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SHORT REPORT

No antibody response in cutaneous manifestations associated with COVID-19: An observational study of 64 cases with microbiological and clinical characterization

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Abstract

Background: The microbiological diagnosis of skin lesions related to COVID-19 is not well known. **Objective:** Perform a microbiological diagnosis in COVID19-related cutaneous manifestations. **Methods:** A cross-sectional study was performed with 64 patients with cutaneous manifestations associated with COVID-19 who underwent serological and nasopharyngeal reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2. **Results:** Out of the 64 patients, 6 patients had positive RT-PCR, with all of them developing SARS-CoV-2 IgG and 4 of them had positive IgM + IgA. Of the 58 patients with negative RT-PCR, 8 cases had positive IgM + IgA and only one of them had IgG seroconversion. Therefore, the infection was demonstrated in 7 cases (10.9%) and was doubtful in 7 other cases (10.9%) who presented negative RT-PCR and presence of IgA + IgM without subsequent seroconversion of IgG. Fifty patients (78.1%) had negative serological tests. The most frequent cutaneous pattern was pseudo-chilblain (48.4%) followed by maculo-papular pattern (26.6%), urticarial lesions (10.9%), vesicular eruptions (6.3%) and livedoid pattern (4.7%). The maculo-papular pattern showed the highest positivity in RT-PCR (3 cases; 17.6%) and serologies (4 cases; 23.5%). Skin lesions developed after the systemic symptoms in most patients (19 cases; 61.3%). **Conclusions:** Microbiological confirmation tests may not be an effective diagnostic technique for COVID-related cutaneous manifestations or that attributed lesions are not related to COVID-19. Confounding factors such as adverse drug reaction, serological cross-reactions with other viruses, the low production of antibodies in asymptomatic or mild forms of COVID-19 or its rapid disappearance, increase diagnostic uncertainty.

KEYWORDS
COVID-19, cutaneous manifestations, RT-PCR, SARS-CoV-2, serological test

1 | INTRODUCTION

Several skin manifestations have been associated with COVID-19, however, the microbiological profile of the patients presenting them has not been sufficiently studied.

2 | MATERIALS AND METHODS

We performed a cross-sectional, single-center study in a Spanish tertiary hospital. The study was conducted between April 22 and June 3, 2020.

The objective of the study was to determine the prevalence of confirmation markers of coronavirus infection in a cohort of patients with cutaneous manifestations suspected of COVID-19, performing nasopharyngeal reverse transcription polymerase chain reaction (RT-PCR) and serological tests for SARS-CoV-2.

We recruited suspicious cases of COVID-19, that met the following criteria: skin lesions belonging to the groups described as related to COVID-19 (pseudo-chilblain, vesicular eruptions, urticarial lesions, maculopapular eruptions, livedo or necrosis and others)[1–4] that were associated with: symptoms compatible with COVID-19 (fever, cough, dyspnea, headache, anosmia, ageusia, myalgia, nausea, vomiting or diarrhea) contact with confirmed or suspected cases of COVID-19. Patients without systemic symptoms and without contact with COVID-19 patients who presented a pseudo-chilblain pattern not associated with exposure to cold, personal history of chilblain lesions or other causes were also included.

Demographics (sex, age) and clinical data (smoking, previous dermatological diseases, duration of suspected skin disorder, systemic symptoms and treatment employed) were collected.

In the 7 days following the consultation, the microbiological tests were performed at the same day: nasopharyngeal swab for the detection of SARS-CoV-2 RNA by RT-PCR (Abbott Real Time SARS-CoV-2 assay, Abbott Park, Illinois; Sensitivity:100 copies/ml; Specificity: 100%), serological tests on blood for SARS-CoV-2 IgM + IgA antibodies (COVID-19 VIRCLIA® IgM + IgA, Vircell, Spain; At 9 days of the infection: Sensitivity:89%; Specificity:100%) and antibodies IgG (chemiluminescent microparticle immunoassay SARS-CoV-2 IgG assay, Abbott Park, Illinois; At 8 days of the infection: Sensitivity: 89%; Specificity: 100%).

We also determine *Mycoplasma pneumoniae* (IgM), and Parvovirus B19 (IgM) antibodies; and a RT-PCR for enterovirus in blood samples. In patients with exudative skin lesions a sample of the exudate was taken for RT-PCR test for SARS-CoV-2. In those patients with negative RT-PCR and positive IgM + IgA antibodies, the serological test for SARS-CoV-2 was repeated 15 days later.

The assessment of the SARS-CoV-2 microbiological results was based on taking the following scenarios as positive: (1) patients with positive nasopharyngeal RT-PCR regardless of the serological test results, (2) patients with positive IgG antibodies (IgG+) (with positive or negative IgM + IgA antibodies), and (3) patients with a first positive IgM + IgA determination without IgG antibodies, who after 15 days developed IgG+ antibodies (seroconversion).

The study followed the ethical guidelines and was approved by the Medical Direction Committee of the Lozano Blesa University Clinical Hospital of Zaragoza. The patients signed informed consent.

3 | RESULTS

Sixty-seven patients with suspicious cutaneous lesions were recruited. Three patients refused to undergo any complementary tests. Therefore, 64 patients were included in our study. Twenty-seven patients (42.2%) were male and 37 (57.8%) were female. Their mean age was 28.09 years old (SD: ±20). The percentage of patients under the age of 6 years old was 7.8%; 43.8% were between 6 and 18 years old, 28.1% were between 18 and 50 years old, and 20.3% were older than 50 years old (Table S1).

The most frequent cutaneous manifestation was pseudo-chilblain (48.4%) in 31 patients, (Figure 1) followed by maculo-papular pattern (26.6%) in 17 patients and urticarial lesions (10.9%) in 7 patients. Four patients (6.3%) presented vesicular pattern and three (4.7%) a livedoid pattern. Others cutaneous manifestations were erythematous-desquamative plaques or dyshidrosis-like lesions in two different patients (3.2%). Pseudo-chilblain lesions were especially frequent in patients between 6 and 18 years old (18 cases, 64.3% of the 28 patients in this age group).

In 19 patients (61.3%) cutaneous eruptions appeared following the resolution of systemic symptoms, 9 cases (29%) manifested at the same time and 3 cases (9.7%) appeared previously.

Serology tests of 64 patients were performed. SARS-CoV-2 IgM + IgA was positive in 12 cases and SARS-CoV-2 IgG was positive in seven patients. Simultaneous positivity to IgM + IgA and IgG immunoglobulins was found in five cases. SARS-CoV-2 RT-PCR of nasopharyngeal swab was positive in six cases and negative in 58 cases (Table 1 and Tables S2 and S3).

Enterovirus RT-PCR of blood samples was negative in all cases. Two patients were positive to *Mycoplasma Pneumoniae* IgM antibodies: one of them had urticarial lesions whereas other presented a maculo-papular eruption. Other three cases were positive to Parvovirus B19 IgM antibodies in which two had a pseudo-chilblain pattern and one a vesicular eruption.

Of the three cases with positive IgM antibodies to parvovirus B19, one presented positive IgG antibodies to SARS-CoV-2. The only case that presented positive IgM antibodies to *M. pneumoniae*, also presented positive SARS-CoV-2 IgA + IgM antibodies that did not present seroconversion of SARS-CoV-2 IgG antibodies in the second determination at 15 days.

Three patients with maculopapular eruptions and positive nasopharyngeal RT-PCR presented histopathological changes consistent with adverse drug reaction.

A SARS-CoV-2 RT-PCR taken from a skin swab was performed in five patients. The results were negative for all of them.

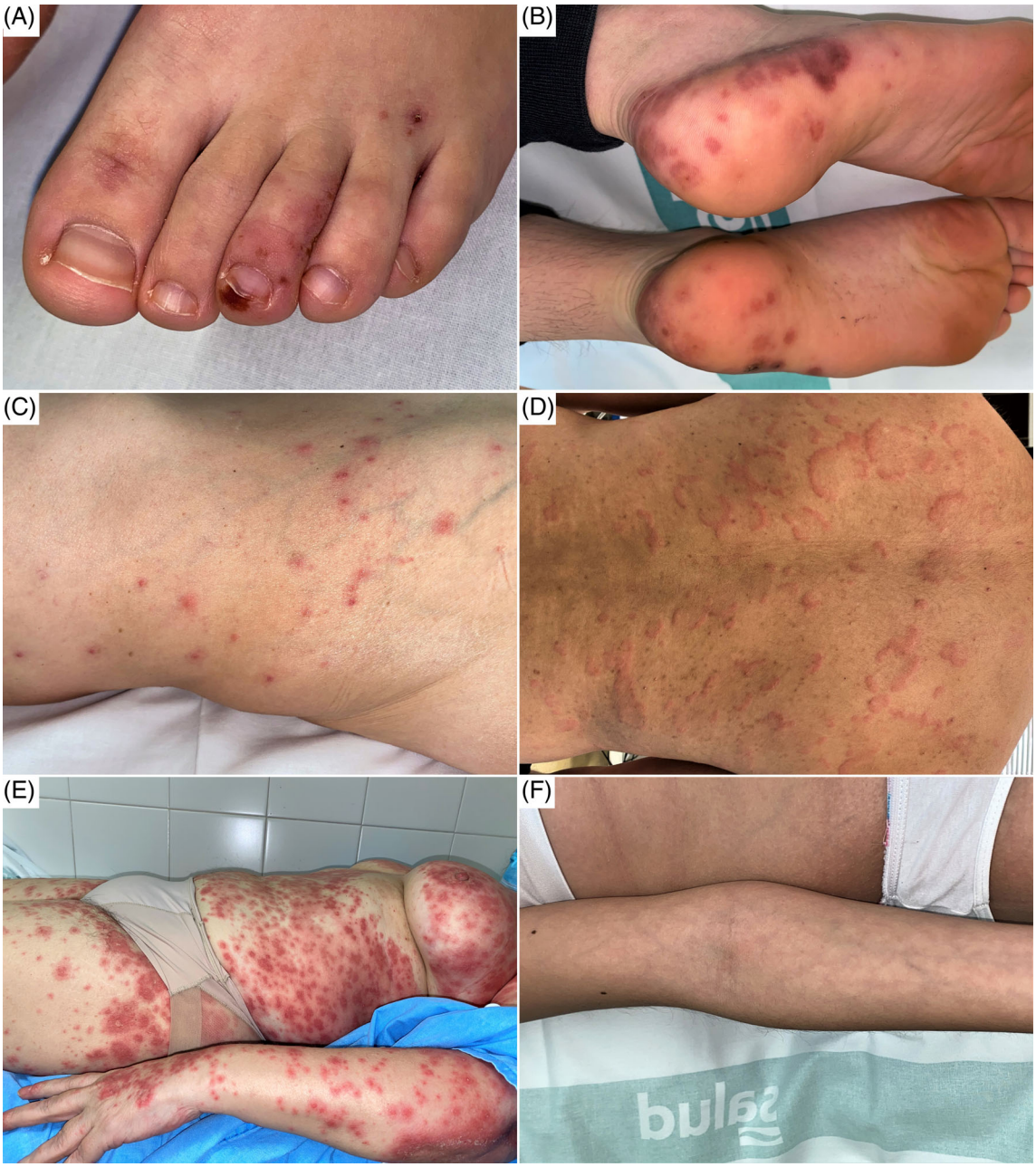

FIGURE 1 COVID-19 related cutaneous manifestations. (A) Pseudo-chilblain lesions on the toes. (B) Pseudo-chilblain lesions located on the soles of the feet. (C) Vesicular pattern: vesicular lesions on the trunk. (D) Urticarial pattern: multiple circumscribed welts on the trunk. (E) Maculopapular pattern: erythematous dianiform macules on the trunk and limbs. (F) Livedoid pattern: livedo reticularis is seen on the upper limbs

4 | DISCUSSION

In our database, the most frequent pattern was the pseudo-chilblain pattern followed by the maculopapular pattern and in the early ages of life, we observed that the pseudo-chilblain pattern is especially frequent as has been observed in other studies.[4,5]

According to our methodological criteria, seven patients (10.9%) had active or past SARS-CoV-2 infection and seven patients (10.9%) were doubtful (positive IgM + IgA antibodies but without subsequent detection of IgG antibodies), which could indicate that they were false-positives.

These findings are consistent with those found in other studies in which serological tests frequently failed to identify active or past infection in patients with lesions related to COVID-19, especially in patients with pseudo-chilblains.[6–9] Furthermore, mild forms of infection, such as those presented by the majority of the patients in our registry, were observed to have lower antibody responses.[6–8,10]

TABLE 1 Results of serological tests and skin manifestations related to SARS-CoV-2

	SARS-CoV-2RT-PCRN (%)		SARS-CoV-2 IgM + IgAN (%)		SARS-CoV-2 IgGN (%)		*Mycoplasma pneumoniae* IgMN (%)		B19 Parvovirus IgMN (%)	
	+	−	+	−	+	−	+	−	+	−
Cutaneous lesions										
Pseudo-chilblain	1 (3.2)	30 (96.8)	3 (9.7)	28 (90.3)	2 (6.5)	29 (93.5)	0 (0)	31 (100)	2 (6.5)	29 (93.5)
Vesicular	1 (25)	3 (75)	1 (25.0)	3 (75.0)	1 (25)	3 (75)	0 (0)	4(100)	1 (25)	3 (75)
Urticarial	1 (14.3)	6 (85.7)	2 (28.6)	5 (71.4)	1 (14.3)	6 (85.7)	1 (14.3)	6 (16.67)	0 (0)	7 (100)
Maculo-papules	3 (17.6)	14 (82.4)	4 (23.5)	13 (76.5)	3 (17.6)	14 (82.4)	1 (5.9)	16 (94.1)	0 (0)	17 (100)
Livedo	0 (0)	3 (100)	2 (66.7)	1 (33.3)	0 (0)	3 (100)	0 (0)	3 (100)	0 (0)	3 (100)
Others	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)
Duration[a]										
<5 d	1 (8.3)	11 (91.7)	3 (25)	9 (75)	2 (16.7)	10 (83.3)	0 (0)	12 (100)	1 (8.3)	11 (91.7)
5–10 d	1 (5.9)	16 (94.1)	2 (11.8)	15 (88.2)	1 (5.9)	16 (94.1)	0 (0)	17(100)	0 (0)	17(100)
>10 d	1 (4)	24 (96)	4 (16)	21 (84)	1 (4)	24 (96)	1 (4)	24 (96)	1 (4)	24 (96)
Temporal relationship of cutaneous manifestations with systemic symptoms										
Before	0 (0)	3 (100)	1 (33)	2 (66.7)	0 (0)	3 (100)	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)
Same time	1 (11.1)	8 (88.9)	1 (11.1)	8 (88.9)	1 (11.1)	8 (98,9)	0 (0)	9 (100)	1 (11.1)	8 (88.9)
After	4 (21.1)	15 (78.9)	7 (36.8)	12 (63.2)	5 (26.3)	14 (73.7)	0 (0)	19 (100)	1 (5.3)	18 (94.7)
No systemic symptoms	1 (3)	32 (97)	3 (9.1)	30 (90.9)	1 (3)	32 (97)	1 (3)	32 (97)	0 (0)	33 (100)

[a]Duration of skin lesions at the time of the serologies were performed. Ten patients did not remember the duration of the skin lesions.

Serological tests as a marker of past infections can also give false-negatives, as 40% of asymptomatic individuals become antibody-seronegative and 12.9% of symptomatic ones become IgG negative in the early convalescence phase.[11]

It must also be taken into account that the majority of patients in the study are young (7.8% under 6 years old, 43.8% between 6 and 18 years old). This young population has frequently negative serologic results for SARS-CoV-2.[12]

These data must be validated with more prospective studies that obtain long-term data because late and low antibody production that occurs in patients with mild or moderate forms of COVID-19, as in our series.[13] In addition, SARS-CoV-2 induces memory T cell responses in antibody-seronegative and antibody-seropositive individuals with asymptomatic or mild COVID-19, so that the fight against this virus could be more related to this mechanism than to the production of antibodies.[14,15]

In conclusion, the low seroprevalence of SARS-CoV-2 antibodies in skin lesions associated with mild cases of COVID-19 reported in our series suggest that serological studies are not an effective technique in the study of these cutaneous manifestations or that attributed lesions are not related to the virus. Immunohistochemical stainings or electron microscopy techniques could help to demonstrate the presence of the SARS-CoV-2 in tissue samples and the direct viral damage.[16–18] However, there are several factors that could contribute to the genesis of these skin lesions like the immune response or the systemic consequences of the infection, rather than to direct damage of the virus to the skin.[19,20]

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: García-Gil, Monte-Serrano, Ara-Martín. *Acquisition, analysis, or interpretation of data*: All authors. *Drafting of the manuscript*: García-Gil, Monte-Serrano, Lapeña-Casado, Villagrasa-Boli, Martínez-Pallás, Bularca, Aldea-Manrique, Ara-Martín. *Critical revisión of the manuscript for important intellectual content*: García-Gil, Monte-Serrano, Lapeña-Casado, Ara-Martín. *Statistical analysis*: Lapeña-Casado, Villagrasa-Boli, Ramírez-Lluch. *Obtained funding*: Ara-Martín. *Administrative, technical or material support*: García-Gil, Monte-Serrano, Benito-Ruesca, Ara-Martín. *Supervision*: García-Gil, Monte-Serrano, Ara-Martín. García-Gil, Monte-Serrano, Lapeña-Casado and Ara-Martín had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy

of the data analysis. García-Gil, Monte-Serrano, Lapeña-Casado and Ara-Martín contributed equally.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

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