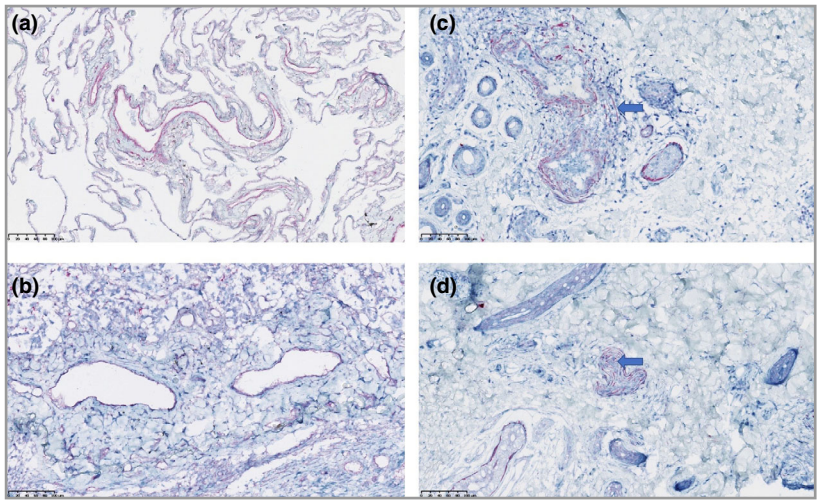

Figure 1 Immunohistochemistry for SARS-CoV-2 [using anti-SARS-CoV-2 NP Antibody (Clone# 6F10) BioVision, Inc. Milpitas, CA, USA]. (a) Surgical pulmonary resection specimen of a patient without COVID-19 who underwent thoracic surgery in 2019 before the COVID-19 pandemic (original magnification × 20). (b) Autopsy pulmonary specimen of a patient with critical COVID-19 (original magnification × 20). Diffuse endothelial staining of pulmonary vessels can be observed in both cases. (c) Skin biopsy specimens of chilblain lesions during the COVID-19 pandemic (original magnification × 20). (d) Skin biopsy specimens of classical chilblains observed in 2015 prior to any cases of COVID-19 (original magnification × 20). Diffuse endothelial staining of dermal vessels is present in both cases.

observed in patients prior to and during the COVID-19 pandemic. The staining was similar in both cases (Figure 1c, d).

We feel that the EM image of a single patient presented by Colmenero *et al.* is not typical of coronavirus particles. Indeed, coronavirus particles have been described by Goldsmith *et al.* as spherical structures clustered within a membrane that separates them from the cytoplasm. Black dots, corresponding to cross-sections through the nucleocapsid, are affixed to the inside of the viral envelope, and the interior of the particles is usually eletron-lucent.[2,3] The structures observed by Colmenero *et al.* seem isolated and free within the cytoplasm, although we would expect to see accumulation of viral particles in membrane-bound areas. Moreover, they are surrounded by dark dots that may be interpreted as spikes of the coronavirus, whereas the spikes would normally be located on the inside of the cisternal space.[3]

Colmenero *et al.* argue that the negative nasopharyngeal and oropharyngeal PCR in six of their patients may be attributed to low positive rates of PCR in children with symptoms of COVID-19. However, several publications confirmed not only negative PCR, but also negative serological tests in patients with chilblains.[4] Additionally, RT-PCR performed on skin biopsy specimens from 21 patients with chilblains failed to detect SARS-CoV-2 RNA.[4]

In light of the questions raised, in our opinion, these findings seem insufficient to establish definitive infection by SARS-CoV-2 or a direct link with COVID-19 in patients with 'COVID toes'.

M. Baeck,[1] D. Hoton,[2] L. Marot[1,2] and A. Herman[1]

[1]Division of Dermatology, and [2]Division of Anatomopathology, Cliniques universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), Avenue Hippocrate 101200,Brussels, Belgium
Email: marie.baeck@uclouvain.be

References

1 Colmenero I, Santonja C, Alonso-Riaño M *et al.* SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of seven paediatric cases. *Br J Dermatol* 2020; **183**:729–37.
2 Goldsmith CS, Tatti KM, Ksiazek TG *et al.* Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis* 2004; **10**:320–6.
3 Goldsmith CS, Miller SE, Martines RB *et al.* Electron microscopy of SARS-CoV-2: a challenging task. *Lancet* 2020; **395**:e99.
4 Herman A, Peeters C, Verroken A *et al.* Evaluation of chilblains as a manifestation of the COVID-19 pandemic. *JAMA Dermatol* 2020; **156**:998–1003.

Funding sources: none.

Conflicts of interest: The authors declare they have no conflicts of interest.

Chilblains and COVID-19: why SARS-CoV-2 endothelial infection is questioned. Reply from the authors

DOI: 10.1111/bjd.19491

Linked Articles: Baeck *et al.* Br J Dermatol 2020; **183**:1152–1153. Colmenero *et al.* Br J Dermatol 2020; **183**:729–737.

Dear Editor, We thank Dr Baeck *et al.*[1] for their interest in our recent article published in the BJD.[2]

The negative reverse-transcription polymerase chain reaction (RT-PCR) in nasopharyngeal swabs in patients with

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.

I. Colmenero,[1] C. Santonja,[2] M. Alonso-Riaño,[3] D. Andina,[4] J.L. Rodríguez-Peralto,[3] L. Requena[5] and A. Torrelo[6]

[1]*Department of Pathology;* [4]*Emergency Department, and* [6]*Department of Dermatology, Hospital Infantil Universitario Niño Jesús, Madrid, Spain;* [2]*Department of Pathology;* [5]*Department of Dermatology, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; and* [3]*Department of Pathology, Hospital Infantil Universitario Niño Jesús, Madrid, Spain*
Email: isabelcolmenero@gmail.com

References

1 Baeck M, Hoton D, Marot L *et al.* Chilblains and COVID-19: why SARS-CoV-2 endothelial infection is questioned. *Br J Dermatol* 2020; **183**:1152–53.
2 Colmenero I, Santonja C, Alonso-Riaño M *et al.* SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultraestructural study of seven paediatric cases. *Br J Dermatol* 2020; **183**:729–37.
3 Andina D, Noguera-Morel L, Bascuas-Arribas M *et al.* Chilblains in children in the setting of COVID-19 pandemic. *Pediatr Dermatol* 2020; **37**:406–11.
4 Magro C, Mulvey JJ, Laurence J *et al.* The differing pathophysiologies that underlie COVID-19 associated perniosis and thrombotic retiform purpura: a case series. *Br J Dermatol* 2020; https://doi.org/10.1111/bjd.19415.
5 Deeks JJ, Dinnes J, Takwoingi Y *et al.* Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev* 2020; **6**:CD013652.

Funding sources: none.

Conflicts of interest: The authors declare they have no conflicts of interest.

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

DOI: 10.1111/bjd.19493

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