Discordant anti-SARS-CoV-2 spike protein and RNA staining in cutaneous perniotic lesions suggests endothelial deposition of cleaved spike protein

Christine J. Ko,[1,2] Malini Harigopal,[2] Jeff R. Gehlhausen,[1] Marcus Bosenberg,[1,2] Jennifer M. McNiff,[1,2] William Damsky,[1,2]

1 Department of Dermatology, Yale Medical School, 333 Cedar St, PO Box 208059, New Haven, CT 06520
2 Department of Pathology, Yale Medical School, 310 Cedar St, New Haven, CT 06511

Key words
SARS-CoV-2, coronavirus, RNA *in situ* hybridization, COVID-19, spike protein

Running title
Anti-SARS-CoV-2 spike protein RNA ISH and IHC

Data available on request due to privacy/ethical restrictions

Corresponding author: Christine J. Ko

Key words
SARS-CoV-2, coronavirus, RNA *in situ* hybridization, COVID-19, spike protein

Abstract
Background: Prior studies have shown the presence of immunohistochemical staining for the SARS-CoV-2 spike protein (SP) in endothelial cells and eccrine epithelium of acral perniosis classified as “COVID toes”. Yet, other studies have been unable to detect SARS-CoV-2 RNA in skin biopsies of “COVID toes” by reverse-transcriptase polymerase chain reaction testing. Objective: In order to address these apparently conflicting findings, we compared detection of SARS-CoV-2 SP, through RNA *in situ* hybridization (ISH) versus immunohistochemistry (IHC), in skin biopsies of acral

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cup.13866

perniotic lesions presenting during the COVID-19 pandemic. Results: Three of six cases showed positive immunohistochemical labeling of endothelial cells, with 1 of 3 cases with sufficient depth also having labeling of eccrine glands, using an anti-SP SARS-CoV-2 antibody. These three cases positive with IHC were negative for SP by RNA ISH. Conclusion: While the gold standard for detection of SARS-CoV-2 in tissue sections has yet to be determined, the detection of SARS-CoV-2 SP alone without spike RNA suggests cleaved SP may be present in cutaneous endothelial cells and eccrine epithelium, providing a potential pathogenetic mechanism of COVID-19 endotheliitis.

Introduction

There are various skin manifestations that have been described in association with COVID-19. The most common patterns are acral perniotic, maculopapular/urticarial/targetoid, vesicular, livedoid, and purpuric (including acral gangrene or retiform purpura).[1,2] Histopathologic findings for vesicular lesions include multinucleated cells and necrotic cells in the epidermis.[3,4] Maculopapular/urticarial/targetoid lesions have been described as having lymphocytic inflammation.[4,5] Perniotic lesions presenting during the COVID-19 pandemic (PDC)

resemble idiopathic perniosis with dermal edema and perivascular and sometimes perieccrine lymphocytic inflammation;[6-14] thrombi may be present.[15,16] Livedoid and purpuric lesions generally show vascular occlusion.[17]

SARS-CoV-2 spike protein has been detected in a variety of these skin lesions, including purpura in COVID-19 hospitalized patients, PDC, and erythema multiforme-like lesions (Table 1).[16-20] For PDC and erythema multiforme-like lesions, patients are generally negative for nasopharyngeal and serologic testing for SARS-CoV-2,[1,2,19] and polymerase chain reaction (PCR) testing for SARS-CoV-2 in the skin of 29 cases of PDC was negative for detectable viral RNA.[9,21] Due to such conflicting data, many experts support that PDC is directly related to SARS-CoV-2 infection of the skin,[1,2] while others suggest that PDC is merely a coincidental increase in idiopathic perniosis.[21] In light of the discordant results of IHC and PCR in literature, negative nasopharyngeal and serologic testing for SARS-CoV-2 in the majority of patients with PDC, and negative anti-SARS-CoV-2 nucleoprotein IHC in six cases of PDC,[22] we tested PDC for spike protein using IHC and RNA *in situ* hybridization (ISH) to clarify if viral spike protein and RNA can be detected in the same tissue sections.

Methods

Six cases of PDC received in our laboratory between mid-May and mid-June, 2020 (with the peak of COVID-19 cases in this geographic area being mid-April, 2020) were

studied using IHC for the SARS-CoV-2 spike protein (Sino Biological, 40150-T62-COV,1:200). All six PDC cases had previously shown negative staining with an anti-SARS-CoV-2 nucleocapsid protein antibody (Thermofisher, mouse monoclonal antibody clone B46F, dilution 1:200).[22] Three of the six PDC cases with positive labeling using an antibody against the SARS-CoV-2 spike protein were also evaluated with RNA ISH using an Advanced Cell Diagnostics anti-SARS-CoV-2 spike protein probe (V-nCoV2019-S, prediluted), performed on the Leica BOND-III platform (Leica, Wetzlar, Germany).

Appropriate positive and negative controls were used. Both RNA ISH and IHC against the spike protein were validated on placental tissue known to be polymerase chain reaction-positive for SARS-CoV-2; nucleoprotein, spike protein, and spike protein RNA were detected in syncytiotrophoblasts. The placental tissue was from a COVID-19-positive patient with severe preeclampsia.[23] Lung from a deceased patient with COVID-19 was also positive for nucleoprotein, spike protein, and spike protein RNA in endothelial cells and macrophages. Placental tissue negative for SARS-CoV-2 by PCR did not stain positively with IHC for nucleoprotein, two different spike protein antibodies, or the RNA ISH probe; and other specimens from non-COVID-19 patients stained negatively with these antibodies and probe. Additionally, eight cases of perniosis diagnosed in 2019 stained negatively for anti-spike protein IHC.

An RNA ISH negative control probe targeting the *B.subtilis* dapB gene showed absent background staining in all tested cases, and a positive control probe to the housekeeping gene peptidylpropyl isomerase B (*PPIB*) to assess RNA integrity was included with each run. These positive and negative controls stained appropriately.

Results

The six cases of PDC presented in mid-May to mid-June of 2020 with 5/6 cases having pernio-like clinical lesions on fingers or toes. One case had a perniotic toe lesion as well as slightly targetoid macules and papules on the legs and dorsal hands. All six cases had perniosis-like histopathologic findings, with perivascular lymphocytes (**Figure 1**); additional findings included interface change (2/6), papillary dermal edema (2/6), and perieccrine inflammation (1 of 3 cases with eccrine glands represented in the specimen). Three of the six cases had IgG and IgM SARS-CoV-2 serologic testing performed; all three were negative.

Three of six cases of PDC (**Table 2**) showed positive staining of endothelial cells for SARS-CoV-2 spike protein by IHC with one of the three with sufficient depth showing staining of eccrine glands (**Figure 2**); these three cases had negative staining by RNA ISH against the SARS-CoV-2 spike protein (**Figure 2**). Staining was observed with the RNA ISH assay in syncytiotrophoblasts of the positive control tissue (placenta). RNA

ISH showed cleaner staining, with very little background signal in control placenta tissue, compared with IHC. Both RNA ISH and IHC showed granular staining.

Discussion

Results of IHC and RNA ISH staining against the SARS-CoV-2 spike protein were discordant in this series of PDC. The presence of spike protein IHC in three cases, negative RNA ISH (**Figure 2**) and nucleoprotein IHC,[22] and absence of SARS-CoV-2 by PCR testing of the skin in 29 cases of PDC[9,21] are compatible with endothelial cell uptake of spike protein fragments rather than the presence of whole virions. Cleavage of spike protein, required for viral endocytosis via angiotensin converting enzyme-2 (ACE2), may result in circulating spike protein fragments that could conceivably bind to ACE2 on endothelial cells of skin and other organs and cutaneous eccrine glands with subsequent receptor-mediated endocytosis. Cleaved spike protein in endothelial cells may then incite vasoconstriction and/or the endotheliopathy associated with vascular occlusion in COVID-19.[24-26]

Eccrine glands are known to express ACE2,[27] providing a mechanism for viral entry, but the exact significance of detecting spike protein in cutaneous eccrine epithelium is unclear. While only one of three cases in this series had sufficient depth to evaluate eccrine glands, detection of spike protein by IHC in eccrine epithelium has been previously reported in PDC,[16,18] purpura in hospitalized patients with COVID-19,[17,20] and erythema multiforme-like lesions.[19] Furthermore, nucleoprotein of SARS-

CoV was detected in eccrine glands on autopsy.[28] Detection of coronavirus proteins in sweat glands could signify the possibility of virus/viral protein excretion into sweat;[18,28] however, further directed investigation would be necessary to evaluate this possibility.

The optimal tissue-based testing method for COVID-19 remains unclear. PCR and RNA ISH are both highly sensitive and specific approaches. PCR for SARS-CoV-2 is reported to detect 0.58 copies/µl of COVID-19 viral RNA with a confidence ≥95%.[29] RNA ISH is able to specifically detect even single molecules within formalin-fixed tissue.[30,31] In contrast, IHC is not generally quantitative and has been shown to be unable to detect low levels of protein expression.[32,33] Furthermore, for reasons that are incompletely understood, IHC is known to sometimes produce nonspecific staining,[34] and an alternative explanation for the discordant spike protein and RNA staining via IHC and RNA ISH is a false positive IHC signal. Notably, IHC and ISH results do not always correlate with the presence of virions as IHC or ISH positivity alone can be due to free viral antigens or degenerating RNA fragments.[35] As both PCR testing[36] and RNA ISH are able to detect a very low number of virions, negative PCR testing of 29 lesions of PDC [9,21] and negative RNA ISH in 3 cases in this series would suggest that SARS-CoV-2 virions are not directly present within the tested samples. Although we cannot exclude the possibility of a false positive IHC spike protein signal, the granular staining pattern, the localization to endothelial cells and eccrine glands, similar findings in other studies (**Table 1**),[16-18] and negative spike protein IHC in eight cases of perniosis diagnosed in

2019 support the interpretation that positive spike protein staining in three cases is not spurious.

One case of PDC was studied by electron microscopy, with detection of 100 nanometer particles with 13 nanometer spikes compatible with SARS-CoV-2.[16] These particles were located within endothelial cells in the skin of a 13-year-old female patient with purpuric papules and plaques on the toes and heels.[16] She did have respiratory symptoms and mild pain in the skin lesions, and the skin biopsy was performed 11 days after onset of the skin lesions.[16] Biopsy findings included a superficial and deep perivascular, perieccrine, and panniculitic lymphocytic infiltrate with vacuolar change and superficial thrombi.[16] The ultrastructural findings in particular support that coronavirus is directly present in this case of PDC; the patient's clinical and histopathologic findings suggest that virions may be more common in painful PDC with corresponding superficial thrombi. It is also unclear if age is a factor. Age ranged from 32-42 in 7 other skin biopsies tested with PCR,[9] and at least four of 13 teenagers were tested with PCR on cutaneous lesions in another study.[21] Ultrastructural examination of more lesions, in children and adults, would be important, with correlation of ultrastructure with testing for various viral proteins and RNA.

SARS-CoV-2 spike protein is expressed on the surface of the virion, and nucleoprotein surrounds RNA internally within the virion. Serologic testing of antibodies against these two proteins have some differences but high sensitivity and specificity for

both.[37,38] Nucleoprotein antibodies are detected slightly earlier than spike protein antibodies.[37] Comparison of IHC using antibodies against the nucleoprotein vs. spike protein in formalin-fixed cutaneous specimens has not been previously performed, to our knowledge. Our results support that IHC of the nucleoprotein is possibly more reliable than that of the spike protein, in our laboratory, if RNA ISH (probe V-nCoV2019-S) is considered a gold standard for detection of virions. Limitations of this study include the small sample size and our inability to test multiple different antibodies from different companies. We were also unable to perform PCR or ultrastructural studies for SARS-CoV-2 directly on these skin biopsies.

IHC and RNA ISH against the SARS-CoV-2 spike protein may have discordant staining. Although our sample size is small, these results support the hypothesis that cleaved SARS-CoV-2 spike protein may be present within endothelial cells and eccrine glands of the skin in the absence of SARS-CoV-2 RNA or other internal proteins. Interaction of cleaved spike protein with ACE2 on endothelial cells could relate to the inflammation, vasoconstriction, and endotheliitis observed in some patients with SARS-CoV-2 infection. Future studies are necessary to confirm this hypothesis.

References

1. Freeman EE, McMahon DE, Lipoff JB, Rosenbach M, Kovarik C, DesaiSR, et al. The spectrum of COVID-19-associated dermatologic manifestations: an international

registry of 716 patients from 31countries. J Am Acad Dermatol 2020; doi: https://doi.org/10.1016/j.jaad.2020.06.1016.

2. Galván Casas C, Català A, Carretero Hernández G, Rodríguez-Jiménez P, Fernández-Nieto D, Rodríguez-Villa Lario A, et al. Classification of the cutaneous manifestations of COVID19: A rapid prospective nationwide consensus study in Spain with 375 cases. Br J Dermatol 2020; https://doi.org/10.1111/bjd.19163.

3. Marzano AV, Genovese G, Fabbrocini G, Pigatto P, Monfrecola G, Piraccini BM, et al. Varicella-like exanthem as a specific COVID-19-associated skin manifestation: multicenter case series of 22 patients. J Am Acad Dermatol 2020; doi: https://doi.org/10.1016/j.jaad.2020.04.044.

4. Gianotti R, Veraldi S, Recalcati S, Cusini M, Ghislanzoni M, Boggio F, et al. Cutaneous clinico-pathological findings in three COVID-19-positive patients observed in the metropolitan area of Milan, Italy. Acta Derm Venerol 2020; 100:adv00124.

5. Marzano AV, Cassano N, Genovese G, Moltrasio C, Vena GA. Cutaneous manifestations in patients with COVID-19: A preliminary review of an emerging issue. Br J Dermatol 2020;doi:10.1111/bjd.19264.

6. Kolivras A, Dehavay F, Delplace D, Feoli F, Melers I, Milone L, et al. Coronavirus (COVID-19) infection-induced chilblains: a case report with histopathologic findings. JAAD Case Reports 2020; https://doi.org/10.1016/j.jdcr.3020.04.011

7. Fernandez-Nieto D, Jimenez-Cauhe J, Suarez-Valle A, Moreno-Arrones OM, Saceda-Corralo D, Arana-Raja A, et al. Characterization of acute acro-ischemic lesions in nonhospitalized patients: a case series of 132 patients during the COVID-19 outbreak. J Am Acad Dermatol 2020; https://doi.org/10.1016/j.jaad.2020.04.093.

8. Landa N, Mendieta-Eckert M, Fonda-Pascual P, Aguirre T. Chilblain-like lesions on feet and hands during the COVID-19 pandemic. Int J Dermatol 2020; doi:10.1111/ijd.14937.

9. Battesti G, El Khalifa J, Abdelhedi N, Ferre V, Bouscarat F, Picard-Dahan C, et al. New insights in COVID-19-associated chilblains: a comparative study with chilblain lupus erythematosus. J Am Acad Dermatol 2020; doi: https://doi.org/10.1016/j.jaad.2020.06.1018.

10. Freeman EE, McMahon DE, Lipoff JB, Rosenbach M, Kovarik C, Takeshita J, et al. Pernio-like skin lesions associated with COVID-19: a case series of 318 patients from 8 countries. J Am Acad Dermatol 2020; doi: https://doi.org/10.1016/j.jaad.2020.05.109.

11. Kanitakis J, Lesort C, Danset M, Jullien D. Chilblain-like acral lesions during the COVID-19 pandemic ("COVID toes"): Histologic, immunofluorescence and immunohistochemical study of 17 cases. J Am Acad Dermatol 2020; https://doi.org/10.1016/jjaad.2020.05.145.

12. Andina D, Noguera-Morel L, Bascuas-Arribas M, Gaitero-Tristan J, Alonso-Cadenas JA, Escalada-Pellitero S, et al. Chilblains in children in the setting of COVID-19 pandemic. Pediatr Dermatol 2020; doi.10.1111/pde.14215.

13. El Hachem M, Diociaiuti A, Concato C, Carsetti R, Carnevale C, Ciofi Degli Atti M, et al. A clinical, histopathological and laboratory study of 19 consecutive Italian paediatric patients with chilblain-like lesions: lights and shadows on the relationship with COVID-19 infection. J Eur Acad Dermatol Venereol 2020, doi:10.1111/jdv.16682.

14. Cordoro KM, Reynolds SD, Wattier R, McCalmont TH. Clustered cases of acral perniosis: Clinical features, histopathology and relationship to COVID-19. Pediatr Dermatol 2020, doi:10.1111/pde.14227.

15. Colonna C, Monzani NA, Rocchi A, Gianotti R, Boggio F, Gelmetti C. Chilblain-like lesions in children following suspected COVID-19 infection. Pediatr Dermatol 2020;https://doi.org/10.1111/pde.14210.

16. Colmenero I, Noguera-Morel L, Hernandez-Martin A, Wiesner T, Rodriguez-Peralto J, Requena L, et al. SARS-CoV-2 endothelial infection causes COVID-19 chilblains: histopathological, immunohistochemical and ultrastructural study of 7 paediatric cases. Br J Dermatol 2020;doi:10.1111/bjd.19327.

17. Magro C, Mulvey JJ, Laurence J, Sanders S, Crowson N, Grossman M, et al. The differing pathophysiologies that underlie COVID-19 associated perniosis and thrombotic retiform purpura: a case series. Br J Dermatol 2020;doi.org/10.1111/bjd.19415.

18. Santoja C, Heras F, Nunez L, Requena L. COVID-19 chilblain-like lesion: immunohistochemical demonstration of SARS-CoV-2 spike protein in blood vessel endothelium and sweat gland epithelium in a polymerase chain reaction-negative patient. Br J Dermatol 2020;doi.org/10.1111/bjd.19338.

19. Torrelo A, Andina D, Santonja C, Noguera-Morel L, Bascuas-Arribas M, Gaitero-Tristan J, et al. Erythema multiforme-like lesions in children and COVID-19. Pediatr Dermatol 2020;doi.10.1111/pde.14246.

20. Magro C, Mulvey J, Berlin D, Nuovo G, Salvatore S, Harp J, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res 2020; doi: 10.1016/j.trsl.2020.04.007.

21. Herman A, Peeters C, Verroken A, Tromme I, Tennstedt D, Marot L, et al. Evaluation of chilblains as a manifestation of the COVID-19 pandemic. JAMA Dermatol 2020; doi:10.1001/jamadermatol.2020.2368.

22. Ko CJ, Harigopal M, Damsky W, Gehlhausen JR, Bosenberg M, Patrignelli R, et al. Perniosis during the COVID-19 pandemic: Negative anti-SARS-CoV-2 immunohistochemistry in six patients and comparison to perniosis before the emergence of SARS-CoV-2. J Cutan Pathol 2020;doi:10.1111/cup.13830.

23. Hosier H, Farhadian S, Morotti RA, Deshmukh U, Lu-Culligan A, Campbell KH, et al. SARS-CoV-2 infection of the placenta. J Clin Invest 2020;doi.10.1172/JCI139569.

24. Iba T, Levy JH, Connors JM, Warkentin TE, Thachil J, Levi M. The unique characteristics of COVID-19 coagulopathy. Crit Care 2020;24(1):360.

25. Goshua G, Pine AB, Meizlish ML, Chang C, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, ross-sectional study. Lancet Haematol 2020;7(8):e575-e582.

26. O'Sullivan J, McGonagle D, Ward SE, Preston RJS, O'Donnell JS. Endothelial cells orchestrate COVID-19 coagulopathy. Lancet Haematol 2020;7(8):e553-e555.

27. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203(2):631-637.

28. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004;203(2):622-630.

29. Genesig Coronavirus (COVID-19) genesig® Real-Time PCR assay: Instructions for Use. Issue 2.0
https://www.who.int/diagnostics_laboratory/eul_0489_185_00_path_covid19_ce_ivd_ifu_issue_2.0.pdf?ua=1

30. Wang F, Flanagan J, Su N, Wang L, Bui S, Nielson A, et al. RNAscope: A novel *in situ* RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 2012;14(1):22-29.

31. Wang H, Su N, Wang L-C, et al. Quantitative ultrasensitive bright-field RNA in situ hybridization with RNAscope. Methods Mol Biol. 2014;1211:201– 212.

32. Sompuram SR, Vani K, Schaedle AK, Balasubramanian A, Bogen SA. Quantitative assessment of immunohistochemistry laboratory performance by measuring analytic response curves and limits of detection. Arch Pathol Lab Med 2018;142(7):851-862.

33. Jensen K, Krusenstjerna-Hafstrom R, Lohse J, Petersen KH, Derand H. A novel quantitative immunohistochemistry method for precise protein measurements directly in formalin-fixed, paraffin-embedded specimens: analytical performance measuring HER2. Mod Pathol 2017;30(2):180-193.

34. Buchwalow I, Samoilova V, Boecker W, Tiemann M. Non-specific binding of antibodies in immunohistochemistry: fallacies and facts. Sci Rep 2011;1:28.

35. Liu J, Babka AM, Kearney BJ, Radoshitzky SR, Kuhn JH, Zeng X. Molecular detection of SARS-CoV-2 in formalin fixed paraffin embedded specimens. JCI Insight 2020;5(12):139042.

36. Maitland NJ, Lynas C. The detection of latent virus infection by polymerase chain reaction. In: Mathew CG (eds). Protocols in human molecular genetics. Methods in molecular biology, vol 9. Springer, Totowa, NJ, 1991, pp 347-354.

37. Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S, et al. Sensitivity in detection of antibodies to nucleocapsid and spike proteins of severe acute respiratory syndrome coronavirus 2 in patients with coronavirus disease 2019. J Infect Dis 2020;222(2):206-213.

38. To KK, Tsang OT, Leung W, Tam AR, Wu T, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20(5):565-574.

Table 1: SARS-CoV-2 spike protein and RNA staining of skin lesions

Study	Control	Other testing performed	Clinical lesion	Biopsy findings	Antibody (Company, Dilution)	Total cases
Spike protein immunohistochemistry in skin						
This study	Placenta (positive) 8 cases of idiopathic perniosis (negative)	3/3 cases: negative IgG and IgM SARS-CoV-2 serology	Pernio-like in 6/6 1 case also with targetoid lesions	Perivascular lymphocytes in all 6; perieccrine inflammation in 1 case with eccrine glands represented	40150-T62-COV (Sino Biological, 1:200)	3/6 +endothelial cells; 1 of 3 with sufficient depth had + eccrine epithelium
Colmenero, *et al*[16]	Lung (positive)	6/6 cases: negative IgG and IgM SARS-CoV-2 serology One case: electron microscopy with detection of ~90 nm particle in	Pernio-like on feet/ toes	Pernio-like with mild interface change, superficial and deep* perivascular and eccrinotropic lymphocytic inflammation; endothelialitis (separation of endothelial cells from the	1A9 (GeneTex, 1:200)	7/7 +endothelial cells and eccrine epithelial cells

		endothelial cells		basement membrane); microthrombi in 4 cases		
Magro, *et al*[17]	Lung (positive) Idiopathic perniosis (negative)	Pernio-like: 1 case with negative nasophary ngeal swab testing; 1 case with negative IgG/IgM serologic testing Purpuric/ thrombotic: 6/6 COVID-19 positive Envelope protein IHC, 1:300, ProSci positive in a pattern similar to spike protein	3 cases pernio-like	Interface dermatitis with superficial and deep perivascular and perieccrine lymphocytes	Spike protein (ProSci, 1:7000)	Pernio-like cases: 3/3 rare positivity in rare inflammator y cells only Thrombotic: 6/6 positive in endothelial cells
			6 cases retiform purpura	Vascular occlusion in mid to deep dermis		
Santoja, *et al*[18]	Lung (positive) Negative controls: Adult tonsil, perniosis from 2019	Negative nasophary ngeal swab testing; negative IgG/IgM serologic testing	Pernio-like, toes and feet	Perivascular and periadnexal lymphocytic inflammation with focal thrombosis	1A9 (GeneTex, dilution not specified)	1 case: +endothelial cells and eccrine secretory and excretory cells
Torrelo, *et al*[19]	Lung (positive)	Negative IgG/IgM serologic testing in 1 case	4 cases, EM-like, and 2 had skin biopsies	Superficial and deep perivascular and perieccrine lymphocytic inflammation	1A9 (GeneTex, dilution not specified)	2/2 cases: +endothelial cells and eccrine epithelium
Magro*, et al*[20]	Lung (positive)	+Naso-pharyngeal swab tests in 5 cases	5 cases of retiform purpura	Vascular occlusion of small and	Spike protein (ProSci, 1:7000)	1 case: +endothelial cells

				medium-sized vessels		
RNA *in situ* hybridization in skin						
This study	See above	See above	See above	See above	V-nCoV2019-S (ACD RNAscope probe, prediluted)	3/3 negative
Magro, *et al*[17]	See above	Purpuric/ thrombotic: 6/6 COVID-19 positive Envelope protein IHC, 1:300, ProSci positive in a pattern similar to spike protein	See above	See above	848561-C3 (ACD RNAscope probe)	Pernio-like cases: 3/3 negative Thrombotic cases: 6/6 negative

EM, erythema multiforme; ACD, Advanced Cell Diagnostics

*6 of 7 cases included subcutaneous fat with extension of inflammation into fat in all 6 cases

Table 2: Clinical Data on Acral Perniotic Lesions Diagnosed During the COVID-19 pandemic, Mid-May to Mid-June, 2020

Case	**Age**	**Gender**	**Biopsy Site**	**Immunohistochemistry**		**RNA *in situ* hybridization to spike protein**
				Nucleoprotein	**Spike protein**	
1	82	Female (F)	Finger	-	+	-
2	62	F	Toe	-	+	-
3	76	Male (M)	Finger	-	+	-
4	61	F	Knee	-	-	ND
5	31	F	Finger	-	-	ND
6			Finger	-	-	ND
Summary	**Mean** 62	**Ratio F:M** 4:1	Fingers > other sites	6/6 negative	3/6 positive	3/3 negative

IHC, immunohistochemistry; ISH, *in situ* hybridization; ND, not done; Cases 1, 4, and 5 had serologic testing for IgM and IgG antibodies against SARS-CoV-2, and all were negative. These cases were previously described in more detail in CUP-O-463-2020, submitted for publication.

Figure legends

Figure 1: Case 3. Acral perniotic lesion presenting during the COVID-19 pandemic histopathologic findings. There is perivascular inflammation in this superficial shave biopsy. Hematoxylin and eosin staining, original magnification 200x.

Figure 2: Acral perniotic lesions presenting during the COVID-19 pandemic (PDC). Staining results of a positive control (placental syncytiotrophoblasts from a COVID-19 patient), showing granular staining that is sharper and cleaner with SARS-CoV-2 anti-spike protein RNA *in situ* hybridization (**A**) compared to anti-spike protein immunohistochemistry (**D**). Two acral perniotic lesions (PDC Case 1, **B,E**; PDC Case 3, **C,F**) presenting during the COVID-19 pandemic stained with anti-SARS-CoV-2 spike protein RNA *in situ* hybridization (ISH) (**B,C**) and an antibody directed against the SARS-CoV-2 spike protein (**E,F**). RNA ISH is negative in **B,C.** There is weak background immunohistochemical staining and darker staining of endothelial cells (**E,F**) and eccrine glands (**E**). All images, magnification 400X.

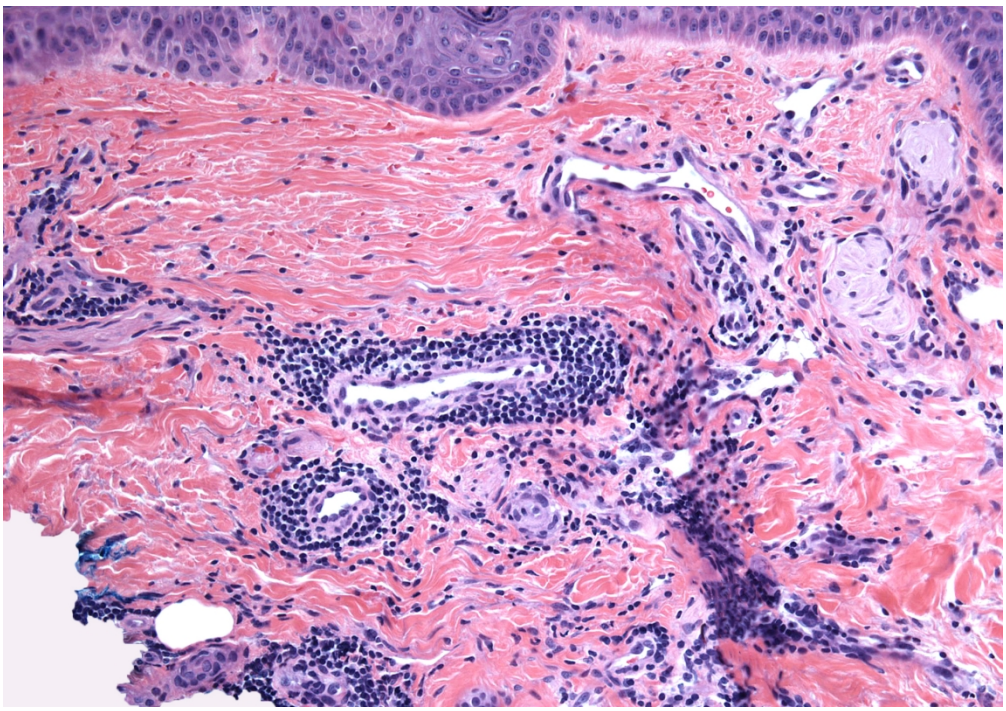

Figure 1: Case 3. Acral perniotic lesion presenting during the COVID-19 pandemic, histopathologic findings. There is perivascular inflammation in this superficial shave biopsy. Hematoxylin and eosin staining, original magnification 200x.

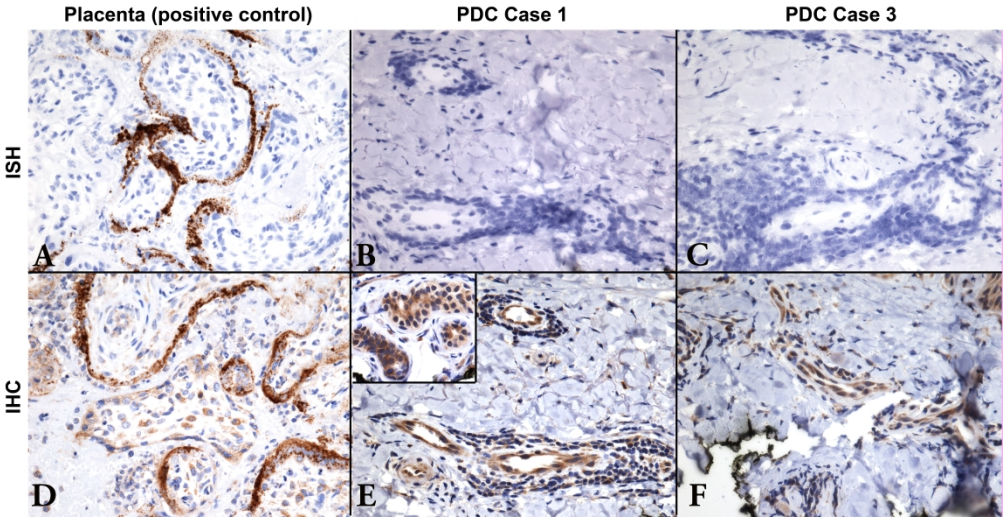

Figure 2: Acral perniotic lesions presenting during the COVID-19 pandemic (PDC). Staining results of a positive control (placental syncytiotrophoblasts from a COVID-19 patient), showing granular staining that is sharper and cleaner with SARS-CoV-2 anti-spike protein RNA in situ hybridization (A) compared to anti-spike protein immunohistochemistry (D). Two acral perniotic lesions (PDC Case 1, B,E; PDC Case 3, C,F) presenting during the COVID-19 pandemic stained with anti-SARS-CoV-2 spike protein RNA in situ hybridization (ISH) (B,C) and an antibody directed against the SARS-CoV-2 spike protein (E,F). RNA ISH is negative in B,C. There is weak background immunohistochemical staining and darker staining of endothelial cells (E,F) and eccrine glands (E). All images, magnification 400X.