coronavirus disease 2019 (COVID-19) chilblains has been extensively acknowledged in the literature; however, a significant proportion of patients had mild systemic symptoms or contact with confirmed or suspected cases.[3]

Magro *et al.*,[4] recently demonstrated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in skin biopsies of three patients with COVID-19-related perniosis by immunohistochemistry (SARS-CoV-2 envelope protein colocalized with SARS-CoV-2 membrane protein) and RNAscope together with evidence of type I interferon signalling activation. The authors propose that a strong type I interferon response may accelerate viral elimination, explaining the reported negativity for RT-PCR and serological tests. Low sensitivity of the serological tests in asymptomatic patients could also explain the negative results. It is unclear whether serological tests can detect the lower antibody levels likely to be seen in mildly symptomatic or asymptomatic patients.[5]

Although limited to the skin of the distal extremities, the vascular damage seen in COVID-19 chilblains is severe enough to produce a lymphocytic vasculitis with endothelial disruption, microthrombosis and localized ischaemia. Why the lesions in these patients are limited to the distal feet and hands is still unknown.

We reaffirm our statement that immunohistochemistry for detection of SARS-CoV/SARS-CoV-2 remains restricted and subject to cautious interpretation. The images provided by Baeck *et al.* show suboptimal nonspecific reactivity. In our research, using an antibody directed against the spike protein of SARS/SARS-CoV-2, after optimization of the staining, we obtained a clean background, and our negative controls showed entirely negative endothelial reactivity. We acknowledge that we have no experience with the SARS-CoV-2 NP antibody used by Baeck *et al.*

The observation that our images show positivity limited to relatively healthy vessels is interesting. In fact, in our cases, not all the vessels showed the same degree of positivity, and heavily inflamed vessels appeared to show a lower expression than mildly inflamed ones. Clearance of viruses by the inflammatory process may be a potential reason for this.

The presence of viral particles on electron microscopy (EM) in endothelial cells is supported by several reports describing virus-like particles in patients with SARS-CoV-2 infection. Two of our coauthors have collaborated in a case series of COVID-19-related cutaneous lesions, which included biopsies of 11 COVID-19-related acroischaemic lesions. EM was performed and demonstrated coronavirus-like particles in three of five cases of COVID-19 chilblains.

Definitive characterization of SARS-CoV-2 virions requires immuno-EM. Unfortunately, we do not have remaining tissue adequately processed to perform this study, and we have not seen any other patient presenting with chilblains since the beginning of May. We are prepared to perform immuno-EM if a second wave of the pandemic causes a new outbreak of similar cases.

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Response to 'No evidence of SARS-CoV-2 infection by polymerase chain reaction or serology in children with pseudo-chilblain'

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Linked Article: Caselli *et al.* *Br J Dermatol* 2020; **183**:784–785.

Dear Editor, We read with interest the article by Caselli *et al.*,[1] which reported a case series of 38 children with chilblain-like lesions (CLLs). Testing for SARS-CoV-2 using polymerase chain reaction (PCR), rapid test serology and enzyme-linked immunosorbent assay (ELISA) for IgA and IgG antibodies yielded negative results in all cases. The authors concluded that their data do not allow them to support the relationship of CLLs with SARS-CoV-2 infection. So far, data in the literature studying CLLs documented a very low percentage of laboratory-confirmed SARS-CoV-2. However, Colmenero *et al.* were able to detect SARS-CoV-2 in endothelial cells of

cutaneous chilblain lesions by immunohistochemistry methods in seven paediatric patients with negative nasopharyngeal swabs.[2]

Recently, we updated our case series[3] with additional serological investigations, collecting results from 32 patients with CLLs (15 female patients, 17 male patients; average age 16·3 years). Data are detailed in Table 1. A SARS-CoV-2 PCR nasopharyngeal swab test was performed in 11 patients yielding two positive cases. These two cases had been screened 3 weeks before the onset of the cutaneous involvement because of fever and contact with patients positive for COVID-19. We tested for detection of SARS-CoV-2 antibodies in 22 patients using a chemiluminescent immunoassay (LIAISON® SARS-CoV-2 IgG kit, DiaSorin®, Saluggia, Italy) at least 14 days after the onset of the cutaneous lesions. Results were consistently negative for specific class IgG antibodies. A new ELISA test was subsequently available (Anti-SARS-CoV-2 ELISA IgM Test, Anti-SARS-CoV-2 ELISA IgG Test ELISA, DIA.PRO Diagnostic Bioprobes, Sesto San Giovanni, Italy) and three previously negative cases were found positive for IgM.

The overall epidemiological and clinical characteristics of CLLs still point to a COVID-19 related condition. Nasopharyngeal swabs are highly dependent on the sampling technique and timing. As far as serology is concerned, in addition to timing, the method used is also a key factor.[4] Serology negativity could be due to the shortcomings of currently available testing methods. Alternatively, a vigorous innate immune response against the virus could hamper the generation of antibodies through the adaptive response. Taken together, our results suggest that CLLs develop in individuals with mild infection, with low and rapid viral shedding, who are unable in most cases to generate detectable specific antibodies. A

Table 1 Clinical and laboratory data for patients with chilblain-like lesions (CLLs)

Case	Age (years)	Sex (M/F)	Localization	Cutaneous symptoms	Systemic symptoms	Onset of CLL after systemic symptoms (days)	PCR swab	Serology	
								IgG[a]	IgM[b]
1	14	F	Feet, hands	None	None	nd	−	−	−
2	15	F	Hands	None	None	nd	np	np	np
3	15	F	Feet	None	None	nd	−	−	−
4	18	F	Feet, hands	None	None	nd	np	−	−
5	13	F	Feet	None	None	nd	np	np	np
6	16	M	Feet	None	Cough	14	np	−	−
7	13	M	Feet	None	None	nd	np	−	−
8	15	F	Hands	None	None	nd	np	np	np
9	14	M	Feet	Itch	Diarrhoea	7	−	−	−
10	11	M	Hands	None	Fever	21	−	np	np
11	14	M	Feet	None	Cold	21	np	−	−
12	17	M	Feet	None	Fever	42	np	np	np
13	12	F	Feet	None	None	nd	np	−	+
14	15	M	Feet, hands	None	None	nd	np	−	+
15	12	F	Feet	Pain	Headache	10	−	−	−
16	8	F	Feet, hands	None	None	nd	−	−	−
17	10	M	Feet	Itch	Fever	56	np	np	np
18	14	M	Feet, hands	None	None	nd	np	−	−
19	16	M	Feet	None	None	nd	np	np	np
20	3	M	Feet, hands	None	Fever	10	np	−	−
21	15	F	Feet, hands	None	None	nd	−	−	−
22	8	M	Hands	None	None	nd	np	−	−
23	14	M	Feet, hands	None	None	nd	−	−	−
24	12	M	Feet	None	Fever	15	−	−	−
25	7	F	Feet, hands	Pain	Fever, headache, ageusia, anosmia	21	+	np	np
26	39	M	Feet	Burning	None	nd	np	−	−
27	23	F	Feet	None	Fever	21	np	−	−
28	25	F	Hands	None	None	nd	np	−	−
29	30	M	Hands	None	None	nd	np	−	−
30	31	M	Hands	None	None	nd	np	np	np
31	23	F	Feet	None	Fever	22	+	np	np
32	31	F	Feet, hands	None	None	nd	np	−	+

M, male; F, female; PCR, polymerase chain reaction; nd, not determined; +, positive; −, negative; np, not performed. [a]Chemiluminescent immunoassay, LIAISON®SARS-CoV-2 IgG kit (DiaSorin®, Saluggia, Italy).[b]Anti-SARS-CoV-2 enzyme-linked immunosorbent assay (ELISA) IgM Test; Anti-SARS-CoV-2 ELISA IgG Test ELISA (DIA.PRO Diagnostic Bioprobes, Sesto San Giovanni, Italy).

better understanding of the underlying immunological mechanism would shed light on the pathogenesis of SARS-CoV-2 and could have relevant implications for the development of an effective vaccine.

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Response to 'No evidence of SARS-CoV-2 infection by polymerase chain reaction or serology in children with pseudo-chilblain'. Reply from the authors

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Linked Articles: Recalcati *et al.* Br J Dermatol 2020; **183**:1154–1156. Caselli *et al.* Br J Dermatol 2020; **183**:784–785. Colmenero *et al.* Br J Dermatol 2020; **183**:729–737.

Dear Editor, Recalcati *et al.* conclude that chilblain-like lesions (CLLs) are part of the spectrum of COVID-19 based on reports of SARS-CoV-2 in endothelial cells of skin biopsies assessed by immunohistochemistry and electron microscopy (EM).[1–3] Nevertheless, the conclusion does not seem to be adequately supported by the data. Recalcati *et al.* expand their previously reported case series to include 32 patients with CLLs. In 21 of 32 cases, no nasopharyngeal swab (NPS) was tested for SARS-CoV-2. Two of 11 patients subjected to molecular testing were positive for SARS-CoV-2, but no serological test was performed to verify the seroconversion. Three patients tested positive for IgM and negative for IgG antibodies without any confirmation of infection through NPS. Again, taken together the diagnostic studies performed confirm that the vast majority of their patients did not test positive for the SARS-CoV-2 genome or for specific IgG. To *et al.* demonstrated that patients with SARS-CoV-2 infection showed an earlier seroconversion for IgG than for IgM. Moreover, they also found a 100% seroconversion for IgG 14 days after the onset of symptoms, but not for IgM.[4] In addition, Van Elslande *et al.* in their study concluded that including IgM antibodies did not improve the diagnostic performance in relation to COVID-19.[5] Therefore, in light of currently available information, the presence of IgM should not be taken as a diagnostic standard given the insufficient level of specificity. The presence of IgM antibodies, not supported by positive NPS and/or seroconversion for specific anti-SARS-CoV-2 IgG antibodies, could be a false-positive result.

To support the conclusion that CLLs are associated with COVID-19, Recalcati *et al.*[1] cite Colmenero *et al.*[3] However, a substantial limitation of that study was the lack of any serological assay performed in their patients. The use of EM morphology is certainly of interest but cannot be taken as a completely satisfactory state-of-the-art assessment of a novel virus. Detection of SARS-CoV-2 using molecular methods in biopsies would certainly offer much more stringent evidence of the presence of the virus in the lesional tissue.

Although we may agree that the cluster of chilblains in children occurred during the pandemic peak and this suggests some correlation, this has not been sufficiently clarified so far and remains intriguing.

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