Research letter

Nailfold videocapillaroscopy and serum vascular endothelial growth factor in probable COVID-19-induced chilblains: a cross-sectional study to assess microvascular impairment

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Dear Editor, COVID-19-induced chilblains represent a type I interferon (IFN)-induced microangiopathy.[1] The objective of this cross-sectional study was to identify whether microangiopathic changes can be detected biologically through the serum levels of vascular endothelial growth factor (VEGF) and clinically through nailfold videocapillaroscopy (NVC).

Between April and July 2020, we investigated serum VEGF levels in 17 patients presenting with probable COVID-19-induced chilblains at the dermatology department of Centre Hospitalier Universitaire Saint-Pierre (Brussels, Belgium) and eight healthy controls (HCs) using enzyme-linked immunosorbent essay (VEGF Quantikine Bio-Techne, R&D Systems, Inc., Minneapolis, MN, USA). Inclusion criteria were: (i) presence of chilblain-like lesions during the COVID-19 pandemic; (ii) no previous history of chilblains; (iii) no exposure to cold before onset of chilblains; (iv) persistence of lesions > 48 h; (v) clinical and histopathological confirmation of chilblains; (vi) no history of lupus erythematosus, systemic sclerosis, photosensitive eruption, or any other dermatosis within the affected area; (vii) antinuclear antibodies ≤ 1: 160 without specific identification. All HCs were included before the COVID-19 pandemic (2018–2019). NVC was performed on 14 patients. All fingers except thumbs, were assessed using a ×200 magnification contact lens (Optilia Mediscope, Vällingby, Sweden). Quantitative parameters were compared with 14 age- and sex-matched HCs, using the capillaroscopic protocol from the European League Against Rheumatism study group on microcirculation in rheumatic diseases.[2]

Table 1 shows demographic data for the included patients and explorative analysis of quantitative NVC parameters. Statistical analysis was performed using StatView version 5·0 (SAS

Table 1 Demographic data of the included patients and explorative analysis of quantitative nailfold videocapillaroscopy parameters

Demographic data of included population	HCs (n = 22)	Patients with chilblains (n = 18)
Age, n (%)		
< 35 years	15 (68)	13 (72)
≥ 35 years	7 (33)	5 (28)
Sex, n (%)		
Male	8 (36)	10 (56)
Female	14 (64)	8 (44)
General symptoms of suspected COVID-19 infection, n (%)	0	11 (61)
Positive COVID-19 serology, n (%)	ND	2 (11)
Positive COVID-19 PCR, n (%)	ND	1 (6)
Participants with VEGF serum level detection, n (%)	8 (36)	17 (94)
Participants with NVC examination, n (%)	14 (64)	14 (78)

NVC quantitative parameters	HCs (n = 14)	Patients with chilblains (n = 14)	P-values
Density			
Capillary density/mm, median (range)	8·38 (7·44–9·75)	6·81 (5·13–9·44)	< 0·001
Dimensions, median (range)			
Number of dilations per mm	0·25 (0–0·95)	1·03 (0–2·75)	< 0·05
Number of regular dilations per mm	0·25 (0–0·95)	0·81 (0–2·19)	0·05
Number of irregular dilations per mm	0 (0–0·19)	0·06 (0–0·75)	< 0·05
Number of dilations/total number of capillaries per mm, %	3·03 (0–12·25)	16·69 (0–46·81)	< 0·05
Morphology, median (range)			
Number of abnormal shapes per mm	0·13 (0–0·63)	0·40 (0–1)	< 0·05
Number of crossings per mm	0 (0–0·19)	0·06 (0–0·31)	< 0·02

(continued)

Table 1 (continued)

NVC quantitative parameters	HCs (n = 14)	Patients with chilblains (n = 14)	P-values
Number of ramifications per mm	0 (0–0·44)	0·12 (0–0·75)	< 0·05
Number of concave tips per mm	0·063 (0–0·19)	0·10 (0–0·38)	> 0·05
Microhaemorrhages			
Number of microhaemorrhages, median (range)	0 (0–0)	0·09 (0–1·38)	< 0·02
Subpapillary venous plexus			
Visibility score, median (range)	0·38 (0–0·94)	0·53 (0–2·25)	< 0·05

HC, healthy controls; ND, not determined; NVC, nailfold videocapillaroscopy; PCR, polymerase chain reaction; VEGF, vascular endothelial growth factor. NVC images were taken at original magnification ×200. The mean of each of the quantitative parameters was calculated at the participant level. The medians of the means are reported in this table. Nonparametric tests were used to determine P-values, the Mann–Whitney U-test was used for continuous variables with a cut-off of $P < 0·05$ for statistical significance. Data are provided as median (ranges) for continuous variables. Standard definitions of capillaroscopic evaluations suggested by the European League Against Rheumatism study group on microcirculation in rheumatic diseases were applied.[2] Capillary crossings were considered abnormal when more than two crossings were observed. The total number of abnormal shapes corresponded with the sum of all capillaries with multiple crossings (more than two), ramifications and concave tips. The subpapillary venous plexus visibility score was graded from 0 to 3 (0, not visible; 1, doubtful visibility; 2, plexus visible only in restricted areas; 3, prominently visible over a wide area).

Institute Inc., Cary, NC, USA). Nonparametric tests were used to determine P-values, and the Mann–Whitney U-test was used for continuous variables ($P < 0·05$). Kendall's rank correlation was used to test the relationship between variables.

Patients with chilblains had significantly decreased capillary density ($P < 0·001$) and increased number of capillary dilations ($P < 0·05$), abnormal capillary shapes ($P < 0·05$) and microhaemorrhages ($P < 0·02$) on NVC compared with HCs. There was a positive correlation between age and capillary density for both HCs and patients with chilblains ($P < 0·05$). While no significant differences in VEGF levels were seen between patients and HCs, VEGF levels in patients with chilblains showed significant positive correlation with age, capillary density and presence of normally shaped capillaries, and significant negative correlation with irregularly dilated capillaries, plexus visibility score and percentage dilated capillaries/total capillaries.

This is the first study on probable COVID-19-induced chilblains where microangiopathic changes, clinically detected on NVC, are correlated biologically with serum levels of VEGF, a known biomarker for vascular homeostasis. Our results show that patients with chilblains had significantly decreased capillary density and significantly increased number of capillary dilations, abnormal capillary shapes and microhaemorrhages on NVC compared with HCs. These findings confirm that reliable NVC signs of microvasculopathy in probable COVID-19-induced chilblains can be identified. This is only the second NVC study that provides evidence of microvasculopathy in probable COVID-19-induced chilblains.[3] There is also limited NVC research in more severe COVID-19 manifestations, such as confirmed COVID-19 pneumonia[4] or multisystem inflammatory syndrome in children, showing signs of systemic microangiopathy in these patients.[5]

VEGF serum levels were determined in 17 of 18 patients with chilblains and showed no significant differences compared with HCs. This absence of significant difference could be consistent with the known, less severe disease course.[6] In our study, VEGF levels in patients with chilblains positively correlated significantly with age and capillary density. Significant negative correlation was seen with irregularly dilated capillaries. These findings suggest that VEGF could be a reliable marker for capillary density and (irregular) capillary dilation in patients with COVID-19 chilblains. Also, in these patients, age could be a protective factor for loss of capillaries through higher VEGF concentrations. This information could be very useful in deciphering the enigma around increased disease prevalence in younger patients.

COVID-19 detection by polymerase chain reaction (PCR) and serology tests is usually negative in patients with COVID-19 chilblains, which was similar to our findings (Table 1).[3,7] Although actual COVID-19 infection could not be confirmed in the majority of our patient cohort, it is widely suspected as the cause of the recent outbreak of chilblains, concurrent with the increase of COVID-19 cases. Furthermore, chilblains have been appearing during the warmer springtime, rather than the usual cold winter period, in young patients lacking a history of Raynaud phenomenon, chilblains or collagen vascular diseases such as lupus erythematosus. Also, viral particles were recently identified within endothelial cells and the secretory portion of eccrine glands through electron microscopy examination indicating COVID-19 causality.[8] Negative PCR results can be explained by the appearance of chilblains in the convalescent phase of the disease and negative serologies are possible through type I IFN activation, inducing rapid viral clearance and also suppression of the humoral antibody response, limiting a cytokine/growth factor (e.g. VEGF) storm.[1]

In conclusion, in this study, characteristics of microvascular impairment were found on NVC in probable COVID-19-induced chilblains.

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Data availability: the data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement: this study has been approved by Le Comité d'Ethique of CHU Saint-Pierre (registration number CE/20-04-19).

References

1 Frumholtz L, Bouaziz JD, Battistella M *et al.* Type I interferon response and vascular alteration in chilblain-like lesions during the COVID-19 outbreak. *Br J Dermatol* 2021; **185**:1176–85.
2 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.
3 El Hachem M, Diociaiuti A, Concato C *et al.* A clinical, histopathological and laboratory study of 19 consecutive Italian paediatric patients with chilblain-like lesions: lights and shadows on the relationship with COVID-19 infection. *J Eur Acad Dermatol Venereol* 2020; **34**:2620–9.
4 Natalello G, De Luca G, Gigante L *et al.* Nailfold capillaroscopy findings in patients with coronavirus disease 2019: Broadening the spectrum of COVID-19 microvascular involvement. *Microvasc Res* 2021; **133**:104071.
5 Çakmak F, Demirbuga A, Demirkol D *et al.* Nailfold capillaroscopy: A sensitive method for evaluating microvascular involvement in children with SARS-CoV-2 infection. *Microvasc Res* 2021; **138**:104196.
6 Kong Y, Han J, Wu X *et al.* VEGF-D: a novel biomarker for detection of COVID-19 progression. *Crit Care* 2020; **24**:373.
7 Gehlhausen JR, Little AJ, Ko CJ *et al.* Lack of association between pandemic chilblains and SARS-CoV-2 infection. *Proc Natl Acad Sci USA* 2022; **119**: e2122090119.
8 Kolivras A, Thompson C, Pastushenko I *et al.* A clinicopathological description of COVID-19-induced chilblains (COVID-toes) correlated with a published literature review. *J Cutan Pathol* 2021; **49**:17–28.