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ORIGINAL RESEARCH

Multicentre retrospective detection of nailfold videocapillaroscopy abnormalities in long covid patients

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ABSTRACT

Background SARS-CoV-2 induces acute non-specific endothelial/microvascular alterations that have been identified by nailfold videocapillaroscopy (NVC). Details on NVC abnormalities in long covid (LC) patients (pts) are unknown.

Methods LC pts without and with systemic sclerosis (non-SSc-LC and SSc-LC), recovered COVID-19 (RC) pts that did not develop LC and healthy matched control subjects (CNT) that underwent NVC examinations were evaluated in a multicentre national study from the Capillaroscopy and Microcirculation in Rheumatic Diseases Study Group of the Italian Society of Rheumatology. Retrospective collection was performed for demographic data, course of SARS-CoV-2 infection, comorbidities, concomitant drugs. NVC alterations were quantified by validated scores. Pre-COVID-19 and post-COVID-19 microvascular status was analysed by NVC.

Results 62 non-SSc-LC pts (49 female/13 male, 51±16 years old), 24 SSc-LC pts (21 female/3 male, 59±17 years old), 23 RC pts (18 female/5 male, 51±18 years old) and 84 CNT (68 female/16 male, 52±12 years old) were analysed. Non-SSc-LC pts showed significantly more dilated capillaries ($p<0.01$, p multivariate<0.01), microhaemorrhages ($p=0.01$, p multivariate<0.05), abnormal shapes ($p<0.05$, p multivariate<0.05) than CNT and of note, lower mean capillary number per linear millimetre ($p<0.01$, p multivariate<0.01) than both RC pts and CTN ($p<0.01$, p multivariate<0.05).
Of highest interest, 16 non-SSc-LC pts showed statistically significantly more dilated capillaries ($p<0.05$) and microhaemorrhages ($p<0.05$) in NVC examinations after COVID-19, compared with pre-COVID-19 status.
Similarly, SSc-LC pts (24) showed significantly lower capillary density ($p=0.01$) and more dilated capillaries ($p<0.01$) in NVC examinations after COVID-19, compared with pre-COVID-19 status.

Conclusions LC pts show more microvascular alterations at NVC as compared with RC patients and CNT, which may contribute to the pathogenesis of persistent organ/systems dysfunction.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ SARS-CoV-2 induces endothelial and capillary damage, that is detectable by nailfold videocapillaroscopy (NVC). NVC shows significant capillary dilations, microhaemorrhages and abnormal shapes in the acute phase of COVID-19 and significant reduction of nailfold capillary density in the short-term recovery phase (3 months).

WHAT THIS STUDY ADDS

⇒ NVC shows significant microvascular damage in long covid (LC) patients compared with matched healthy controls. Dilated capillaries, microhaemorrhages, abnormal shapes and reduced capillary density are still detectable in LC patients 12 months after acute SARS-CoV-2 infection.

⇒ NVC demonstrates normalisation of nailfold capillary density in recovered COVID-19 patients without LC symptoms 12 months after acute SARS-CoV-2 infection.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ NVC reveals significant persistence of microvascular damage in LC patients.

⇒ The involvement of microcirculation is a matter of further investigation in LC pathogenesis and related systemic symptoms.

INTRODUCTION

Long covid (LC) is a widespread clinical chronic condition due to the 'continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation'.[1]

Indeed, LC presents with a variety of symptoms, including general systemic manifestations (malaise, asthenia, muscle pain) and organ-specific involvement, such as the central and/or peripheral nervous system (memory disturbances, cognitive impairments, tremors, paraesthesia), the respiratory system (dyspnoea, cough, shortness of breath) and the cardiovascular system (tachycardia, orthostatic hypotension, chest tightness) among others.[2]

The pathophysiology of SARS-CoV-2 infection involves early damage to endothelial and respiratory epithelial cells, triggering a proinflammatory response.[3] In favourable cases, inflammation allows the elimination of the pathogen and clinical recovery. In unfavourable cases, ineffective viral clearance stimulates a hyperinflammatory response, leading to extensive endothelial damage, prothrombotic phenomena and organ complications, primarily affecting the respiratory and central nervous systems.[4]

After recovery, uncomfortable symptoms of SARS-CoV-2 infection or new clinical manifestations can develop. The persistence of the virus in target cells and tissues is thought to sustain a proinflammatory response, leading to microvascular dysfunction, immune dysregulation, tissue hypoxia and then clinical manifestations of LC.[5]

Nailfold videocapillaroscopy (NVC) is a reliable and safe technique for studying microcirculation, actually mainly in connective tissue diseases (CTDs).[6 7] However, NVC has also been used to assess microvascular damage in COVID-19. During the acute phase of SARS-CoV-2 infection, several non-specific abnormalities have been detected, that is, capillary dilations, microhaemorrhages and pericapillary oedema.[8 9]

In the post-COVID-19 recovery phase, a reduction in the absolute number of capillaries per linear millimetre has been identified as a distinguishing feature of COVID-19 survivors compared with a control healthy matched population.[10 11]

However, to date, no NVC studies focused on the type and intensity of microvascular abnormalities in LC patients.

Therefore, the study group (SG) on Capillaroscopy and Microcirculation in Rheumatic Diseases of the Italian Society of Rheumatology (CAPSIR) has promoted a multicentre Italian retrospective study on the role of NVC in LC patients (CAPSIR_2 project), in agreement and with the consultancy of the equivalent European Alliance of Associations for Rheumatology (EULAR) SG on Capillaroscopy and Microcirculation in Rheumatic Diseases, to evaluate the presence (or not) of microvascular damage in LC patients, compared with recovered COVID-19 (RC) patients that did not develop LC and matched healthy controls (CNT). Additionally, when available, pre-COVID-19 and post-COVID-19 NVC examinations were collected in the same patient to further characterise the microvascular damage inducible by SARS-CoV-2.

MATERIALS AND METHODS

Online surveys for participation of Italian rheumatology centres

Two national exploratory online surveys were recently emailed to the members of CAPSIR SG to gauge the interest of Italian rheumatological teams in participating in the CAPSIR_2 project: 'Study on the role of capillaroscopy in long covid patients'.

The four expert centres (equally distributed between North, Centre, East and South Italy) that up to now contributed actively to the investigation were: Unit of Internal Medicine and Nephrology, Ospedale dell'Aquila (L'Aquila, IT), Unit of Allergology and Clinical Immunology, Ospedale di Teramo (Teramo, IT), Department of Precision Medicine, 'Luigi Vanvitelli' University of Campania (Napoli, IT) and, as leading centre, the Laboratory of Experimental Rheumatology and Academic Division of Clinical Rheumatology, University of Genova (Genova, IT).

Patients and controls

The study used mainly a retrospective cross-sectional design, validated using the Strengthening the Reporting of Observational Studies in Epidemiology checklist for observational study (online supplemental file 3).[12]

In addition to the checklist, a Consolidated Standards of Reporting Trials-like diagram was adopted to show the recruitment of patients (online supplemental file 2).[13]

Three different cohorts of patients with available NVC examinations were enrolled: LC patients without and with systemic sclerosis (SSc) (non-SSc-LC and SSc-LC), RC (patients that did not develop LC) and matched healthy CNT.

Indeed, LC was defined according to the WHO as 'the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation'.[1]

Therefore, patients with SSc were all recruited from the same centre (Napoli, IT) and were classified according to the 2013 American College of Rheumatology/EULAR classification criteria.[14] All SSc diagnoses preceded SARS-CoV-2 infection.

Healthy CNT were recruited from the NVC outpatient clinics of the participating centres, specifically individuals undergoing the examination for Raynaud's phenomenon (RP), peripheral paraesthesia or autoantibody positivity without clinical correlation. None of the controls met the classification criteria for the most common autoimmune CTDs of rheumatologic interest (systemic lupus erythematosus, antiphospholipid syndrome, idiopathic inflammatory myopathies, Sjögren's syndrome, SSc and mixed CTD).[15–19]

Inclusion and exclusion criteria for all enrolled subjects are detailed in online supplemental file 3.

Demographic data were retrospectively collected for the three groups of subjects, including age, sex at birth, ethnicity, smoking habits, concomitant diseases

(comorbidities), SARS-CoV-2 vaccination(s) and treatments. Additionally, information on the presence of RP and autoantibody serological status (antinuclear antibodies—ANA, extractable nuclear antigens—ENA, anticardiolipin antibodies IgM and IgG, anti-beta2-glycoprotein IgM and IgG) was included when available.

For LC and RC patients, time and clinical manifestations at the time of SARS-CoV-2 infection (influenza-like symptoms, pneumonia, thrombotic events and/or acute central nervous system involvement) were retrospectively collected.

On the other hand, manifestations related to LC were categorised into clinical domains: general (fatigue, myalgia, arthralgia, sweats, fever, chills and hair loss), psychoaffective (post-traumatic stress disorder, mood disorders, sleep disorders, anxiety and depression), neurological (memory impairment, cognitive impairment, tremor, paraesthesia, movement disorders, hearing impairment, tinnitus, headache, vertigo, ageusia and anosmia), respiratory (dyspnoea, cough, chest pain and interstitial lung disease), cardiovascular (angina pectoris, arrhythmia, tachycardia and orthostatic hypotension) and gastrointestinal (loss of appetite, nausea, vomiting, diarrhoea, constipation and abdominal pain).

NVC examination

NVC was performed by the same well-trained rheumatologist in each centre (FC for L'Aquila, EA for Teramo, GC for Napoli and CP for Genova) using an optical probe equipped with a video camera and a 200× magnification lens, connected to image analysis software. All patients and CNT underwent NVC examination, avoiding smoking and alcohol consumption before the test and acclimating in the test room for 15 min at a temperature of 22°C.[20] Patients with recent hand/finger trauma and nail-biters were excluded from NVC assessment, as well as subjects with nail polish or who had their cuticles pushed back.

NVC was performed as per routinary and standard clinical indications, as RP, acrocyanosis, 'cold hand' with paraesthesia.

For each subject, two images of the middle region of the nailfold bed (excluding the thumbs) were acquired, along with the following NVC parameters: dilated capillaries (capillary diameter increased to 20–50 µm), giant capillaries (uniformly dilated capillaries with a diameter ≥50 µm), microhaemorrhages (dark deposits indicative of hemosiderin accumulation), neoangiogenesis (abnormal shapes) and the absolute capillary count per linear millimetre (normal density defined as ≥7 capillaries).[20] A validated algorithm was used to differentiate 'non-scleroderma' patterns from 'scleroderma' patterns.[21] A validated semiquantitative scoring system was applied to assess NVC abnormalities, with the following scale: no abnormalities=0; capillary alterations/reduction affecting <33%=1; 33–66%=2; and >66% per linear millimetre=3.[22]

Statistical analysis

Statistical analysis was performed using the Datatab Statistics Calculator. Continuous variables were reported as mean and SD or as the median, as appropriate, while categorical variables were expressed as counts and percentages. The two-tailed Mann-Whitney U test was used to compare values between two independent groups, while the Wilcoxon signed-rank test was performed for paired variables. Relationships between groups and NVC capillary characteristics were explored using appropriate univariate analyses and subsequently investigated with a multinomial logistic regression model, with the healthy CNT group as the reference category. All analyses were adjusted for age, sex, smoking habits, RP and comorbidities. Spearman's rank correlation was applied to analyse relationships between variables. Multiple comparisons were corrected using the Benjamini-Hochberg method to control the false discovery rate. A p value of ≤0.05 was considered statistically significant.

RESULTS

Demographic characteristics of LC patients, RC patients (no LC) and healthy matched CNT

NVC examinations of a total number of 86 LC patients (66 females and 16 males, 56±15 mean years old), 23 RC patients (18 females and 5 males, 51±18 mean years old) and 84 healthy matched CNT (68 females and 16 males, 52±12 mean years old) were collected from all the centres.

Among 86 LC patients, 24 were affected by SSc (from participating centres following patients with SSc and executing regular standard NVC analysis). Consequently, the whole LC population was further divided into two different subgroups: 62 were non-SSc-LC patients (49 females and 13 males, 51±16 years old) and 24 were SSc-LC patients (21 females and 3 males, 59±17 years old). Only 6% of all LC patients were unvaccinated.

Non-smoker subjects were the majority in all the SGs (84.5% in non-SSc-LC, 79.2% in SSc-LC, 82.6% in RC and 88.1% in CNT cohorts).

A mild influenza-like form of SARS-CoV-2 infection was detected in 75.8% of non-SSc-LC patients, while only 24.2% of them developed pneumonia. In contrast, all the other groups of LC patients experienced influenza-like syndrome.

The most represented clinical domains of LC were constitutional (91.9% in non-SSc-LC and 70.8% in SSc-LC patients), neurological (67.7% in non-SSc-LC and 41.7% in SSc-LC patients), respiratory (50.0% in non-SSc-LC and 29.2% in SSc-LC patients) and psychoaffective (46.8% in non-SSc-LC and 8.3% in SSc-LC patients).

The most frequent comorbidities were musculoskeletal diseases (37.1% in non-SSc-LC, 37.5% in

SSc-LC, 56.5% in RC, 15.5% in CNT cohorts) and cardiovascular diseases (30.6% in non-SSc-LC, 50.0% in SSc-LC, 26.1% in RC, 23.8% in CNT cohorts).

The most common concomitant medications included antiplatelets (9.7% in non-SSc-LC, 58.4% in SSc-LC, 8.7% in RC, 11.9% in CNT cohorts), angiotensin receptor blockers (14.5% in non-SSc-LC, 8.3% in SSc-LC, 8.7% in RC, 9.5% in CNT cohorts), glucocorticoids (8.1% in non-SSc-LC, 29.2% in SSc-LC, 17.4% in RC, 2.4% in CNT cohorts) and vitamin D analogues (32.2% in non-SSc-LC, 45.8% in SSc-LC, 34.8% in RC, 8.3% in CNT cohorts).

Demographic data of all subjects is reported in table 1.

Table 1 Demographic data of long covid (LC) patients without systemic sclerosis (non-SSc-LC), LC patients with systemic sclerosis (SSc-LC), recovered COVID-19 patients that did not develop LC (RC) and healthy matched controls (CNT)

	Non-SSc-LC patients	SSc-LC patients	RC patients	CNT
Patients/subjects, n	62	24	23	84
Age (years, mean±SD)	55±16	59±17	51±18	52±12
Females, n (%)	49 (79.0)	21 (87.5)	18 (78.3)	68 (80.9)
Males, n (%)	13 (21.0)	3 (12.5)	5 (21.7)	16 (19.1)
Caucasian ethnicity, n (%)	61 (98.4)	24 (100.0)	23 (100.0)	84 (100.0)
Current smokers, n (%)	9 (14.5)	5 (20.8)	4 (17.4)	10 (11.9)
Raynaud's phenomenon, n (%)	39 (63.0)	24 (100.0)	17 (73.9)	69 (82.1)
Autoantibodies				
Antinuclear antibody (ANA) positivity, n (%)	15 (24.2)	24 (100.0)	4 (17.4)	3 (3.6)
Antiphospholipid profile positivity, n (%)	4 (6.4)	0 (0.0)	0 (0.0)	4 (4.8)
Acute COVID-19 symptoms				
Influenza-like, n (%)	51 (82.3)	24 (100.0)	23 (100.0)	–
Pneumonia, n (%)	11 (17.7)	0 (0.0)	0 (0.0)	–
Thrombotic events, n (%)	4 (6.4)	0 (0.0)	0 (0.0)	–
Long covid clinical domains				
Constitutional, n (%)	57 (91.9)	17 (70.8)	–	–
Neurological, n (%)	42 (67.7)	10 (41.7)	–	–
Respiratory, n (%)	31 (50.0)	7 (29.2)	–	–
Psychoaffective, n (%)	29 (46.8)	2 (8.3)	–	–
Gastrointestinal, n (%)	21 (33.9)	1 (4.2)	–	–
Cardiovascular, n (%)	19 (30.6)	0 (0.0)	–	–
Comorbidities				
Musculoskeletal, n (%)	23 (37.1)	9 (37.5)	13 (56.5)	13 (15.5)
Cardiovascular, n (%)	19 (30.6)	12 (50.0)	6 (26.1)	20 (23.8)
Endocrinological, n (%)	23 (37.1)	8 (33.3)	0 (0.0)	23 (27.4)
Gastroenterological, n (%)	8 (12.9)	10 (41.7)	3 (13.0)	5 (6.0)
Respiratory, n (%)	9 (14.5)	3 (12.5)	4 (17.4)	3 (3.6)
Concomitant drugs				
Antiplatelets, n (%)	6 (9.7)	14 (58.4)	2 (8.7)	10 (11.9)
Calcium channel blockers, n (%)	6 (9.7)	14 (58.4)	0 (0.0)	4 (4.8)
Angiotensin receptor blockers, n (%)	9 (14.5)	2 (8.3)	2 (8.7)	8 (9.5)
Proton pump inhibitors, n (%)	13 (20.7)	20 (83.3)	6 (26.1)	6 (7.1)
Beta-blockers, n (%)	8 (12.9)	3 (12.5)	0 (0.0)	7 (8.3)
Immunosuppressants, n (%)	2 (3.2)	12 (50.0)	5 (21.7)	1 (1.2)
Glucocorticoids, n (%)	5 (8.1)	7 (29.2)	4 (17.4)	2 (2.4)
Vitamin D analogues, n (%)	20 (32.2)	11 (45.8)	8 (34.8)	7 (8.3)

*Antiphospholipid profile positivity means positivity for any of the following: lupus anticoagulant test, anticardiolipin antibodies (IgG or IgM) or anti-beta2-glycoprotein antibodies (IgG or IgM). Most common comorbidities and concomitant drugs have been listed.

High prevalence of non-specific NVC abnormalities in non-SSc-LC compared with all controls

The NVC examinations of non-SSc-LC patients, RC patients and CNT were considered to evaluate microcirculatory damage. Most patients/subjects were ANA-negative (75.8% in the LC cohort, 82.6% in the RC cohort, 96.4% in the CNT cohort) and antiphospholipid profile negative (93.6% in LC, 100% in RC and 95.2% in CNT cohorts), with no cases of clinically overt antiphospholipid syndrome.

Most patients/subjects had experienced RP (63.0% in LC, 73.9% in RC, 82.1% in CNT) at the time of the NVC examination, in particular, it was performed 17±13 mean months after COVID-19 in non-SSc-LC patients and 12±7 mean months after COVID-19 in RC patients.

All semiquantitative scores for the NVC non-specific capillary abnormalities were significantly higher in non-SSc-LC patients compared with healthy CNT: dilated capillaries (1.3±0.7 vs 0.7±0.7, p<0.01, p values multivariate<0.01), microhaemorrhages (0.5±0.6 vs 0.01±0.1, p=0.01, p values multivariate<0.05), abnormal shapes (0.3±0.6 vs 0.2±0.4, p<0.05, p values multivariate<0.05) and of note, the mean capillary number per linear millimetre was significantly lower (8.8±1.5 vs 10.0±1.5, p<0.01, p values multivariate<0.05). Absence of differences was found regarding the parameters related to giant capillaries (0.02±0.1 vs 0.0±0.0, p=1).

On the other hand, RC patients did not show significant differences in semiquantitative scores of giant capillaries (0.0±0.0 vs 0.0±0.0, p=1), microhaemorrhages (0.2±0.4 vs 0.01±0.1, p=0.9), abnormal shapes (0.2±0.4 vs 0.2±0.4, p=0.1) and mean capillary number per linear millimetre (9.9±0.9 vs 10.0±1.5, p=0.7) when compared with healthy matched CNT on an average time of 12 months after SARS-CoV-2 infection. Of note, only capillary dilations were still more significantly expressed in RC patients only versus healthy matched CNT (1.3±0.4 vs 0.7±0.7, p<0.01, p values multivariate<0.05).

Moreover, and once again of great value, a statistically significant difference was observed concerning the reduction of the mean capillary number per linear millimetre that was significantly higher in the non-SSc-LC patients (8.8±1.5 vs 9.9±0.9, p<0.01, p values multivariate<0.01) compared to RC patients. Interestingly, a non-significant trend was noted regarding increases in microhaemorrhages (0.5±0.6 vs 0.2±0.4, p=0.06) and abnormal shapes (0.3±0.6 vs 0.2±0.4, p=0.06), while no significant differences were observed for dilated capillaries (1.3±0.7 vs 1.3±0.4, p=0.5) or giant capillaries (0.02±0.1 vs 0.0±0.0, p=0.9).

Finally, a correlation was investigated between the reported microvascular abnormalities and the clinical domains of LC, and capillary dilations were found to significantly correlate with respiratory (r(60)=0.3, p=0.01) and cardiovascular involvement (r(60)=0.3, p<0.05). No statistically significant results emerged from the correlation between NVC features and other LC clinical domains.

All NVC findings are reported in table 2 and NVC examples of three different patients' cohorts are shown in figure 1.

Evidence for microvascular damage progression from pre-COVID-19 to post-COVID-19 in NVC examinations in non-SSc-LC and SSc-LC

As mentioned, patients were collected from centres managing CTDs and NVC examinations pre-COVID-19 and post-COVID-19 were available for 22 non-SSc-LC patients (19 females and 3 males, 51±16 mean years old) and for 24 SSc-LC patients (21 females and 3 males, 59±17 mean years old). Demographic characteristics are reported in online supplemental table I.

However, non-SSc-LC patients were ANA-negative (72.7%), while both above-mentioned cohorts had a negative antiphospholipid profile. Moreover, all patients in both cohorts suffered from RP at the time of the NVC examination.

Therefore, the most recent NVC examination before COVID-19 (38±13 months for non-SSc-LC patients and 36±25 months for SSc-LC patients) was compared with the post-COVID-19 NVC analysis (7±2 months for non-SSc-LC patients and 11±3 months for SSc-LC patients).

Of note and of interest, SSc-LC patients (who already have altered basal NVC due to the SSc status) showed significantly lower mean capillary number per linear millimetre (7.0±2.5 post-COVID-19 vs 8.0±2.3 pre-COVID-19, p=0.01) and significantly more dilated capillaries (1.6±0.7 post-COVID-19 vs 1.2±0.8 pre-COVID-19, p<0.01) after COVID-19.

However, no differences were observed for giant capillaries (0.9±0.9 post-COVID-19 vs 0.75±0.8 pre-COVID-19, p=0.5), microhaemorrhages (0.5±0.7 post-COVID-19 vs 0.3±0.5 pre-COVID-19, p=0.9) or abnormal shapes (0.5±0.7 post-COVID-19 vs 0.3±0.6 pre-COVID-19, p=0.2) at semiquantitative scoring.

Similarly, non-SSc-LC patients showed statistically significant differences, but generally with better basal NVC scores than SSc-LC patients, in mean capillary number per linear millimetre (8.5±1.6 post-COVID-19 vs 9.2±1.0 pre-COVID-19, p<0.01), dilated capillaries (1.3±0.7 post-COVID-19 vs 0.6±0.7 pre-COVID-19, p<0.05) and microhaemorrhages (0.7±0.6 post-COVID-19 vs 0.3±0.5 pre-COVID-19, p<0.05). No differences were observed for giant capillaries (0.0±0.0 post-COVID-19 vs 0.0±0.0 pre-COVID-19, p=1) or abnormal shapes (0.04±0.2 post-COVID-19 vs 0.0±0.0 pre-COVID-19, p=0.2).

Of note, after excluding six ANA-positive patients from the analysis, the statistical significance of the increase in capillary dilations (1.3±0.7 post-COVID-19 vs 0.6±0.7 pre-COVID-19, p<0.05) and the number of microhaemorrhages (1.2±0.8 post-COVID-19 vs 0.5±0.7 pre-COVID-19, p<0.05) after SARS-CoV-2 infection was confirmed, while the reduction in mean capillary number per linear millimetre (9.2±1.1 post-COVID-19 vs 9.6±0.6 pre-COVID-19, p=0.2), although present, did not reach statistical significance (low numbers).

Table 2 Semiquantitative scores (0–3) of nailfold videocapillaroscopy (NVC) abnormalities of long covid (LC) patients without systemic sclerosis (non-SSc-LC), recovered COVID-19 patients that did not develop LC (RC) and healthy matched controls (CNT)

				P values (univariate)			P values (multivariate)			P values (FDR)		
	Non-SSc-LC patients (n=62)	RC patients (n=23)	CNT (n=84)	LC versus CNT	RC versus CNT	LC versus RC	LC versus CNT	RC versus CNT	LC versus RC	LC versus CNT	RC versus CNT	LC versus RC
Time between COVID-19 and NVC examination (months, mean±SD)	17±13	12±7	–	–	–	–	–	–	–			
NVC findings												
Mean capillary number per linear millimetre (mean±SD)	8.8±1.5	9.9±0.9	10±1.5	<0.01	n.s.	<0.01	<0.01	–	<0.01	<0.01	<0.01	<0.01
Dilated capillaries (0–3) (mean±SD)	1.3±0.7	1.3±0.4	0.7±0.7	<0.01	<0.01	n.s.	<0.01	<0.05	–	<0.01	–	–
Abnormal shapes (0–3) (mean±SD)	0.3±0.6	0.2±0.4	0.2±0.4	<0.05	n.s.	n.s.	<0.05	–	–	<0.05	–	–
Microhaemorrhages (0–3) (mean±SD)	0.5±0.6	0.2±0.4	0.01±0.1	0.01	n.s.	n.s.	<0.05	–	–	<0.05	–	–
Giant capillaries (0–3) (mean±SD)	0±0	0±0	0±0	n.s.	n.s.	n.s.	–	–	–	n.s.	–	–

*A p value of ≤0.05 was considered statistically significant.
†Multivariate analysis included adjustment for age, sex, smoking habit, Raynaud's phenomenon and comorbidities.
‡Multiple comparisons were corrected using the Benjamini-Hochberg method to control the false discovery rate (FDR).
n.s., non-significant.

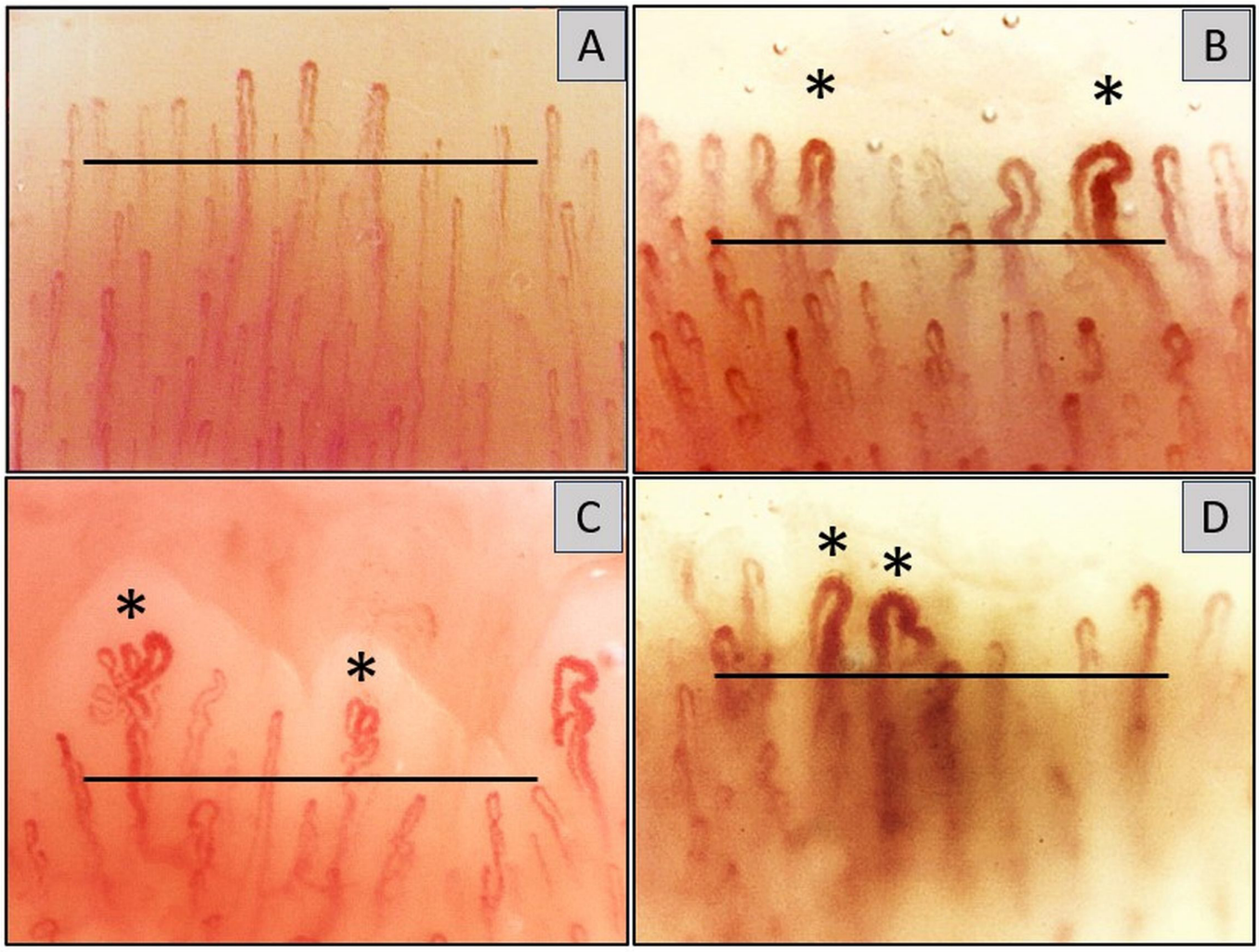

Figure 1 Nailfold videocapillaroscopy picture of a control healthy subject (A), a recovered COVID-19 patient without long covid (B) and two long covid patients without systemic sclerosis (C–D). In picture A (control healthy subject, male, 60 years old), normal hairpin capillaries without dilations, no microhaemorrhages, normal capillary density (12 capillaries per linear mm) and no abnormal shapes are present. In picture B (recovered COVID-19 patient without long covid, male, 53 years old), dilated capillaries are highlighted by asterisks and capillary density is normal (9 capillaries per linear mm). In picture C (long covid patient without systemic sclerosis, male, 42 years old), two abnormal shapes (neoangiogenesis) are highlighted by asterisks and capillary density is reduced (5 capillaries per linear mm). In picture D (long covid patient without systemic sclerosis, female, 39 years old), two dilations are highlighted by asterisks and capillary density is 8 capillaries per linear mm. In each image, the black horizontal line represents a linear millimetre. Magnification 200×. Original images from CP, Laboratory of Experimental Rheumatology and Academic Clinical Division at University of Genova.

All NVC findings pre-COVID-19 and post-COVID-19 are reported in table 3.

DISCUSSION

Present retrospective multicentre national investigation evidenced, in a detailed NVC analysis from experienced teams, significant non-specific microvascular abnormalities, mainly capillary dilations and microhaemorrhages, in LC patients up to 18 months after recovery from the acute SARS-CoV-2 infection.

On the contrary, RC patients without symptoms of LC showed a complete recovery of NVC microvascular status and capillary density, except for non-significant but persisting capillary dilations after 1 year from the acute infection.

To be considered, the study accounted for the main confounding factors (age, sex, smoking habit, presence of RP, comorbidities) in the different populations to strengthen the statistical significance of the whole results. In fact, among these confounding factors, it has been demonstrated that female sex hormones (particularly 17β-estradiol) are associated with increased vasoconstriction of cutaneous arterioles in response to environmental cold, due to the activation of α2C-adrenoreceptors expressed by vascular smooth muscle cells resulting in more frequent damage to endothelial cells.[23 24] Moreover, cigarette smoking has been associated with a higher

Table 3 Analysis of pre-COVID-19 and post-COVID-19 semiquantitative score of nailfold videocapillaroscopy (NVC) abnormalities in a subgroup of long covid (LC) patients without SSc (non-SSc-LC) and in LC patients with SSc (SSc-LC) for whom a NVC performed before COVID-19 was available

NVC findings	Non-SSc-LC patients ANA positive and negative (n=24)		P values	Non-SSc-LC patients ANA negative (n=16)		P values	SSc-LC patients (n=24)		P values
	Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19	
Mean capillary number per linear millimetre (mean±SD)	9.2±1	8.5±1.6	<0.01	9.6±0.6	9.2±1.1	n.s.	8±2.3	7±2.5	0.01
Dilated capillaries (0–3) (mean±SD)	0.6±0.7	1.3±0.7	<0.05	0.6±0.7	1.3±0.7	<0.05	1.2±0.8	1.6±0.7	<0.01
Abnormal shapes (0–3) (mean±SD)	0±0	0.04±0.2	n.s.	0±0	0.1±0.3	n.s.	0.3±0.6	0.5±0.7	n.s.
Microhaemorrhages (0–3) (mean±SD)	0.3±0.5	0.7±0.6	<0.05	0.5±0.7	1.2±0.8	<0.05	0.3±0.5	0.5±0.7	n.s.
Giant capillaries (0–3) (mean±SD)	0±0	0±0	n.s.	0±0	0±0	n.s.	0.75±0.8	0.9±0.9	n.s.

n.s., non-significant.

frequency of capillary dilations, even in the absence of giant capillaries, in chronic smokers.[25]

In addition, the pre-COVID-19 and post-COVID-19 intrapatient NVC analysis, performed in the investigation, provided interesting data on microcirculatory damage outcomes in the same patient. In fact, even if SARS-CoV-2 seems to alter the microcirculation both in patients with primary and secondary RP (SSc-related), most LC patients in the study suffered from primary RP (prior to SARS-CoV-2 infection), which is not usually associated with microvascular damage.[7] The increase in capillary dilations and microhaemorrhages appears to be independent of ANA positivity, whereas ANA-positive patients exhibit a greater reduction in capillary density, suggesting that SARS-CoV-2 could act as a further trigger for immune-mediated endothelial damage. On the other hand, SSc is a complex and rare CTD that is characterised by endothelial damage as a key driver of its pathogenesis.[26 27]

Therefore, in SSc-LC patients, existing microvascular abnormalities were found to worsen after SARS-CoV-2 infection, but it is reasonable to also consider the pathological progression of the disease itself, and the potential effects of reduced vasoactive or immunosuppressive therapies in concomitance with the acute COVID-19 condition.[28 29]

In any case, this investigation suggests new microcirculatory damage in all patients with SARS-CoV-2 infection as evaluated by NVC.

As already reported, after an initial acute phase characterised by NVC capillary dilations, pericapillary oedema, microhaemorrhages and reduced capillary density per linear millimetre, COVID-19 survivors still show a reduction in capillary density after 3 months from acute infection.[8 10]

Here, the investigation reports for the first time that NVC abnormalities fully recover in RC patients without LC, except for capillary dilations.

Dilated capillaries are usually non-specific alterations observed at NVC, but they require monitoring when several capillaries show a diameter greater than 30 µm in the presence of a RP, since possible further evolution might be expected versus SSc patterns.[30] On the other hand, microcirculatory damage persists in LC patients, since the NVC features of the acute phase can still be detected after more than 1 year from acute SARS-CoV-2 infection.

No 'scleroderma patterns' were found in non-SSc-LC patients, except for a patient with isolated giant and ANA positivity, which will need to be monitored in the future.[21] This suggests that microvascular damage induced by SARS-CoV-2 is not similar to that observed in SSc.

Therefore, it is reasonable to hypothesise that the persistence of endothelial/microvascular damage itself may contribute to the pathogenesis of LC clinical manifestations.[31 32]

Endothelial damage in LC patients seems to originate from different pathological events, for example, a

procoagulant state induced by the release of proinflammatory cytokines.[33] An important mediator of endothelial damage is type I interferon produced by plasmacytoid dendritic cells. Although type I interferon plays a crucial antiviral role, its aberrant production promotes hyperinflammatory syndrome through the stimulation of the cGAS-STING (cyclic GMP-AMP synthase - Stimulator of Interferon Genes) pathway in macrophages adjacent to endothelial cells.[34 35]

Furthermore, neurological and psychoaffective symptoms are very frequent in LC patients and damage to the blood–brain barrier has been identified in patients with neurocognitive symptoms together with abnormal immune response, clot formations and neuroinflammation.[36]

In addition, neurological symptoms of LC have been associated with reduced cerebral blood flow in the left frontal–temporal gyrus as a result of the interaction of the virus with more than 2000 genes expressed by neurons of these brain regions.[37]

On the other hand, capillary rarefaction (loss of capillaries) and reduced blood flow have been detected in sublingual and retinal microcirculation of LC patients using videomicroscopy.[38 39]

Notably, even cutaneous microcirculation is significantly impaired in LC patients, as demonstrated by flow-mediated skin fluorescence techniques.[40]

Therefore, the current multicentre investigation aligns with the very recent clinical evidence reported in the literature and provides a comprehensive description of the detailed NVC abnormalities of microcirculation observed in LC patients, supporting the presence of diffuse endothelial/microvascular damage.

A previous study reported that NVC during SARS-CoV-2 infection does not predict the development of LC, as no differences in the number of 'abnormal' versus 'normal' NVC examinations were found between patients who developed LC and those who did not.[41]

On the other hand, it is important to note that authors considered NVC investigations as 'abnormal' if 'more than 1 morphologic abnormality in at least 2 different nail bed examinations' was present. However, NVC abnormalities were identified with possible presence of 'giant capillary or>50% tortuous or>10% elongated capillary or bleeding area or neoangiogenesis or avascular areas plus the presence of another capillaroscopic abnormality'.[41] Such specific NVC parameters (in particular, giant capillaries and neoangiogenesis-abnormal shapes) are usually observed until now only in CTDs including SSc with overtime progression.[42]

It is therefore reasonable to assume that the different parameters used to assess microcirculation may explain the differing results obtained, especially considering that we analysed NVC performed before SARS-CoV-2 infection rather than during COVID-19. A prospective study with three points of observation (baseline, during/right after COVID-19 and after recovery or LC development) will be planned to better clarify the significance of these observations.

The current study is not without limitations.

The retrospective nature may limit the generalisability of the results, although collaboration in data collection and analysis from several expert national centres helped to counteract this intrinsic bias.

Interpretation of NVC changes can be subject to inter-rater variation, but all the centres are certified members of CAPSIR and EULAR SGs on microcirculation in rheumatic diseases, using a standardised evaluation of NVC parameters.[20] Moreover, inter-rater/intrarater agreement in NVC interpretation, assessed through the proportion of agreement and k coefficients, demonstrated strong reliability in a previous large international study.[43]

Specifically, the k coefficients were found to be 0.96 and 0.95 for capillary loss, 0.84 and 0.95 for giant capillaries, 0.90 and 0.95 for microhaemorrhages and 0.64 and 0.65 for capillary ramifications (abnormal shapes), confirming reliability of qualitative and semiquantitative NVC assessments across different raters and centres.[43]

Data regarding the treatment of SARS-CoV-2 infection in the acute phase are lacking, both for those who overcame the infection at home and for those who were hospitalised for pneumonia. However, among concomitant therapies, it is interesting to note the widespread and significant use of vitamin D analogues in LC and RC patients, widely prescribed during the pandemic for the ancillary effects on immunoregulation exerted by this secosteroid also in COVID-19.[44–46]

Most patients presented with a mild form of SARS-CoV-2 infection, as expected from a study population consisting of approximately 80% women, who are known to have a better response and outcomes to COVID-19 (gender effect).[47] As a matter of fact, a significantly higher risk of mortality was/is observed in male COVID-19 patients.[47]

However, to date, the symptoms of LC appear to develop independently of the severity of the acute infection and related stratified risk factors (gender effect, duration/intensity of acute phase and type/efficacy of treatments).[48]

The frequent overlap between LC clinical domains in the patients considered for the investigation did not allow us to clearly associate the NVC abnormalities with a prevalent symptom cluster, even if capillary dilations seem to correlate with respiratory and cardiovascular domains of LC. Serum analyses of endothelial damage biomarkers are lacking to further strengthen the reported observations, even if reliable blood markers of LC have not yet been identified.[49]

Furthermore, it will be a further target to compare NVC abnormalities in COVID-19 with those induced by other viral infections characterised by an acute inflammatory phase (eg, Epstein-Barr virus infection or cytomegalovirus) to identify similarity or differences.[50] Comparative studies are needed to determine whether SARS-CoV-2 has a unique vascular impact.

In conclusion, for the first time, the investigation shows significant endothelial/microvascular damage induced by SARS-CoV-2 infection in LC patients, using the detailed NVC scoring of microvascular status from multiple centres, and reveals the persistence of significant, although non-specific NVC alterations, even more than a year after recovery from COVID-19.

Incoming research is focussing on prospective longitudinal studies, standardised NVC methodologies, integration with serologic and endothelial biomarkers and comparative viral studies to determine whether post-COVID-19 and LC microvascular changes are clinically meaningful or simply a transient postinfectious phenomenon.

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