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Original Contribution

Cutaneous manifestations in patients with coronavirus disease 2019: clinical and histological findings☆,☆☆

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Summary The clinical spectrum of coronavirus disease 2019 is getting wider with the exponential increase of patients worldwide. Initially described with flu-like symptoms, variable cutaneous manifestations have been reported, with only few histopathological descriptions. Detection of the virus in cutaneous samples has been assessed in very few cases until now, and the causative role of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has not been proven for every type of cutaneous manifestations yet. We aimed to describe histological features of cutaneous eruptions occurring concomitantly to SARS-CoV-2 infection and assess by immunochemistry and *in situ* hybridization using RNAscope validation techniques the presence of the virus in skin lesions. We retrieved all skin biopsies received in the departments of pathology and dermatopathology, University Hospital of Strasbourg, performed in hospitalized SARS-CoV-2–infected patients presenting concomitant cutaneous manifestations since March 2020. *In situ* hybridization and immunostaining using a polyclonal SARS nucleocapsid protein antibody were performed on each sample. Skin biopsies from six patients presenting morbilliform eruption concomitant to SARS-CoV-2 infection were available for evaluation. All six samples showed varying degrees of spongiosis, perivascular inflammatory infiltrates of the dermis, and, for some of them, discrete interface dermatitis. *In situ* hybridization and immunohistochemistry were negative in all cutaneous samples. Morbilliform rash concomitant to SARS-CoV-2 infection is characterized by mild and unspecific histopathological features with no detectable viral RNA and protein and appears then not to be directly caused by the virus. Even if, at least for a few cases, the differential diagnosis with drug hypersensitivity reaction can be difficult, these cutaneous eruptions seem to rather correspond to paraviral rashes.

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1. Introduction

The coronavirus disease 2019 (COVID-19) has been declared by the World Health Organization as a pandemic in March 2020. Initially described with flu-like symptoms, the clinical spectrum of this infectious disease caused by a new coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is getting wider with the exponential increase of patients worldwide. Cutaneous manifestations in patients with COVID-19 have been first reported by Recalcati [1] in March 2020 as erythematous rash, urticarial, and chickenpox-like vesicles. Since then, many descriptions of skin eruptions occurring concomitantly to SARS-CoV-2 infection have been described, with six main clinical patterns: urticarial rash, confluent maculopapular rash, papulovesicular exanthema, chilblain-like acral pattern, livedo reticularis pattern, and purpuric *vasculitis* pattern [2]. Maculopapular eruption is the most common pattern described ranging from 23% to 47% of cases [3,4]. Until now, only a few histopathological examinations of skin samples have been reported with usually unspecific pathological lesions such as mild perivascular lymphocytic infiltrate, slight spongiosis, and discreet lichenoid and vacuolar interface dermatitis [5,6]. However, these results remain sparse and require additional database confirmed. Nevertheless, based on these findings, several questions arise regarding the impact and the presence of SARS-CoV-2 in the skin.

We aimed to describe histological features of cutaneous eruption occurring concomitantly to SARS-CoV-2 infection and assess by immunochemistry (IHC) and *in situ* hybridization (ISH) using RNAscope validation techniques the presence of the virus in skin lesions.

2. Materials and methods

2.1. Clinical data

We retrieved all skin biopsies received in the departments of pathology and dermatopathology, University Hospital of Strasbourg, performed in hospitalized SARS-CoV-2−infected patients presenting concomitant cutaneous manifestations since March 2020. All patients had a nasopharyngeal swab positive for SARS-CoV-2 before the occurrence of rash. Clinical course of the disease and rash, as well as history of recent drug intakes and blood hypereosinophilia, was reported using the patient's medical file. Each skin lesion was clinically examined by dermatologists, directly or by photographs. Each patient has been informed of this study and expressed an oral nonopposition.

2.2. Microscopic examination

Two experienced pathologists, trained in dermatopathology, blindly analyzed all the cutaneous samples. IHC assays were performed on a Ventana Benchmark ULTRA platform (Ventana Medical Systems, Roche tissue diagnostics, Tucson, AZ, USA) using formalin-fixed paraffin-embedded specimens sectioned at five microns onto positively charged glass slides. IHC antigen retrieval was performed using Cell Conditioning 1 (CC1 − Ventana) for 36 min at 95°C. Specimens were incubated with a polyclonal SARS nucleocapsid protein antibody (200-401-A50; Rockland Immunochemicals, Inc, USA − dilution 1/500) for 32 min at room temperature, followed by revelation with the ultraView Universal DAB detection kit at room temperature. The specimens were then counterstained with hematoxylin [7,8]. For ISH assay, we used RNAscope technology with a SARS-CoV-2 spike probe to COVID-19 coronavirus as previously described [9]. Both assays had been validated in our laboratory on lung samples from SARS-CoV-2−infected patients, where viral RNA and protein were both found in pneumocytes. We used these samples as positive controls (Fig. 1).

3. Results

We identified six SARS-CoV-2−infected patients with skin biopsies available for evaluation. Four were performed in intensive care unit−hospitalized patients and two in conventional medicine unit−hospitalized patients (Table 1). Clinically, all six patients presented maculopapular rash of the trunk with variable extension to the limbs (Fig. 2). The latency between rash and onset of extracutaneous COVID-19 symptoms ranged from 5 to 35 days (mean 23 days). Five patients had been treated with antibiotics during the month preceding the rash. Three of them had blood hypereosinophilia concomitant with their rash. One patient did not use any new medication before the occurrence of the rash.

All six samples showed the same histopathological pattern including predominant spongiosis and mild perivascular lymphocytic infiltrate of the dermis associated, for some cases with discrete interface dermatitis with basal cell vacuolization and exceptional apoptotic keratinocytes for one case (Table 2). No virally induced cytopathic alterations or intranuclear inclusions were seen (Fig. 3A−C). ISH and IHC for SARS-CoV-2 were negative in all cutaneous samples (Fig. 3D and E).

4. Discussion

Histopathologic evaluation of tissue from SARS-CoV-2−infected patients is critical in advancing our understanding of the pathogenesis of this disease and to assess the distribution of SARS-CoV-2 within different organs and tissue [10]. Until now, the best-described histopathologic features have involved the lungs, which show various degrees of diffuse alveolar damage, severe capillary congestion, and, occasionally, superimposed bronchopneumonia

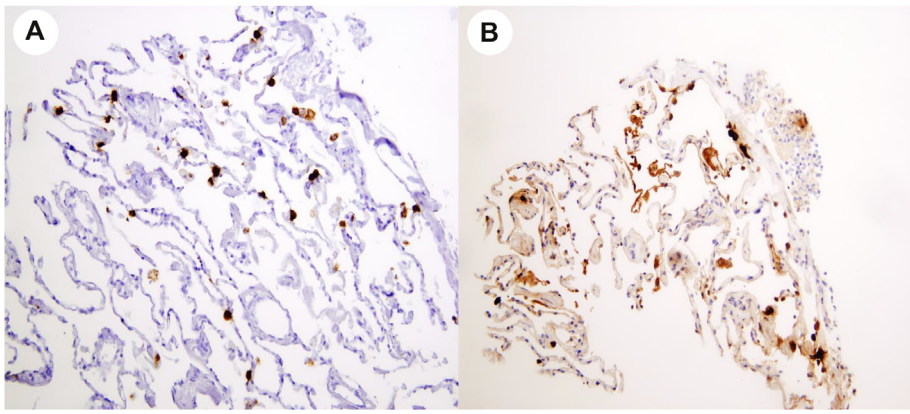

Fig. 1 SARS-CoV-2 mRNA detection in pneumocytes by *in situ* hybridization (A) and viral nucleocapsid protein detection in pneumocytes by immunohistochemistry (B).

Table 1 Clinical data of patients with COVID-19 presenting cutaneous manifestations.

Patients	Hospital unit	Extracutaneous manifestations related to COVID-19	Cutaneous manifestations occurring concomitantly to COVID-19	Delay between first COVID-19 extracutaneous symptoms and rash onset (days)	Drugs used before the occurrence of the rash	Delay between initiation of each treatments and the onset of the rash (days)	Hypereosinophilia (>0,5G/L)
Patient 1	ICU	Severe bilateral pneumonia requiring orotracheal intubation	Maculopapular exanthema of trunk and shoulders	25	Ceftriaxone Azithromycine Hydroxychloroquine Tazocilline Linézolide Valaciclovir	25 20	Yes
Patient 2	ICU	Severe bilateral pneumonia requiring oxygen therapy	Diffuse maculopapular exanthema	20	Amoxicilline/ clavulanic acid Ceftriaxone Spiramycine Azithromycine Hydroxychloroquine	20 3	No
Patient 3	CMU	Flu-like symptoms	Maculopapular exanthema of abdomen and limbs and bilateral livedo of knees Pruritus	5	Paracetamol	0	No
Patient 4	CMU	Flu-like symptoms	Maculopapular exanthema of trunk and limbs with slight pruritus	30	Ceftriaxone Azithromycine	30	Yes
Patient 5	ICU	Severe bilateral pneumonia requiring orotracheal intubation	Maculopapular exanthema of trunk and limbs	35	Ceftriaxone Azithromycine Linézolide Tazocilline Aciclovir	35 25	No
Patient 6	ICU	Severe bilateral pneumonia requiring oxygen therapy	Diffuse maculopapular exanthema Pruritus	30	Ceftriaxone Spiramycine Hydroxychloroquine Tazocilline Linézolide	20 5	Yes

ICU, intensive care unit; CMU, conventional medicine unit; COVID-19, coronavirus disease 2019.

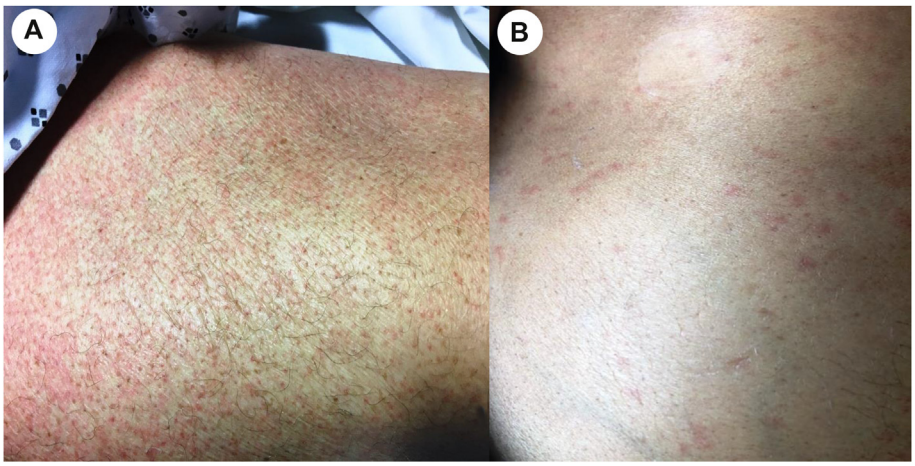

Fig. 2 Maculopapular exanthema of the limb (A) and trunk (B).

Table 2 Histopathologic clues which could help for the differential diagnosis between viral exanthem and drug-induced reaction.

Patients	Dermal eosinophilic infiltrate	Vacuolar change of basal keratinocyte	Apoptotic keratinocyte	Lymphocytic exocytosis
Patient 1	Discrete	No	No	No
Patient 2	No	Rare	No	Discrete
Patient 3	No	Rare	No	Discrete
Patient 4	No	Rare	Rare	Discrete
Patient 5	No	Rare	No	Discrete
Patient 6	Discrete	No	No	Discrete

[11]. Postmortem biopsy study reported that various organs such as the liver and heart, even when the presence of SARS-CoV-2 was assessed by real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) assay, showed only mild, nonspecific histopathologic changes by light microscopic examination [12].

To date, only few studies have been carried out on histopathological features of cutaneous manifestations occurring concomitantly to COVID-19. According to a systematic review published in late June 2020, only 23 cases of skin biopsy were reported to this date [5]. The histopathological features described are usually mild and unspecific and combine various degrees of spongiosis and lymphocytic exocytosis in the epidermis, basal vacuolar changes with interface dermatitis, lymphocytic perivascular and interstitial infiltrate of the dermis associated with dermal congestion, and extravasation of red blood cells [6,13−15]. Viral detection on skin samples using RT-PCR techniques on fresh skin biopsy specimens was performed in two cases. Results were negative [16,17]. A published article provides protocols of immunohistochemical and *in situ* hybridization assays that can be used for tissue identification of the virus and assessment of its distribution. Authors report that eight autopsies lung samples and one placenta showed cell positivity for SARS-CoV-2. Ten renal biopsies were also evaluated and were all negative [18]. A recent article, published in October 2020, reported histopathological features of seven cases of COVID-19 chilblains demonstrating typical findings of pernio-like lesions (combining variable degrees of lymphocytic vasculitis associated with purpura, superficial and deep perivascular lymphocytic inflammation with perieccrine accentuation, edema, and mild vacuolar interface damage). SARS-CoV-2 immunohistochemistry was positive in endothelial cells, and epithelial cells of eccrine glands and coronavirus particles were found in the cytoplasm of endothelial cells on electron microscopy. These findings could support a causal role of SARS-CoV-2 in chilblain lesions. Given our findings, it could mean that the pathophysiology of COVID-19 cutaneous manifestations is different depending on its type [19].

SARS-CoV-2 is a single-stranded RNA virus composed of 16 nonstructural proteins (named as NSP 1−16) whose pathophysiology is not yet fully understood. At the respiratory level, the current hypothesis is that the virus enters the cells via the angiotensin-converting enzyme 2 (ACE2) expressed on type I and II alveolar epithelial cells [20]. Many studies show that ACE2 is expressed in several organs and tissues including the skin, especially in the basal layer of the epidermis, endothelial cells of dermal blood vessels, and eccrine adnexal tissue [21,22]. Moreover, SARS-CoV-2 RNA has been detected in blood samples from a significant number of patients [23]. This brought many authors to assume of a direct viral effect to explain cutaneous manifestations. However, it is now well reported

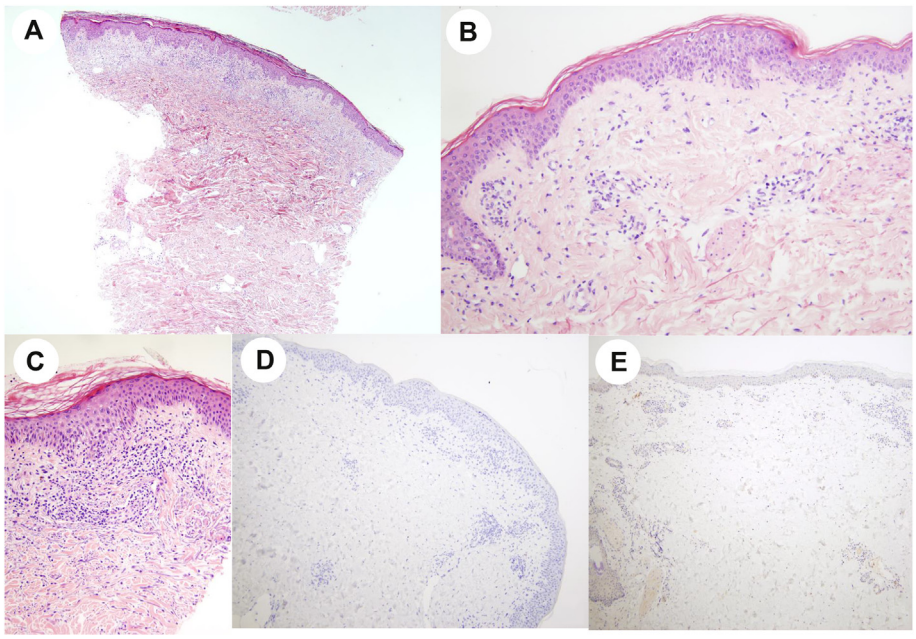

Fig. 3 Histopathological features in skin biopsy: Slight spongiosis (A, HE ×4), mild perivascular lymphocytic infiltrate of the dermis (B, HE ×20), and discrete basal cell vacuolization (C, HE ×20). *In situ* hybridization (ISH) using RNAscope technology (D, ×10) and immunostaining using a polyclonal SARS nucleocapsid protein antibody (E, ×4), both negative.

that SARS-CoV-2 infection can cause overactive immune responses that may induce immunopathological conditions, named as *cytokine storm* [24]. Cytokines could reach not only the lung but all other organs, including the skin. By stimulating inflammatory cells, cytokines could promote eruptions such as erythema, urticarial lesions, vesicles, and others. In this view, rashes may be paraviral due to cytokines [20]. This hypothesis is consistent with the fact that no viral RNA or protein can be detected neither in the lungs after the very early stage of the disease nor in skin lesional samples [16,17]. Histopathological features of cutaneous manifestation reported in our study and in the literature are mild and unspecific. Mild interface dermatitis associated with spongiosis and perivascular inflammatory infiltrate can be observed in various eruptions such as paraviral rash or toxic drug reaction. Indeed, hospitalized patients with SARS-CoV-2 infection are usually treated with several antibiotics (penicillin or azithromycin) and/or antiviral drugs, well known to be responsible of cutaneous toxic reaction.

Drug hypersensitivity reaction and viral infections are the most common cause of morbilliform eruptions, with significant and often problematic clinical overlap. Drug-induced rashes usually develop within three days to three weeks after initiation of a novel drug and may last up to two weeks after cessation of attributable treatment. Blood hypereosinophilia can be a weak argument in favor of drug-induced rash. Histopathological features of these two types of eruptions are also overlapping. Subtle microscopic clues in favor of drug hypersensitivity reaction are eosinophilic infiltrate in the dermis, lymphocytic exocytosis, basal cell damage with apoptotic keratinocytes, and usually more marked lymphocytic dermal infiltrate than in viral exanthema [25]. In our cohort, five patients had a novel drug initiation before the onset of the rash. For three of these patients, the delay between initiation of treatment and rash (comprised between three days and three weeks) could be compatible for drug hypersensitivity cutaneous reaction. Among them, patient 1 and patient 6 had blood hypereosinophilia (Table 1). Under light microscope, patient 1 had slight dermal eosinophilic infiltrate and neither basal cell damage, apoptotic keratinocytes nor lymphocytic exocytosis. Patient 2 had slight lymphocytic exocytosis, rare vacuolar change of basal keratinocyte, and neither dermal eosinophilic infiltrate nor apoptotic keratinocytes. Patient 6 had slight dermal eosinophilic infiltrate and lymphocytic exocytosis with neither basal cell damage nor apoptotic keratinocytes (Table 2). Therefore, for these three patients, there are no clear-cut arguments in favor of drug hypersensitivity reaction rather than viral exanthem. For the three other patients, viral exanthem remains the first hypothesis, with even greater confidence for the patient who did not use any new medication before the occurrence of the rash.

Our study was made on a small cohort of patients and concern only skin biopsy performed on maculopapular rash. To confirm our findings, further investigations on a larger number of patients with various cutaneous manifestations are necessary.

5. Conclusion

Morbilliform rash concomitant to SARS-CoV-2 infection is characterized by mild and unspecific histopathological features with no detectable viral RNA and protein and appears then not to be directly caused by the virus. Even if, at least for a few cases, the differential diagnosis with drug hypersensitivity reaction can be difficult, these cutaneous eruptions seem to rather correspond to paraviral rashes.

Author contributions

All authors whose names appear on the submission contributed substantially to the conception and design of the study, the acquisition of data, or the analysis and interpretation of the data.

Data availability

All available data are published in the current manuscript.

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