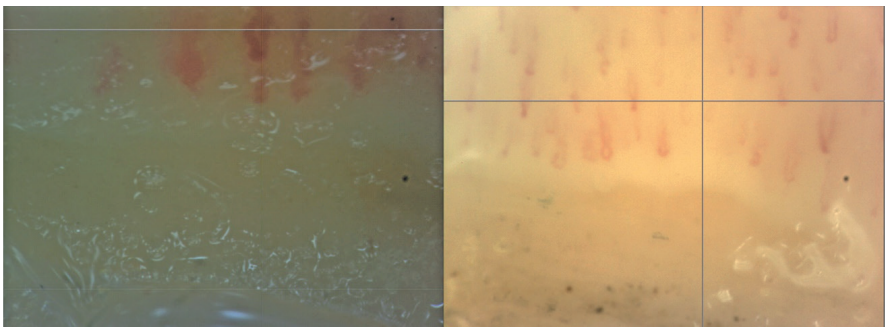


Fig. 1. Example of a nailfold capillaroscopic image of a severe systemic sclerosis patient before (left: mean visual analogue scale score of two observers 88 mm) and after (right: mean Visual Analogue Scale score of two observers 42 mm) treatment with haematopoietic stem cell transplantation.
Both images are captured at a 200x magnification, vertical lines indicate a 1 mm distance from the left side.

No significant differences in the use of vasoactive medications were noted between the groups at follow-up, with 8 (44%) patients receiving calcium channel blockers in the HSCT group and 9 (43%) in the control group (*p*=0.92). One patient in each group was treated with endothelin receptor antagonists (*p*=0.91).

Capillary density after treatment was comparable between groups (median cap/mm 5.5 in HSCT *vs*. 4.5 in controls, *p*=0.30) but VAS for microangiopathy (39 *vs*. 69, *p*=0.01) and number of giant capillaries [0 (range 0–1) *vs*. 1 (range 0–2), *p*<0.01] were lower in the HSCT group.

From baseline to follow-up, 33% of HSCT patients showed improvement from scleroderma pattern to normal NC (Fig. 1), compared to 6% of controls (*p*=0.15). Significant improvement in semiquantitative scores for capillary loss (-0.5 *vs*. 0.0, *p*<0.05) and disorganisation (-0.8 *vs*. 0.0, *vs*.<0.05) was observed in individuals treated with HSCT (Table II). Change in capillary density (+1.0 *vs*. +0.5, *p*=0.46) and VAS scores (-20 *vs*. -3, *p*=0.05), although numerically in favour of the HSCT group, were not significantly different between groups.

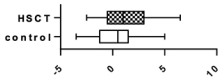
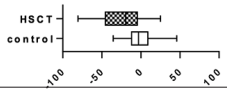
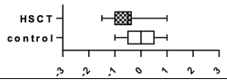
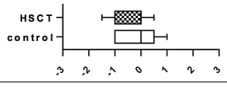
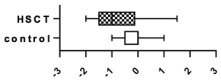
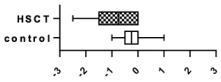
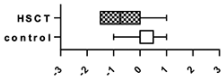
Discussion

Using NC images from two large prospective cohorts, we assessed the evolution of microangiopathy in patients with severe SSc treated with HSCT. Two years after the procedure, we observed less capillary loss, a decrease in number of giant capillaries and a lower degree of disorganisation. In 33% of transplanted patients, NC pattern changed to normal, while this was observed in only 6% of controls. Our data are consistent with previous research hypothesising a role of HSCT in remodelling microvascular architecture (8-10). Aschwanden *et al*. (8) described early NC improvements occurring one month after HSCT. Conversely, we were able to evaluate modifications over two years after the procedure, suggesting that the benefit in terms of peripheral microcirculation might be long-lasting.

Since also control patients received immunosuppressive treatment, an important point would be to elucidate which part of the HSCT regimen might be responsible for more pronounced effect. Microvascular normalisation could be a consequence of regeneration of endothelial precursor cells. The origin of rarefaction of capillaries in this scenario would be based on deficiencies in endothelial precursors, which indeed have been shown to be involved in early stages of the disease (19). Alternatively, HSCT could induce loss of cells responsible for inhibition of endothelial regeneration (20). However, contrasting results can be found in literature, with data highlighting persistently high levels of circulating angiogenic factors after HSCT, suggesting that mechanisms associated with aberrant angiogenic expression are not abated by the transplantation procedure (10). Further research is warranted to unveil the molecular background promoting vascular remodelling after HSCT.

Limitations of our study include the small sample size and the significant differences retrieved between patients

Table II. Change in NC parameters between baseline and follow-up of SSc-patients treated with either haematopoietic stem cell transplantation (n=18) or otherwise (n=21).

		Numbers *	Median (range)	*p*-value **
Normalisation of NC pattern		HSCT = 5/15 control = 1/18		0.15
Change in NC parameters				
Δ capillary density (mm)		HSCT n=15 control n=21	+1.0 (-2.5 to +6.5) +0.5 (-3.5 to +5.0)	0.46
Δ VAS (0-100 mm)		HSCT n=15 control n=19	-20 (-81 to +25) -3 (-36 to +46)	0.05
Δ capillary loss		HSCT n=14 control n=18	-0.5 (-1.5 to +1.0) +0.0 (-1.0 to +1.0)	0.04
Δ haemorrhages		HSCT n=13 control n=18	0.0 (-1.5 to 0.5) 0.0 (-1.0 to +1.0)	0.18
Δ dilatations		HSCT n=12 control n=15	-1.0 (-2.0 to +1.5) 0.0 (-1.0 to +1.0)	0.09
Δ giants		HSCT n=12 control n=16	-0.8 (-2.5 to 0.0) -0.3 (-1.0 to +1.0)	0.49
Δ disorganisation		HSCT n=12 control n=17	-0.8 (-1.5 to +1.0) 0.0 (-1.0 to +1.0)	<0.05

* Numbers differ according to evaluability. Whiskers represent min to max.
***p*-values corrected for multiple testing (Holm-Bonferroni method): Δ capillary loss $p=0.04$, Δ disorganisation $p<0.05$.

and controls in terms of age and DLCO. This is not surprising considering that only patients fulfilling the definition of severe SSc were included, that age >65 years is an exclusion criterion for HSCT, and that there were more patients with severe ILD in the HSCT group than in the control group. We acknowledge that the proportion of patients presenting a late scleroderma pattern at the baseline NC was high in our study, even if the median disease duration was relatively short. However, it is important to highlight that disease duration was defined as the time since development of the first sign of skin thickening, whereas microangiopathy may appear long before skin thickening (21). There is limited literature evidence about NC patterns in selected populations of patients with early progressive SSc. Most data regarding the evolution of the scleroderma pattern evaluated the timing of transition from early to late pattern in unselected cohorts of SSc patients, suggesting median intervals between 3 and 5 years, but possibly shorter in individuals with anti-topoisomerase I antibodies, as the majority of our patients are (5, 22). Therefore, our findings are difficult to compare with current literature but we would not consider them unexpected given the characteristics of the included patients. Additionally, a potential concern in our study is the timing of follow-up NC. We recognise that 3 months is probably a short period to expect changes in microangiopathy after the introduction of an immunosuppressive therapy and, presumably, also after HSCT. Since our study has a retrospective design, the timing of follow-up NCs, which were performed for clinical practice, was not the same in all patients. For this reason, we took a minimum time of 3 months but, when available, later NCs were considered, namely the last available images were scored. Moreover, we decided to use similar methods applied by other studies investigating changes of microangiopathy after HSCT or cyclophosphamide. Miniati *et al*. (9) evaluated patients three months after the beginning of treatment. Aschwanden *et al*. (8) assessed their patient at one month, while Santana-Gonçalves *et al*. did the first NC 6 months after the procedure (10). Since the number of patients included in our study was already limited, we decided to take a cut-off of 3 months in order not to miss relevant information but also to increase the statistical power of our analysis. Follow-up NC was performed before 6 months in only 3 patients of the HSCT group and 4 of the control group. The risk of bias related to early reassessment is, therefore, low. In conclusion, our study corroborates previous observations about the beneficial effects of HSCT on microvasculature in SSc patients. We contribute relevant data to the currently limited knowledge about the evolution of vasculopathy after HSCT, suggesting a more profound impact of the procedure on the vascular niche beyond immune ablation.

References

1. GABRIELLI A, AVVEDIMENTO EV, KRIEG T: Scleroderma. *N Engl J Med* 2009; 360: 1989-2003. https://doi.org/10.1056/nejmra0806188
2. MARIE I, GEHANNO JF: Environmental risk factors of systemic sclerosis. *Semin Immunopathol* 2015; 37: 463-73. https://doi.org/10.1007/s00281-015-0507-3
3. CIAFFI J, VAN LEEUWEN NM, SCHOONES JW, HUIZINGA TWJ, DE VRIES-BOUWSTRA JK: Sex hormones and sex hormone-targeting therapies in systemic sclerosis: A systematic literature review. *Semin Arthritis Rheum* 2020; 50: 140-8. https://doi.org/10.1016/j.semarthrit.2019.07.007
4. DI BATTISTA M, BARSOTTI S, ORLANDI M *et al*.: One year in review 2021: systemic sclerosis. *Clin Exp Rheumatol* 2021; 39 (Suppl. 131): S3-12. https://doi.org/10.55563/clinexprheumatol/izadb8
5. SULLI A, PAOLINO S, PIZZORNI C *et al*.: Progression of nailfold capillaroscopic patterns and correlation with organ involvement in systemic sclerosis: a 12 year study. *Rheumatology* (Oxford) 2020; 59: 1051-8. https://doi.org/10.1093/rheumatology/kez374

6. BOULON C, AIOUAZ S, BLAISE S *et al*.: Correlation between capillaroscopic classifications and severity in systemic sclerosis: results from SCLEROCAP study at inclusion. *Clin Exp Rheumatol* 2019; 37 (Suppl. 119): S63-8.
7. KAYSER C, SEKIYAMA JY, PRÓSPERO LC, CAMARGO CZ, ANDRADE LE: Nailfold capillaroscopy abnormalities as predictors of mortality in patients with systemic sclerosis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): S103-8.
8. ASCHWANDEN M, DAIKELER T, JAEGER KA *et al*.: Rapid improvement of nailfold capillaroscopy after intense immunosuppression for systemic sclerosis and mixed connective tissue disease. *Ann Rheum Dis* 2008; 67: 1057-9. https://doi.org/10.1136/ard.2007.082008
9. MINIATI I, GUIDUCCI S, CONFORTI ML *et al*.: Autologous stem cell transplantation improves microcirculation in systemic sclerosis. *Ann Rheum Dis* 2009; 68: 94-8. https://doi.org/10.1136/ard.2007.082495
10. SANTANA-GONÇALVES M, ZANIN-SILVA D, HENRIQUE-NETO Á *et al*.: Autologous hematopoietic stem cell transplantation modifies specific aspects of systemic sclerosis-related microvasculopathy. *Ther Adv Musculoskelet Dis* 2022; 14: 1759720x221084845. https://doi.org/10.1177/1759720x221084845
11. VAN LAAR JM, FARGE D, SONT JK *et al*.: Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. *JAMA* 2014; 311: 2490-8. https://doi.org/10.1001/jama.2014.6368
12. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23: 581-90. https://doi.org/10.1002/art.1780230510
13. SMITH V, HERRICK AL, INGEGNOLI F *et al*.: Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. *Autoimmun Rev* 2020; 19: 102458. https://doi.org/10.1016/j.autrev.2020.102458
14. CUTOLO M, SULLI A, PIZZORNI C, ACCARDO S: Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000; 27: 155-60.
15. WILDT M, WUTTGE DM, HESSELSTRAND R, SCHEJA A: Assessment of capillary density in systemic sclerosis with three different capillaroscopic methods. *Clin Exp Rheumatol* 2012; 30 (Suppl. 71): S50-4.
16. CIAFFI J, VAN LEEUWEN NM, HUIZINGA TWJ, DE VRIES-BOUWSTRA JK: Cumulative endogenous estrogen exposure is not associated with severity of peripheral microangiopathy in patients with systemic sclerosis. *Clin Exp Rheumatol* 2019; 37 (Suppl. 119): S82-7.
17. CIAFFI J, VAN LEEUWEN NM, HUIZINGA TWJ, DE VRIES-BOUWSTRA JK: Smoking and systemic sclerosis: influence on microangiopathy and expression of anti-topoisomerase I antibodies in a monocentric cohort. *Clin Exp Rheumatol* 2020; 38 (Suppl. 125): 25-8.
18. MEDSGER TA, JR, BOMBARDIERI S, CZIRJAK L, SCORZA R, DELLA ROSSA A, BENCIVELLI W: Assessment of disease severity and prognosis. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S42-6.
19. DEL PAPA N, PIGNATARO F: The role of endothelial progenitors in the repair of vascular damage in systemic sclerosis. *Front Immunol* 2018; 9: 1383. https://doi.org/10.3389/fimmu.2018.01383
20. FLEMING JN, NASH RA, MCLEOD DO *et al*.: Capillary regeneration in scleroderma: stem cell therapy reverses phenotype? *PLoS One* 2008; 3: e1452. https://doi.org/10.1371/journal.pone.0001452
21. BELLANDO-RANDONE S, DEL GALDO F, LEPRI G *et al*.: Progression of patients with Raynaud's phenomenon to systemic sclerosis: a five-year analysis of the European Scleroderma Trial and Research group multicentre, longitudinal registry study for Very Early Diagnosis of Systemic Sclerosis (VEDOSS). *Lancet Rheumatol* 2021; 3: e834-e43. https://doi.org/10.1016/s2665-9913(21)00244-7
22. SULLI A, PIZZORNI C, SMITH V, ZAMPOGNA G, RAVERA F, CUTOLO M: Timing of transition between capillaroscopic patterns in systemic sclerosis. *Arthritis Rheum* 2012; 64: 821-5.