


## CONCISE COMMUNICATION

# Dermatological manifestations during COVID-19 and histological picture: Description of two clinical cases

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## ABSTRACT

It is not yet entirely clear what is the relevance of skin symptoms and what clinical implications are related to their appearance in COVID-19 patients. We describe two cases of COVID-19-associated pneumonia, which presented skin manifestations in advanced stage of illness, when nasopharyngeal swabs became negative for SARS-CoV-2. The first case presented erythematous, maculopapular lesions; the second developed petechial, vesicular and blood-encrusted lesions on the limbs. Histopathology documented perivascular lymphocytic infiltrates, with prevalent CD4+ T-cells in both patients. The research of SARS-CoV-2 in tissues with real time RT-PCR was negative. Basal keratinocytes displayed C4d deposits in one case, who developed laboratory signs indicative of a pro-coagulative condition at the same time as the skin rash. Skin manifestations during SARS-CoV-2 infection seem to be clinically relevant and further studies are necessary to assess if they are linked to systemic complications, lack of viral clearance or cascades of immune responses induced by the virus, even in patients affected by mild pneumonia.

**Key words:** COVID-19, inflammation markers, rash, SARS-CoV-2 infection, skin biopsy.

## BACKGROUND

Reports focused on dermatological symptoms of COVID-19, which accompanies other more common symptoms, are piling up in the literature. However, a disease-specific skin picture has not been identified so far.

This article attempts to shed light on the relevance of skin manifestations in COVID-19 patients by describing two cases of COVID-19 pneumonia observed at "L. Spallanzani" Institute, which presented skin manifestations at an advanced stage of clinical course.

## CASE PRESENTATIONS

### Case 1

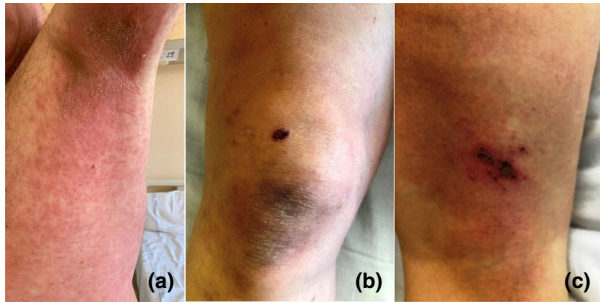
A 48-year-old man affected by hypothyroidism was admitted for pneumonia at day 6 from symptoms onset. He did not report drug intolerance. Oxygen saturation was normal. Nasopharyngeal swab was positive for SARS-CoV-2, CRP 2.67 mg/dL (normal value (nv) < 0.6); D-dimer 904 ng/mL (nv < 300); ferritin 370 ng/mL (nv < 336); full blood count

showed lymphocytopenia 880/mm<sup>3</sup> (reference range, 1000–4500). He underwent treatment with ceftriaxone IV, hydroxychloroquine and LPV/r orally. On day 11, he complained of articular pain and at day 14 manifested a widespread, erythematous, maculopapular, confluent rash, with initial flaking of face and scalp and following suberythrodermic diffusion (Fig. 1a). Ceftriaxone was discontinued. Lopinavir and hydroxychloroquine were suspended on day 17. Nasopharyngeal swabs (day 14 and 15) became negative. On day 19 rash worsened, with itching and burning and further spreading to the limbs. Skin punch biopsy was performed. Full blood count did not show eosinophilia; neutrophils 12,910/mm<sup>3</sup>; lymphocytes 1880/mm<sup>3</sup>, CRP 5.91 mg/dL, ferritin 645 ng/mL. HIV antibodies, autoantibodies and blood cultures were negative.

D-dimer increased abruptly (7803 ng/mL) and enoxaparin was started subcutaneously (day 20). CT pulmonary angiogram did not document pulmonary embolism. Oral prednisone 0.5 mg/kg OD was added for one week. Itching and burning improved; erythema reduced with diffuse, abundant desquamation. He was discharged on day 23. At day 33 visit, D-dimer was normal, rash persisted on hips, thighs and elbows, pale,

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**Figure 1.** (a) Case 1, day 20. Skin rash with initial seborrheic-like flaking of the face and scalp and following diffusion to suberythrodermic appearance; (b,c) Case 2, day 39. Petechial and crusty lesions on the lower limbs

with minimal flaking. He was subjected to skin biopsy, which was negative for SARS-CoV-2 with real time RT-PCR technique (Appendix S1).

## CASE 2

A 72-year-old man, diabetic, hypertensive, affected by chronic kidney failure, having a positive nasopharyngeal swab for SARS-CoV-2, was admitted for pneumonia at day 15 from onset of respiratory symptoms. Blood gas analysis showed PaO<sub>2</sub>/FiO<sub>2</sub> ratio 258 (nv ≥ 400); CRP 5.71 mg/dL; D-dimer 1264 ng/mL; ferritin 1600 ng/mL; full blood count showed 1060 lymphocytes/mm<sup>3</sup>.

He received enoxaparin SC; ceftriaxone and methylprednisolone IV; hydroxychloroquine and LPV/r orally. On day 19 he developed respiratory failure (PaO<sub>2</sub>/FiO<sub>2</sub> ratio 128 mmHg), treated with non-invasive ventilation (NIV). Tocilizumab was added IV. Nasopharyngeal swabs for SARS-CoV-2 were negative (day 15 and 34). He improved and NIV was stopped on day 33.

On day 39, when he was taking only intravenous saline and routine treatment for comorbidities, petechial lesions of suspected vasculitic origin were detected at limbs, some of which covered with a blood crust, slightly itchy. The most recent lesion, detectable on palpation, of bright erythematous-purple color was removed using a 6 mm punch (Fig. 1b,c). Real time RT-PCR for SARS-CoV-2 in tissue was negative. Full blood count did not show eosinophilia; neutrophils 2210/mm<sup>3</sup>; lymphocytes 1990/mm<sup>3</sup>, platelets 88,000/mm<sup>3</sup>; CRP 0.07 mg/dL, ferritin 1339 ng/mL, D-dimer 552 ng/mL; Quantiferon TB Plus was indeterminate; anti-HIV negative. Serologic tests for VZV and HSV 1-2 were not indicative of active infection. Skin lesions spontaneously improved and at day 54 visit they had lost the blood crust and were dry and slightly depressed.

## Histopathology

In both patients (Appendix S1) we observed mild spongiosis in the epidermis, cuffs of lymphocytes and macrophages around vessels and skin appendages, without vascular damage (Fig. 2:

Case 1,a; Fig. 3: Case 2,a). Neutrophils and eosinophils were not present in the infiltrates.

Immunohistochemistry (Appendix S1) showed prevalent CD4+ T-cells in cellular infiltrates, while less lymphocytes were CD8+ (Figs 2c,d and 3c,d); CD68+ macrophages were observed throughout the dermis, especially in perivascular areas (Figs 2E, 3E). Immunohistochemistry showed also C4d deposition along dermoepidermal junction in Case 1: basal keratinocytes displayed C4d deposits, whereas C4d was absent on endothelial cells (Fig. 2B). In Case 2 faint and disperse C4d staining was present (Fig. 3B); FXIIIa signal was observed in inflammatory cells and fibroblasts (Fig. 2F, 3F) of perivascular infiltrates and papillary dermis.

## DISCUSSION AND CONCLUSIONS

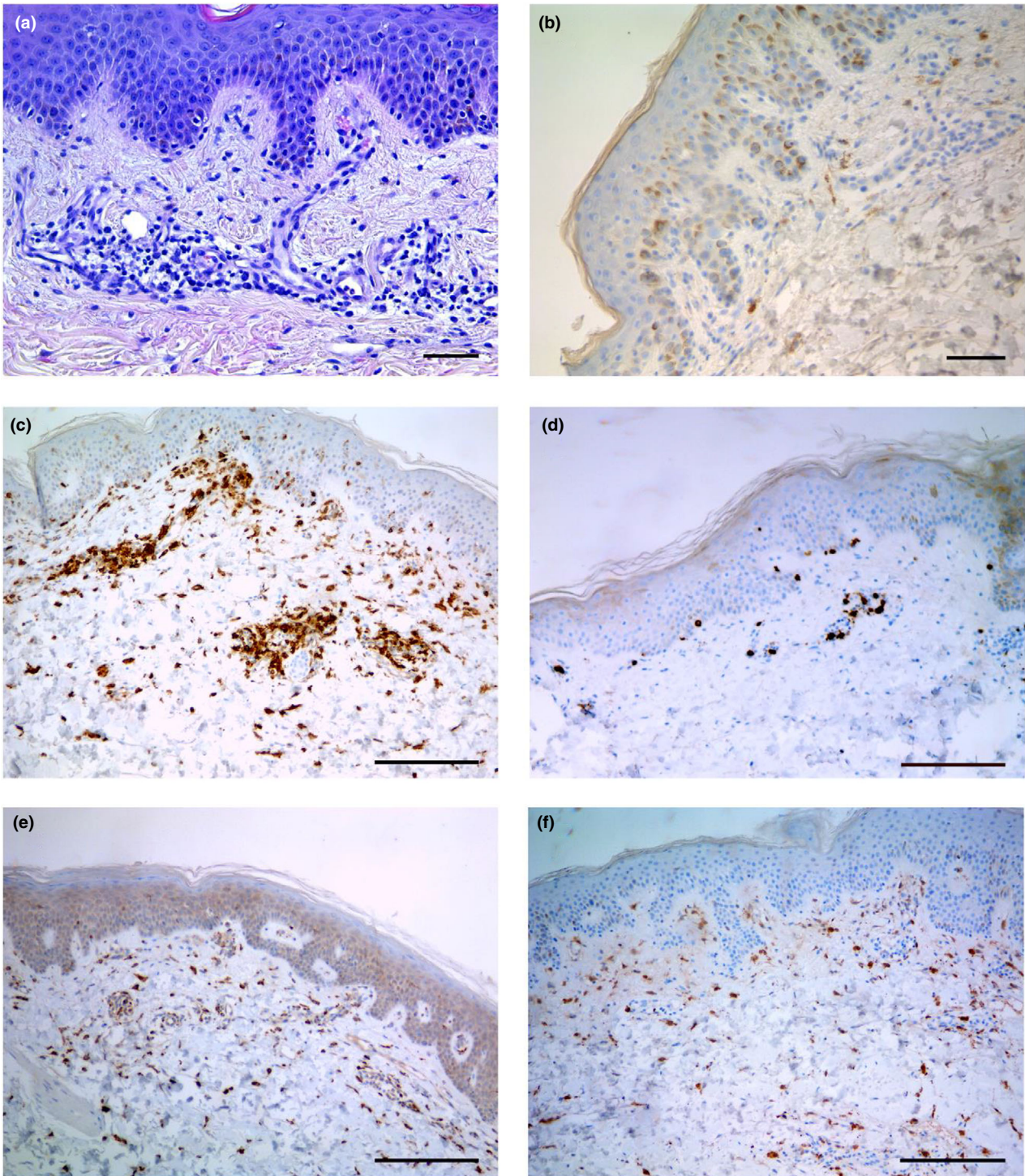
We describe two cases of COVID-19 pneumonia that experienced skin manifestations at an advanced stage of disease. Although the aspect of the cutaneous lesions was quite different, histological presentation was similar. In patient 1, the rash was associated with the increase of inflammation markers.

The initial report from Wuhan<sup>1</sup> probably underestimated the prevalence of dermatological manifestations during disease. Recent contributions indicate that skin manifestations often develop after the onset, with a histological picture of pauci-inflammatory thrombotic cutaneous vascular disease, similarly to that observed in lung, kidney and small bowel of patients deceased of COVID-19.<sup>2,3,4</sup>

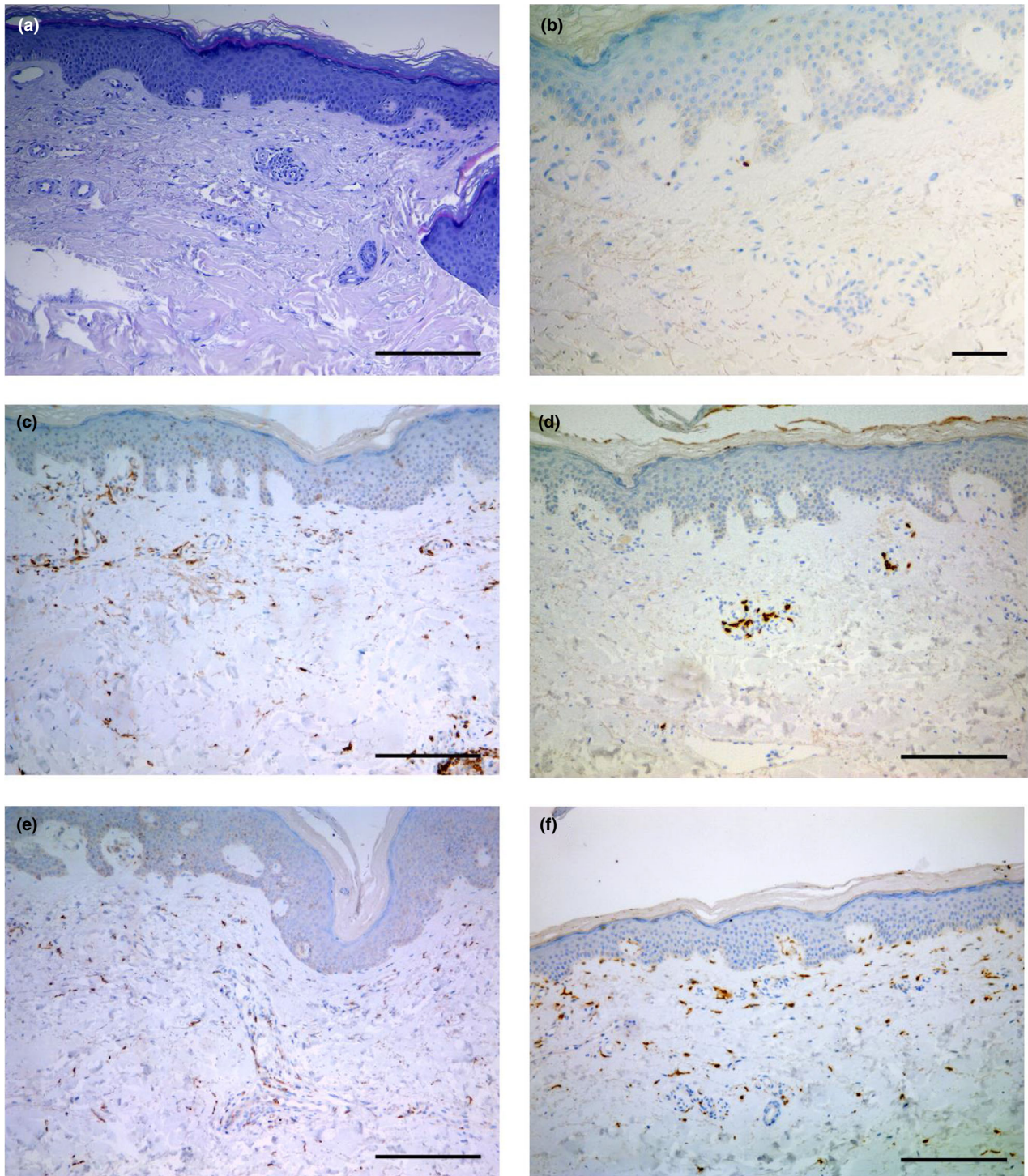
Among hospitalized COVID-19 patients, Recalcati<sup>5</sup> found 20% with skin manifestations: diffuse urticaria, erythematous and varicelliform rash. Galván Casas *et al.*<sup>6</sup> selected a series of 375 COVID-19 patients with skin manifestations, without mentioning localized or diffuse erythema, that we observed in Case 1.

Gianotti *et al.*<sup>7</sup> described the histopathology of COVID-19 skin manifestations as a superficial perivascular dermatitis, characterized in the epidermis by mild spongiosis or by dyskeratotic, ballooning and necrotic keratinocytes with lymphocytic exocytosis and nests of Langhans cells, and in the dermis by thrombosed vessels, extravasated red blood cells and perivascular lymphocytic infiltrates.

Quoting Magro *et al.*,<sup>2</sup> which demonstrated the presence of SARS-CoV-2 spike proteins in endothelial cells of cutaneous blood vessels, Gianotti hypothesized a possible interaction of the virus with keratinocytes. This hypothesis is now supported by the discovery of the expression of ACE2, the spike protein receptor, on human skin keratinocytes, which can then be infected with SARS-CoV-2.<sup>8</sup> Gianotti and Reymundo<sup>7,9</sup> wondered whether SARS-CoV-2 could be found in skin biopsies of patients with rash, but Ahouac<sup>10</sup> and ourselves tried the PCR method with no success, probably because the biopsies were performed at an advanced stage of disease. Gianotti affirmed that perivascular infiltrates of patients with skin manifestations during COVID-19 are mainly composed by CD8+ cytotoxic lymphocytes and eosinophils. In our cases with favorable evolution and at advanced stage of the disease, we did not find eosinophils, which could have suggested an allergic-type



**Figure 2.** Skin slides Case 1. (a) H&E-stained sections reveal an extensive dermal-epidermal inflammatory perivascular infiltrate; (b) basal keratinocytes display C4d deposits, note absence of C4d on endothelial cells. (c,d) Most lymphocytes are CD4+ cells, while fewer lymphocytes are CD8 positive. (e) CD68+ macrophages were observed in various locations throughout the dermis, particularly in the perivascular areas. (f) FXIIIa is expressed in both inflammatory cells and fibroblasts. Bars: a, b = 14  $\mu$ m; c, d, e, f = 50  $\mu$ m



**Figure 3.** Skin slides Case 2. (a) H&E-stained sections reveal lymphocytic inflammation targeting the vessels wall of the derma, without significant wall damage; (b) faint C4d staining is present; (c, d) most lymphocytes are CD4+ cells, while fewer lymphocytes are CD8 positive; (e) CD68+ macrophages were observed particularly in the perivascular areas; (f) FXIIIa is expressed in both inflammatory cells and fibroblasts. Bars: a, c, d, e, f = 50  $\mu$ m; b = 14  $\mu$ m

reaction to drugs (urticarial vasculitis), nor did we find features of immune complex leukocytoclastic vasculitis, but we found a picture of perivascular lymphocytic infiltrates, as also described by Ahouach et al,<sup>10</sup> a characteristic typically observed in superficial perivascular dermatitis of erythematous eruptions.

Immunohistochemistry showed that both populations of T-lymphocytes were represented in perivascular infiltrates. CD4+ T-cells were prevalent in perivascular cuffs, while less lymphocytes were CD8+ (Figs 2c,d and 3c,d).

However, we might also expect a different situation in patients with severe disease, in which high-level viremia and extreme ACE2 receptor expression, causing secretion of IFN, cytokines and activation of the complement system, could be associated to CD8+ cytotoxic lymphocytes massive migration from the vascular compartment. Such different balance of T-cells subsets might be associated with the presence of other cell populations, for example neutrophils,<sup>2</sup> and should be the object of further studies.

In Case 1 we observed deposition of C4d along dermoepidermal junction and absence of intravascular deposits, with an increase in the expression of FXIIIa, as occurs in inflammatory conditions.<sup>11</sup>

The observation of C4d deposits in basal keratinocytes is suggesting the possibility of complement activation in basal layers of epidermis, where keratinocytes are ACE2 receptor positive.<sup>8</sup> The activation of complement system, principally via the lectin pathway, may imply the co-activation of coagulation cascade by mean of MBL-associated serine proteases (MASPs).<sup>12</sup>

Inevitably, the uncertainty in distinguishing between drug-induced eruption and viral eruption in our cases persists, but the clinical observation of patients and the fact that keratinocytes and sweat gland cells are carriers of ACE2 receptors and can therefore become infected, suggest that SARS-CoV-2 can cause rashes, either directly, via invasion of resident epithelial cells or via an immune-mediated mechanism.<sup>13</sup>

Further research should be conducted to better define what is the relevance of skin symptoms and if the pathogenesis of cutaneous damage during COVID-19 disease is different in mild and severe cases.

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FP and GG reviewed the manuscript. All Authors read and approved the manuscript

**CONFLICT OF INTEREST:** On behalf of all authors, the corresponding author states that there is no conflict of interest.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

The authors had the patients' written consent for publication of this case report including the personal and clinical details and any accompanying images.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Methods.