Research letters

Analysis of T-cell responses directed against the spike and/or membrane and/or nucleocapsid proteins in patients with chilblain-like lesions during the COVID-19 pandemic

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Dear Editor, A range of cutaneous manifestations have been described in association with SARS-CoV-2 infection during the COVID-19 pandemic.[1] Among them, chilblain-like lesions (CLL) occurred more frequently than expected. A direct link was demonstrated thanks to the visualization of viral particles in the skin endothelial cells by electron microscopy,[2] which however was further questioned.[3] An indirect link was highlighted with high prevalence of seropositivity in patients with CLL compared with the general population.[4] However, numerous publications still question the link between CLL and SARS-CoV-2·[5] Herein, we assessed this association in a cohort of 50 patients with CLL. The patients were aged 32 years (interquartile range 27–43), 29 (58%) had suggestive extracutaneous COVID-19 symptoms and 20 (40%) had been in close contact with people with confirmed COVID-19. We performed SARS-CoV-2 reverse-transcription polymerase chain reaction (RT-PCR) for direct viral assessment, SARS-CoV-2 serology for humoral response, and interferon (IFN)-γ release assay for cellular T-cell response.

Firstly, at recruitment, real-time RT-PCR testing for SARS-CoV-2 was performed using a nasopharyngeal swab for all patients ($n = 50$), and in the skin ($n = 6$) and the stools ($n = 3$) of some patients. RT-PCR was negative for all samples tested.

COVID-19 serological tests were performed using three different techniques at recruitment and 14 days later: (i) IgG Abbott Architect COVID test (Abbott Laboratories, Libertyville, IL, USA); (ii) IgG and IgA enzyme-linked immunosorbent assay (ELISA) nucleocapsid COVID test (EUROIMMUN, Lübeck, Germany); and (iii) flow spike IgA and IgG detection.[6] The serological results were compared with those from a separate cohort of three patients with RT-PCR-confirmed mild COVID-19. The serological tests were all negative in the CLL group, except for four positive and four doubtful IgA ELISA anti-SARS-CoV-2 tests at the first visit. All three samples from the COVID-19 group were positive.

IFN-γ release assay was performed using ELISpot. Briefly, cryopreserved peripheral blood mononuclear cells were thawed and stimulated with pooled overlapping peptides spanning the SARS-CoV-2 spike, nucleocapsid and membrane protein (each at 2 mg mL^{-1}; Miltenyi Biotec, Bisley, UK). The response was compared between patients with CLL, patients with RT-PCR-confirmed mild COVID-19, and healthy control samples collected before the pandemic. We detected reactive T-cell responses directed against the spike and/or membrane proteins and/or nucleocapsid in 33% of CLL, 31% of healthy control and 100% of COVID samples. The CLL samples had higher levels of spot-forming unit than healthy controls, although not significantly (median 23·3, interquartile range 3·3–98·3 vs. −12·2, interquartile range −21–170, $P = 0·37$) (Figure 1).

Although SARS-CoV-2 is known to elicit a strong antibody response towards both surface and nucleocapsid peptides during systemic and pulmonary severe and mild disease, little is known about the humoral response in asymptomatic patients. Moreover, specific T-cell response has been less studied, but it has recently been shown that when asymptomatic, patients more frequently display a T-cell response than a humoral response.[7]

Given that chilblains are described as a later manifestation of COVID-19,[1] it is unsurprising that the patients had negative PCR results. Moreover, RT-PCR can give false negatives if the amount of viral genome is insufficient or if the correct time window of viral replication is missed. Discrepancies between studies concerning positive serologies, some reporting seropositivity in up to 30% of patients with CLL,[4] may be explained by many factors. Among them are different sensitivity, different timing between onset of disease and blood collection, and searching for only certain isotypes. Therefore, we used three different serology techniques to improve sensitivity, collected blood at two timepoints and searched for IgA anti-SARS-CoV-2 antibodies, as they have been shown to be associated with vasculitis manifestations. The S-Flow assay has the advantage of capturing all anti-SARS-CoV-2 S protein antibodies and providing excellent sensitivity. Nevertheless, none had a positive serology, consistently with previous reports.[5]

We and others have previously demonstrated that the cellular infiltrate plays a key role in the pathogenesis of CLL, and more particularly type I IFN, T helper 1 polarization and cytotoxic infiltration, highlighting the role of the cellular response.[4] However, we demonstrate for the first time that patients with CLL have the same specific T-cell response towards either the S, N or M protein as healthy controls. This could be explained by a pre-existing cross-reactive CD4 T-cell memory towards common-cold coronaviruses, as

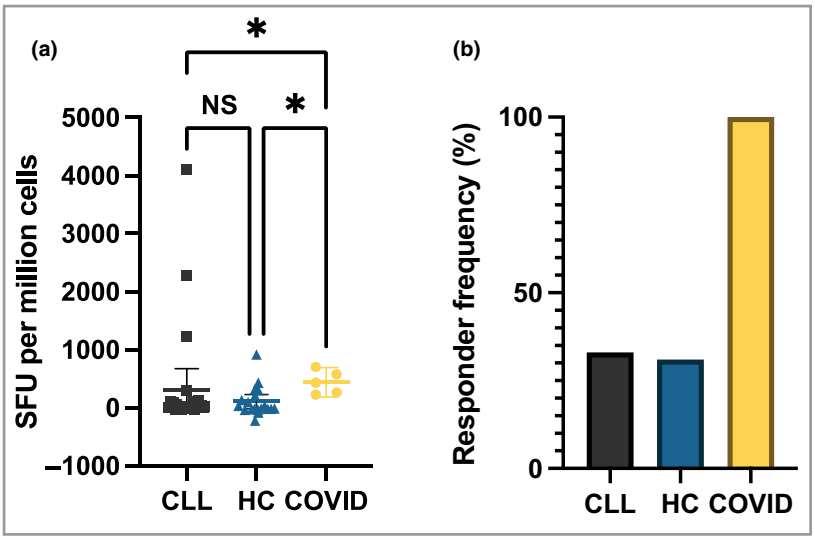

Figure 1 SARS-CoV-2-specific T-cell response. Peripheral blood mononuclear cells (PBMCs) isolated from patients with chilblain-like lesions (CLL; n = 27), patients with COVID (n = 5) and healthy controls (HC; n = 19) were stimulated *ex vivo* with overlapping peptides spanning the immunogenic domains of the SARS-CoV-2 S, M and N proteins in an interferon-γ ELISpot assay. Negative control wells lacked peptides, and positive control wells included a CEF (Cmv–Ebv–inFluenzae virus) peptide pool. (a) Interferon-γ-producing spot-forming units (SFU) per 10^6 PBMCs in response to the peptide mix. Responses were normalized to the positive control using background subtraction. Each dot represents one donor. The horizontal line represents the median, and the error bars represent the interquartile range. (b) Frequencies of donors responding to the peptide mix in each group. Antigen-specific responses were considered positive when the number of SFU per 10^6 PBMCs was above 50 after background subtraction. *$P < 0{\cdot}05$, Kruskal–Wallis rank-sum test with Dunn's post hoc test for multiple comparisons. NS, not significant.

described previously in up to 20–50% of people, or by the absence of a link between CLL and SARS-CoV-2. Further studies should be conducted studying specific CD4 and CD8 responses towards different peptides separately. For example, cross-reactivity with common-cold coronaviruses seems more important in the context of nucleocapsid-specific CD4 T cells.[8] Our data therefore do not demonstrate the role of SARS-CoV-2 in the pathogenesis of CLL through specific T-cell activation.

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C. Cassius,[1,2] M. Merandet,[2] L. Frumholtz,[1] D. Bergerat,[2] A. Samri,[3] C. Grolleau,[1,2] L. Grzelak,[4] O. Schwartz,[4] N. Yatim,[1,5] P. Moghadam,[1] L. Jaume,[1] M. Bagot,[1,2] J. Legoff,[6,7] C. Delaugerre,[6] J.-D. Bouaziz,[1,2] H. Le Buanec[2] and on behalf of Saint-Louis CORE (COvid REsearch)

[1]*Dermatology Department, AP-HP, Hôpital Saint-Louis, Paris, F-75010;* [2]*Université de Paris, Human Immunology, Pathophysiology, Immunotherapy, INSERM U976, Institut de Recherche Saint-Louis, Paris, F-75010, France;* [3]*Sorbonne Université, INSERM 1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, Paris, F-75013, France;* [4]*Virus and Immunity Unit, Institut Pasteur, Paris, F-75015;* [5]*Translational Immunology Lab, Institut Pasteur, Paris, F-75015, France;* [6]*Virology Department, AP-HP, Hôpital Saint-Louis, Paris, F-75010, France; and* [7]*Université de Paris, Team Insight, INSERM U976, Institut de Recherche Saint-Louis, Paris, F-75010, France*

Email: *charles.cassius@aphp.fr*

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1 Members of the French National Union of Dermatologists-Venereologists (SNDV) and member of Saint-Louis CORE.

Teledermoscopy: a helpful detection tool for amelanotic and hypomelanotic melanoma

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Dear Editor, Amelanotic and hypomelanotic melanomas (AHMs) comprise 2–8% of all melanomas. They are usually diagnosed late and are more advanced at diagnosis compared with pigmented melanomas (MMs). As a result, AHMs are associated with higher mortality and morbidity.[1]

Some reports have described the dermoscopic features of amelanotic melanomas (AMMs) and hypomelanotic melanomas (HMMs) to aid the diagnosis of such lesions.[2–4] These include polymorphous vessels, usually in irregular distribution (dotted, linear and/or coiled vessels), milky-red areas, short white lines, grey dots and granules.[3,4] However, the lack of pigment in these lesions often renders their referrals as 'routine', commonly as suspected basal cell carcinomas (Figure 1a–c).

With the surge of telemedicine over the past two decades, and several studies reporting its reliability and concordance with face-to-face diagnoses,[5] we launched a 'store and forward' teledermatology service in 2013. A Nikon D300S camera (Nikon, Tokyo, Japan) and Heine® Delta 20T dermatoscope (Heine Optotechnik, Herrsching, Germany) were used for clinical and dermoscopic images, taken by trained photographers in our Medical Illustration Department. The images were interpreted by dermatology consultants with a special interest in skin cancer and dermoscopy. Each clinician has a separate teledermatology list, with an average reporting time of 5–7 min per case. This service was established for 'routine' lesion referrals, excluding urgent suspected cancer, which should be referred via the 2-week wait face-to-face pathway.

During the 6-year period (2014–2019), our teledermatology service received > 16 000 referrals. Of these, 165 melanomas were identified, all of which were excised urgently owing to suspicious teledermoscopy features; 49 patients (30%) (27 male patients; 22 female patients) had AMM (n = 28) and HMM (n = 21). The remaining 116 melanomas were pigmented (n = 101, 61%) or partially pigmented (pigment occupying < 25% of the lesion) (n = 15, 9%).

The diagnostic concordance between the clinical review of the dermoscopic images and histology was 53·1% (40·8% of the lesions labelled as 'suspicious' and 12·3% labelled as 'likely AHM'). Other suggested diagnoses included basal cell carcinoma (14·3%), squamous cell carcinoma (10·2%), atypical seborrhoeic keratosis (6·1%) and lentigo maligna (LM) (4·1%). However, all these lesions were excised urgently as the dermoscopic patterns were nonspecific and AHM could not be excluded. Interestingly, one patient presented with a flat nonpigmented pink-orange patch on the neck, which was confirmed to be a hypomelanotic LM melanoma with a Breslow thickness of 0·4 mm (Figure 1d, e). Other less frequently proposed differentials included Bowen disease, dermatofibroma, haemangioma and pyogenic granuloma (all at 2%).

The median age for patients with AHM was 67 years (range 16–87). Most of these melanomas were on the arms (n = 15) and back (n = 10). The rest were on the head and neck (n = 8), legs (n = 8), shoulders (n = 4), abdomen (n = 2), chest (n = 1) and buttock (n = 1).

Three of the 49 lesions were *in situ* AHM. The majority of the invasive AHM (n = 27, 59%) were of the superficial spreading subtype. The other subtypes included nodular AHM (n = 14, 29%), LM melanoma (n = 2, 4%) and desmoplastic melanoma (n = 3, 6%). The median Breslow thickness of invasive AHM was higher than that of MM [1·7 mm for AHM (interquartile range = 3·50) vs. 0·98 mm (interquartile range = 1·23) for MM].

Awareness of the dermoscopic features of AHM is vital for the detection and treatment of these difficult to diagnose melanomas. Marchetti et al. demonstrated the difficulty in diagnosing amelanotic tumours despite the addition of dermoscopy, where concordance was lower for this cohort of lesions.[6] In our experience, reviewing the images on a computer monitor was very helpful for visualizing these features, which are often subtle and sometimes difficult to detect on direct dermoscopic examination. Additionally, in a study by Pizzichetta et al., dermoscopy has shown a higher sensitivity and specificity in diagnosing AHM compared with clinical examination (89% and 96% for dermoscopy vs. 65% and 88% for clinical examination, respectively).[7] This was reflected in our study as we were able to diagnose 49 AHMs via our routine teledermoscopy referral system.

A limitation of our study is that we cannot report the diagnostic accuracy of the detected melanomas. However, the aim of our study was primarily to demonstrate that with good teledermoscopy images we were able to identify AHMs in patients who would have otherwise remained on the routine

