

infection has not been found.<sup>5</sup> This is the first description of the second wave, and cases of CLL are probably expected to increase again.

The reason of this phenomenon is still unknown, but two different hypotheses could be advanced.

The first and most accepted one supports a relationship with SARS-CoV-2 infection, whose contact would induce in young patients a higher innate more than cell-mediated immune response with consequent fast clearance of antibodies and appearance of CLL.<sup>6–10</sup> The second less likely hypothesis is due to immobility; indeed, in Italy the first outbreak was observed during the lockdown and the second outbreak is now occurring during the soft lockdown as well. Young guys are the most affected by these measures because they are not attending schools and spending most of their time sat down watching monitors or TV. The lack of mobility could create a decreased blood flow with consequent appearance of CLL. Although interesting, against this second hypothesis is that frostbite is not among the cutaneous signs observed in paraplegic or wheelchair-immobilized patients.

We would like to share these new data about the second outbreak and need to wait what is going to happen in the next future in order to understand whether the Italian CLL will be followed by the rest of Europe.

### Acknowledgements

patients in this manuscript have given written informed consent to the publication of their case details.

### Funding source

None.

### Conflict of interest

The authors have no financial obligations or conflict of interest to declare.

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DOI: 10.1111/jdv.17145

## Absence of SARS-CoV-2 RNA detection in tissue samples of COVID-19-related cutaneous lesions analyzed by real-time RT-PCR

### Editor

Despite the increasing knowledge of COVID-19-related skin lesions, few studies have attempted to demonstrate the presence of the virus in skin lesions by real-time reverse transcriptase polymerase chain reaction (RT-PCR).<sup>1,2</sup>

The objective of this research was to determine through RT-PCR whether SARS-CoV-2 was present in skin biopsies of patients with cutaneous manifestations related to COVID-19.

A single-centre case series study was performed. We included samples from skin biopsies of 14 patients with cutaneous manifestations related to COVID-19 between April and May 2020. The biopsies were processed embedded in paraffin in five patients, immersed in physiological saline (fresh) in three patients and both samples (paraffinated and fresh) in six patients. This implies that 20 biopsies (11 paraffinated and 9 fresh) were analysed. (Table 1).

Each specimen was sent for virological investigation to the Respiratory Virus and Influenza Unit of the National Microbiology Center (ISCIII, Madrid, Spain). The biopsies were processed within 24 h. RNA from the homogenized skin tissue of the

**Table 1** Results of SARS-CoV-2 RT-PCRs from skin tissue. Microbiological and histological studies

Case	Cutaneous manifestation	Age	Sex	Medical history	Systemic symptoms	RT-PCR nasopharyngeal	SARS-CoV-2 serology	Clinical evolution time Cutaneous/Systemic symptoms	Cutaneous biopsy Histological study	RT-PCR Smear from the vesicles	RT-PCR tissue (in Fresh)	RT-PCR tissue (in paraffin)
1	Pseudo-chilblain	66	M	Antisynthetase syndrome	Dry cough	N	N	3 weeks/2 weeks	Lichenoid dermatitis. DIF: Granular C3 deposit	N	N	N
2	Vesicular eruption	52	F	Family history of dry cough and fever	Dry cough Headache	N	N	4 weeks/2 days	Lymphocytic vasculitis	N	N	N
3	Pseudo-chilblain	10	M	NO	Dry cough	N	N	4 weeks/1 week	Perivascular lymphocytic dermatitis and vacuolar degeneration in epidermis	NP	NP	N
4	Acral purpuric lesions	18	M	Papulovesicular eruption and cough in the previous 3 weeks	Headache	N	N	5 days/3 weeks	Lymphocytic vasculitis	NP	NP	Inhibited
5	Vesicular eruption	30	F	NO	Dry cough	N	N	1 weeks/4 weeks	Superficial perivascular dermatitis with vacuolar damage	N	NP	N
6	Acral purpuric lesions (Erythema multiforme)	12	M	NO	NO	N	N	4 days/No systemic symptoms	Epidermal necrosis, perivascular lymphocytic dermatitis and microthrombosis.	NP	NP	Inhibited
7	Maculopapular eruption (Erythema multiforme)	55	F	NO	Pneumonia	Positive	Positive IgG	6 days/No systemic symptoms	Interface dermatitis and eosinophilic infiltrates	NP	NP	Inhibited
8	Livedo reticularis	42	F	NO	NO	N	Positive IgM + IgA	8 days/No systemic symptoms	Superficial lymphocytic dermatitis	NP	N	N
9	Livedo reticularis	12	M	Brother with COVID-19 and acrocyanosis after the infection.	Fever Thrush	N	N	3 weeks/6 weeks	Chronic perivascular inflammatory component. DIF: C4c deposits in the epidermal basement membrane	NP	N	NP
10	Livedo reticularis	10	F	NO	Headache Fever Asthenia	N	Positive IgM + IgA	3 days/2 weeks	Chronic inflammatory component DIF: Linear deposit in basal and perivascular membrane	NP	N	NP
11	Pseudo-chilblain	57	M	NO	NO	N	N	2 weeks/No systemic symptoms	Superficial perivascular dermatitis	NP	N	Inhibited
12	Vesicular eruption	45	F	In contact with a COVID-19 patient.	Headache	N	N	4 weeks/4 weeks	Superficial mild perivascular dermatitis	NP	N	N
13	Urticarial lesions	42	F	NO	Dry cough, dyspnoea, headaches, dysguesia and asthenia	Positive	Positive IgM + IgA and IgG	2 weeks/4 weeks	DIF: Negative for C1q, C3, C4c, fibrinogen, IgA, IgG, IgM Papillary dermis oedematous mild chronic inflammatory infiltrate	NP	N	N
14	Granuloma annulare	53	F	In contact with a COVID-19 patient	Headache dysguesia and arrosmia	Positive	Positive IgG	4 weeks/4 weeks	Interstitial granuloma annulare	NP	N	NP

DIF, direct immunofluorescence; ESR, erythrocyte sedimentation rate; F, female; M, male; N, negative; NP, not performed.



**Figure 1** COVID-19 related cutaneous manifestations. (a) Pseudo-chilblain pattern: pernio-like lesions on the toes (Case 3). (b) Acral purpuric lesions (erythema multiforme type) located on the soles of the feet. (Case 6) (c) Vesicular pattern: vesicular lesions on the trunk. (Case 2). (d) Urticarial pattern: multiple annular welts on the trunk and extremities. (Case 13) (e) Maculo-papular pattern: erythematous maculopapular lesions on the trunk and extremities. (f) Livedoid pattern: livedo reticularis is seen on the upper limbs. (Case 10).

biopsies, deparaffinated skin or fresh biopsies was extracted by using the QIAamp Mini Elute Virus spin kit in an automated extractor (QIAcube, Qiagen, Valencia, CA). SARS-CoV-2 detection was performed by multiplex RT-PCR real-time assays based on published RT-PCRs designed for E and N genes.<sup>3</sup> In cases where both types of samples were available, fresh and paraffin-embedded tissue, the RT-PCR assays were performed simultaneously in order to compare both results. Furthermore, histological studies were performed.

SARS-CoV-2 nasopharyngeal RT-PCR, serologies for specific SARS-CoV-2 IgA + IgM and IgG antibodies were conducted.

Serologies were also performed for Parvovirus B19, Cytomegalovirus, Epstein-Barr virus and *Mycoplasma pneumoniae*. An RT-PCR for enterovirus (Coxsackievirus, Poliovirus and Echovirus) in blood was also performed.

For the patients that presented vesicles or blisters, a skin swab of the content was taken for the performance of SARS-CoV-2 RT-PCR.

The most prevalent lesions were pseudo-chilblain or acral purpura in five cases (35.7%), followed by vesicular eruptions in three cases (21.4%), livedo reticularis or acrocyanosis in three cases (21.4%), maculopapular eruption in one case (7.1%),

urticarial eruption in one case (7.1%) and granuloma annulare in one case (7.1%) (Fig. 1).

The nasopharyngeal smear for SARS-CoV-2 RT-PCR was positive in three cases (21.4%) prior to the onset of skin symptoms, and negative in 11 cases (78.6%).

In the serological studies, nine out of 14 cases (64.3%) presented negative serological tests. Two cases (14.3%) were positive for IgM + IgA antibodies with a negative nasopharyngeal RT-PCR, two cases (14.3%) were positive for IgG with previously positive nasopharyngeal RT-PCR and one case (7.1%) was positive for both IgM + IgA and IgG antibodies, with a previously positive nasopharyngeal RT-PCR.

Most cases presented negative results in nasopharyngeal RT-PCR and serological tests. These data are consistent with other studies that have failed to demonstrate active or past infections.<sup>2,4-6</sup>

The serologies for other viruses and RT-PCR for enterovirus were negative.

Similar to other studies, the result of the RT-PCR of the vesicles obtained by smear was negative in the three cases performed.<sup>7</sup>

The histopathological studies frequently showed lymphocytic infiltrates, especially superficial and perivascular, similar to the findings reported in the literature.<sup>8,9</sup>

Of the nine skin biopsy samples submitted fresh for SARS-CoV-2 RT-PCR, all were negative.

Of the 11 samples of skin biopsies submitted in paraffin for SARS-CoV-2 RT-PCR, seven biopsies (63.6%) were negative and in four biopsies (36.4%) the RT-PCR reaction was inhibited.

We can observe that the technique practiced in paraffin biopsies usually produces an inhibition of the reaction (36.4% of cases) because the RNA suffers damage during fixation in formaldehyde and in the subsequent paraffinization.

The absence of SARS-CoV-2 virus detection, as identified by RT-PCR, leads us to consider that these skin lesions related to COVID-19 may be attributed to a collateral effect of the activation of the immune system rather than being a direct effect of the virus, or that these skin lesions are not related to the infection.

### Acknowledgement

The patients in this manuscript have given written informed consent to publication of their case details.

### Funding sources

This study was funded by the Department of Dermatology of the Lozano Blesa University Clinical Hospital and the Health Research Group GIISA002 of Health Research Institute IIS Aragón.

### Conflict of interest

The authors declare that they have no conflict of interest.

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DOI: 10.1111/jdv.17146

### LETTERS TO THE EDITOR

## Successful treatment of recalcitrant genital lichen planus with secukinumab

Editor,

Sir, Lichen planus (LP) is an inflammatory autoimmune disease that affects both skin and mucosae. Genital erosive LP is a severe form of mucosal lichen planus, frequently resistant to treatment.