Archives of Oral Biology 136 (2022) 105374

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Review

Morphological and tissue-based molecular characterization of oral lesions in patients with COVID-19: A living systematic review

Felipe Martins Silveira [a,b], André Luiz Rodrigues Mello [c], Laura da Silva Fonseca [c], Luíse dos Santos Ferreira [c], Laura Borges Kirschnick [d], Manoela Domingues Martins [b,d], Lauren Frenzel Schuch [d], José Alcides Almeida de Arruda [e], Ciro Dantas Soares [d,f], Alexandre de Oliveira Sales [f], Ronell Bologna-Molina [a], Alan Roger Santos-Silva [d], Ana Carolina Uchoa Vasconcelos [c,*]

[a] Molecular Pathology Department, School of Dentistry, Universidad de la República, Montevideo, Uruguay
[b] Department of Oral Pathology, School of Dentistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil
[c] Diagnostic Center for Oral Diseases, School of Dentistry, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil
[d] Department of Oral Diagnosis, Piracicaba Dental School, Universidade Estadual de Campinas, Piracicaba, São Paulo, Brazil
[e] Department of Oral Surgery, Pathology and Clinical Dentistry, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[f] Division of Anatomic Pathology, Getulio Sales Diagnósticos, Private Pathology Service, Natal, Rio Grande do Norte, Brazil

ARTICLE INFO

Keywords:
COVID-19
Oral pathology
Molecular pathology
Pandemics
SARS-CoV-2

ABSTRACT

Objective: This living systematic review aims to integrate the morphological and tissue-based molecular characterization of oral lesions occurring in individuals infected by COVID-19 (OLICs).
Materials and Design: This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. PubMed, Web of Science, Scopus, Ovid, Embase, and LILACS were searched to identify reports on OLICs with morphological and/or tissue-based molecular data.
Results: Four studies reporting five cases were included. Three patients were male, and the mean age of the individuals was 47.6 years. The most reported anatomical location was the palate (n = 4), whereas ulcers were the most frequent clinical presentation (n = 3). Histopathologically, all cases revealed cell vacuolization and exocytosis in the epithelial layer. In the mesenchymal layer, inflammatory cell infiltrate and thrombi/microvascular thrombosis were observed in three cases. Immunohistochemical reactions were performed in two cases. Both cases were negative for HHV-1, HHV-2, and CMV. One case revealed positivity for SARS-CoV-2 spike protein. No other molecular tests were found for the characterization of OLIC.
Conclusions: The pathological characteristics of OLICs are still unspecific. However, with the ongoing COVID-19 pandemic and well-documented new cases, whether OLICs are due to coinfections or has a primary origin can be determined.

1. Introduction

Severe acute respiratory syndrome (SARS) coronavirus 2 (CoV-2) was first detected in Wuhan, China, in December 2019, and soon after a global pandemic, as labeled by the World Health Organization (WHO), began (World Health Organization, 2022). As of July 2021, there were 190,169,833 confirmed cases of coronavirus disease 19 (COVID-19), and the disease had caused approximately 4086,000 deaths worldwide (World Health Organization, 2022). The SARS-CoV-2 virus leads to severe respiratory disturbance that may develop into acute respiratory syndrome. Symptoms are characterized by a wide range of clinical presentations, varying from headache, fever, and dry cough to severe pneumonia in more severe cases. The respiratory system seems to be the most affected, although some of the infected individuals present symptoms and signs at other distant places in the body, such as the oral and perioral regions (Tang et al., 2020). Thrombotic complications seem to be frequent in COVID-19 (Hanff, Mohareb, Giri, Cohen, & Chirinos, 2020).

Abbreviations: OLICs, oral lesions occurring in individuals infected by COVID-19.

* Correspondence to: Centro de Diagnóstico das Doenças da Boca – CDDB – Faculdade de Odontologia, Universidade Federal de Pelotas., R. Gonçalves Chaves, 457, room 607, Pelotas, Rio Grande do Sul 96015-560, Brazil.
E-mail address: carolinauv@gmail.com (A.C.U. Vasconcelos).

https://doi.org/10.1016/j.archoralbio.2022.105374
Received 17 December 2021; Received in revised form 9 February 2022; Accepted 10 February 2022
Available online 12 February 2022

The prevalence of oral manifestations in individuals infected by SARS-CoV-2 is virtually unknown. Concerns have been raised about whether the oral manifestations are caused by SARS-CoV-2, a pattern of different viruses that take advantage of systemic weaknesses during COVID-19, or even an adverse reaction to therapies used to control the disease. Recent literature has reported that oral lesions are usually symptomatic, presenting as ulcers, erosions or macules (Santos, Normando, Carvalho da Silva, Acevedo, De Luca Canto & Sugaya, 2021a; Santos et al., 2021b). The tongue and labial mucosa are the most common locations, and there is no sex predilection (Iranmanesh, Khalili, Amiri, Zartab, & Aflatoonian, 2021). Clinically, the oral manifestations of COVID-19 can make a differential diagnosis with aphthous stomatitis, herpetiform lesions, necrotizing periodontal disease, angina bullosa-like, angular cheilitis, and Melkerson-Rosenthal syndrome.

To date, there is no definitive evidence in the specific microscopic findings of oral lesions occurring in individuals infected with COVID-19 (OLICs) that suggest etiopathogenesis. In turn, only a few histopathological studies of OLIC have been published elsewhere. Studies have generally shown unspecific histopathological properties such as vascular ectasia and perivascular lymphocytic infiltrate in biopsies from oral lesions of COVID-19 patients. Additionally, the SARS-CoV-2 spike protein was positive by immunohistochemistry in inflammatory endothelial cells and keratinocytes, and acinar and ductal cells of the minor salivary glands in one study (Cruz Tapia, Peraza Labrador, Guimaraes, & Matos Valdez, 2020; Soares, Carvalho, Carvalho, Carvalho, & Almeida, 2020; Soares, Mosqueda-Taylor, de Carvalho, & de Almeida, 2021). Recent studies have established angiotensin-converting enzyme 2 (ACE2) as the functional host receptor for SARS-CoV-2 (Bourgonje et al., 2020).

The OLICs have become an important field of research once their morphological and molecular characterizations are not well defined. In this sense, a living systematic review (LSR) is the recommended methodology to incorporate scientific evidence that is continuously emerging (Elliott, Synnot, Turner, Simmonds, Akl & McDonald, 2017). From this perspective, this LSR aims to incorporate the available data on the demographic, clinical, morphological and tissue-based molecular characterization of OLICs.

2. Materials and methods

2.1. Eligibility criteria

Case reports and case series on OLICs confirmed by polymerase chain reaction (PCR) tests and presenting the histopathological report and/or tissue-based molecular characterization (i.e., immunohistochemistry, electron microscopy, immunofluorescence, or PCR) of the lesion were included in this LSR. No restrictions were imposed on the date of publication, language or geographic region where the study was conducted. Bibliographic reviews, systematic reviews, editorial reviews, meeting/congress abstracts, experimental studies, *in vitro* or *ex vivo* studies, and articles in which it was not possible to access the full text were excluded.

2.2. Information sources and search strategies

Electronic searches were undertaken in August 2021 in PubMed (National Library of Medicine), Web of Science (Clarivate Analytics), Scopus (Elsevier), Ovid (Wolters Kluwer), Embase (Elsevier), and LILACS (Virtual Health Library). The complete scheme for the search strategy in the PubMed database is provided in Supplementary Table 1. Specific searches were tailored to each database. The retrieved references were exported to EndNote software (Clarivate Analytics, Philadelphia, USA), and duplicates were removed upon identification. To identify any eligible articles that may not have been retrieved by the search strategy, hand searches were conducted to check all references of the included articles. The same search strategy will be updated every 12 months for two years, and we will take at least one month to apply the review process for study selection, data collection, risk of bias, and synthesis of results.

2.3. Selection process

The selection was performed in two phases by two authors (L.S.F. and A.L.R.M.). In phase 1, the two authors independently reviewed the titles and abstracts of all references according to the eligibility criteria. If the title or abstract fulfilled the eligibility criteria, the study was incorporated into phase 2. In this phase, the same eligibility criteria were applied to the full text of the articles. Possible disagreements between the two authors were resolved by a third author (A.C.U.V.) – a senior lecturer in Oral Pathology & Oral Medicine. The final selection was made based on the full text of the publication, ensuring that only studies that did not meet the eligibility criteria were excluded. The calibration of the reviewers was verified by assessing the agreement among the three reviewers (L.S.F., A.L.R.M., and A.C.U.V.) regarding the evaluation of titles/abstracts of the first 50 references retrieved during the searches. A kappa value of 0.93 was achieved, i.e., excellent agreement between reviewers.

2.4. Data extraction and data items

The following data, when available, were obtained from each reference and recorded on a standardized form: continent of publication, sex, age, skin color, systemic condition, general systemic conditions, serological tests, pharmacological management for COVID-19, and status, and number of OLICs/patients, anatomical location, concomitant skin lesions, fundamental lesion, symptomatology, time of progression, histopathological features, immunohistochemistry, electron microscopy, immunofluorescence, PCR, follow-up, and lesion treatment. In cases where information was lacking, unclear, incomplete or not described in the studies, contact was made with the authors of the respective studies. If the contact was not effective, the data were disregarded or indicated as not informed. Doubts during the data extraction process were resolved through discussion among the authors.

2.5. Critical appraisal of the included studies

The methodological quality of the included articles was assessed using the Joanna Briggs Institute – University of Adelaide tool for case reports or case series (Gagnier et al., 2013). The case reports included were assessed according to the following parameters: clear description of the patient's demographic characteristics, medical history and presentation as a timeline, clear description or presentation of the patient's current clinical condition, clear description of the diagnostic tests and assessment methods, clear description of the treatment provided, information on the postintervention clinical condition, identification or report of adverse events, and lessons provided by the case report. The case series articles included were assessed according to the following parameters: clear statement of the criteria for inclusion, condition measured in a standard and reliable way, use of valid methods for identification of the condition, consecutive and complete inclusion of participants, clear reporting of the demographics and clinical information of the participants, clear reporting of the outcomes and of the demographic information regarding study site(s)/clinic(s), and use of appropriate statistical analysis. In each article included, the parameters could be rated as "yes" (low risk of bias), "no" (high risk of bias) or "not applicable".

2.6. Other information

This systematic review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page, McKenzie, Bossuyt, Boutron, Hoffmann & Mulrow, 2021). A protocol was drafted and registered with the International Prospective Register of Systematic Reviews (PROSPERO), protocol number:

CRD42020214584.

2.7. Synthesis methods

Descriptive statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 25.0, Armonk, US).

3. Results

3.1. Study selection

The electronic searches yielded 1188 references. After the removal of 55 duplicates, the inclusion and exclusion criteria were applied to 1133 references. After the exclusion of 1110 references according to the eligibility criteria, a total of 23 articles were selected for full-text assessment. Of these, 21 articles were excluded for the following reasons: the study did not present the histopathological characteristics of the oral lesions and the oral lesion developed before the COVID-19 infection. Finally, two articles fulfilled the eligibility criteria, and two articles were identified in the manual search process. Therefore, a total of four studies reporting five cases of OLICs with histopathological records were included in this systematic review (Ansari, Gheitani, Heidari, & Heidari, 2021; Cruz Tapia et al., 2020; Soares et al., 2020, 2021). A flowchart of the selection process of the studies is illustrated in Fig. 1.

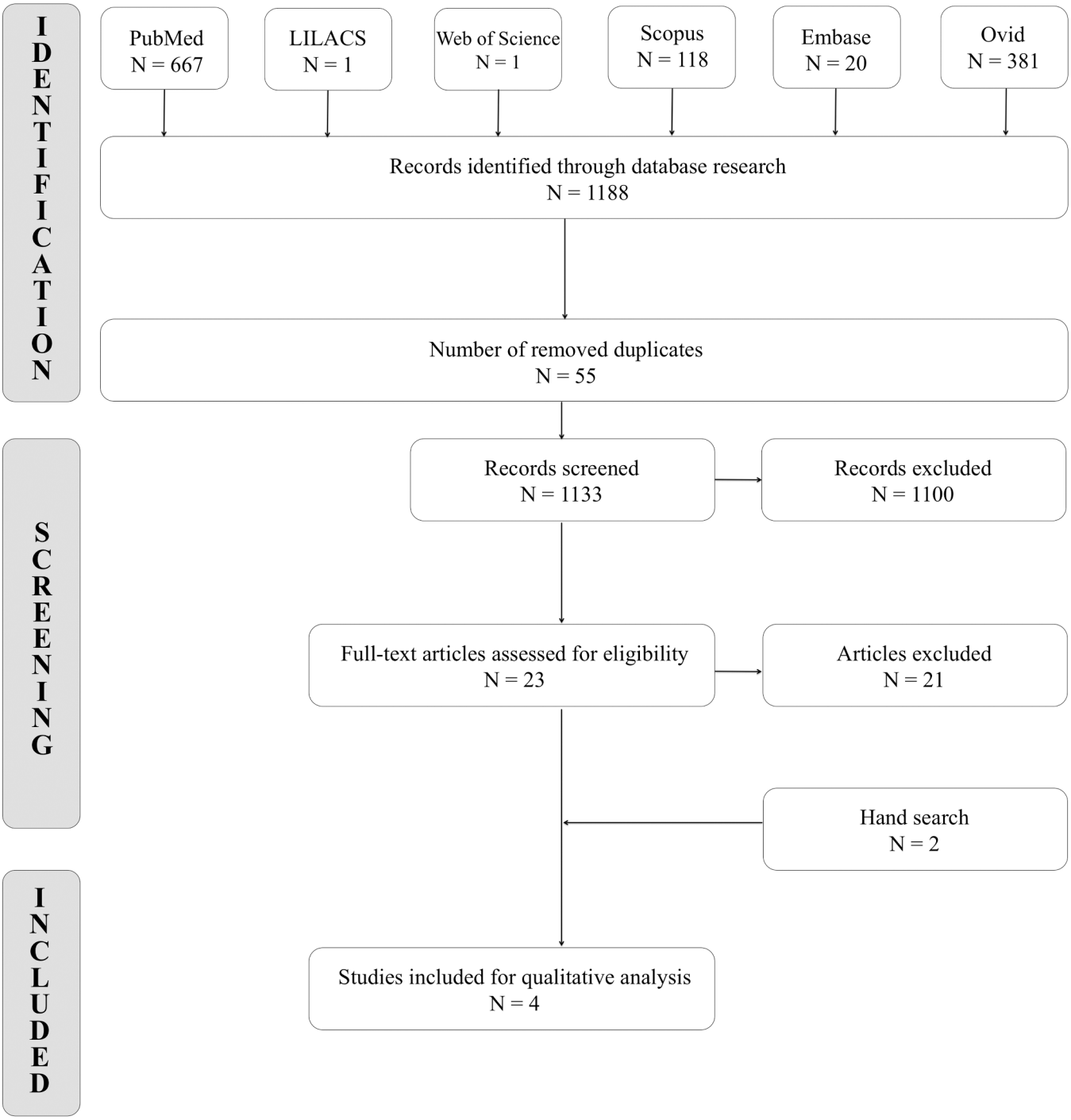

Fig. 1. Flowchart of the selection process.

3.2. Demographic and clinicopathological features and treatment of the affected individuals

Considering the few included studies in this LSR in this first approach, the results were presented based in a descriptive statistics. In this sense, the data is qualitative described for each result. The demographic and clinical data of the affected patients are displayed in Table 1. Articles from three countries were included. Cases were reported from Iran (n = 2/40%), Brazil (n = 2/40%), and Mexico (n = 1/20%). Three patients were male (60%), and two were female (40%). The mean age of the individuals was 47.6 years (range: 23–75 years). All individuals (n = 5/100.0%) were white. The following associated systemic diseases were reported in three cases: diabetes (n = 2/40%), hypertension (n = 2/40%), and coronary heart disease (n = 1/20%). Of the informed cases, the general systemic conditions were fever (n = 3/23%), shortness of breath (n = 2/15.4%), cough (n = 2/15.4%), hypoxia (n = 1/7.7%), vesiculobullous lesions on the skin (n = 1/7.7%), myalgia (n = 1/7.7%), dysgeusia (n = 1/7.7%), headache (n = 1/7.7%), and burning mouth (n = 1/7.7%). Serological tests were performed in two patients: HSV was negative in both cases (n = 2/100%). The drugs used for COVID-19 management were supportive treatment [analgesic and anti-thermal drugs] (n = 5/50%), antibiotic therapy (n = 2/33.3%), and antivirals (n = 1/16.7%). Regarding the status of the patients, all of them (n = 5/100%) were alive.

3.3. Clinical, morphological and molecular characterization, and treatment of oral lesions

The features and molecular characterization of the OLICs are displayed in Table 2. All OLICs involved multiple lesions, and the anatomical location most commonly affected was the palate, with 3 cases (37.5%), followed by the lips and tongue, with two cases (25%)

Table 1
Demographic, clinicopathological features, and treatment of the individuals.

Variable	n (%)
Continent (n = 5)	
America	3 (60.0)
Asia	2 (40.0)
Sex (n = 5)	
Male	3 (60.0)
Female	2 (40.0)
Age (years)	
Mean DS	47.6 (± 19.3)
Range	23–75 years
Skin color (n = 5)	
White	5 (100.0)
Systemic condition (n = 5)[a]	
Diabetes	2 (40.0)
Hypertension	2 (40.0)
Coronary heart disease	1 (20.0)
General systemic conditions (n = 13)[a]	
Fever	3 (23.0)
Shortness Of Breath	2 (15.4)
Cough	2 (15.4)
Hypoxia	1 (7.7)
Vesicobullous lesions on skin	1 (7.7)
Myalgia	1 (7.7)
Dysgeusia	1 (7.7)
Headache	1 (7.7)
Burning mouth	1 (7.7)
Serological tests (n = 2)	
HSV Negative	2 (100.0)
Pharmacological management to COVID-19 (n = 6)[a]	
Supportive treatment	3 (50.0)
Antibiotic therapy	2 (33.3)
Antiviral	1 (16.7)
Status (n = 5)	
Alive	5 (100.0)

[a] Some cases presented more than one feature.

Table 2
Clinical, morphological and molecular characterization, and treatment of the oral lesions.

Variable	n (%)
Number of OLICs / patient	
Single	–
Multiple	5 (100.0)
Anatomical location (n = 8)[a]	
Palate	3 (37.5)
Lips	2 (25.0)
Tongue	2 (25.0)
Buccal mucosa	1 (12.5)
Concomitant skin lesions (n = 5)	
Yes	2 (40.0)
N.I.	3 (60.0)
Fundamental lesion (n = 6)[a]	
Ulcer	3 (50.0)
Macule	2 (33.3)
Bullous	1 (16.7)
Symtoptomatology (n = 4)	
Burning mouth	1 (25.0)
Pain	2 (50.0)
Dysphagia	1 (25.0)
Evolution time (days)	
Mean (DS)	5.0 (± 2.0)
Range	3–7 days
Histopathological features[b]	
Epithelial Layer	
Vacuolization	5 (100.0)
Exocytosis	
Ulcer	5 (100.0)
2 (40.0)	
Mesenchymal Layer	
Inflammation	3 (60.0)
Thrombi/microvascular thrombosis	3 (60.0)
Hemorrhage	
Necrosis	2 (40.0)
1 (20.0)	
Immunohistochemistry	
Soares et al. (2020)	
HHV-1	–
HHV-2	–
CMV	–
Treponema pallidum	–
CD20[c]	+
CD68[c]	+
CD163[c]	+
CD138[c]	+
CD4[c]	+
CD34[c]	+
CD3[c]	+
CD8[c]	+
Soares et al. (2021)	
HSV-1	–
HSV-2	–
HHV-3	–
CMV	–
SARS-CoV-2	+
Electron microscopy	
–	–
Immunofluorescence	
–	–
Polymerase chain reaction	
–	–
Follow up (days) (n = 5)	
Mean (DS)	12.8 (± 7.4)
Range	7–21 days
Lesion treatment (n = 6)[a]	
Topical analgesic, antibiotic and corticotherapy	3 (50.0)
Systemic corticotherapy	2 (33.3)
Supportive treatment	1 (16.7)

N.I., not informed
[a] Some cases presented more than one characteristic
[b] Described according to presence or absence reported in the histopathological records or observed in the provided figures of the cases
[c] Positivity in inflammatory cells or endothelial cells

each. One case (12.5%) occurred in the buccal mucosa. Two cases reported concomitant skin lesions. Ulcer was the fundamental lesion present in three cases (50%), macule in two cases (33.3%) and bullous in one case (16.7%). Four individuals had symptomatology, such as pain (n = 2), burning mouth (n = 1), and dysphagia (n = 1). The mean time of progression was 5 (± 2.0) days. Regarding the treatment for OLICs, topical agents were used in three cases (30%), systemic corticotherapy was employed in two cases (33.33%) and supportive treatment was employed in one case (16.7%).

3.4. Morphological analysis

Regarding the histopathological findings of the OLICs, in the epithelial layer all cases (n = 5/100%) presented vacuolization and exocytosis. The presence of ulcer was described in two (40%) cases. In the mesenchymal layer, inflammatory cell infiltration was observed in three cases (60%), presence of thrombi/microvascular thrombosis in three cases (60%), and presence of hemorrhage in two cases (40%). Focal areas of necrosis were reported in one case (20%). The complete histopathological description of each of the cases included in this systematic review is summarized in Supplementary Table 2.

3.5. Immunohistochemical analysis

Immunohistochemical reactions were performed in two cases. A study (Soares et al., 2020) reported focal positivity to CD20, CD68, CD163, CD138, and CD4 (inflammatory cells). The same authors also showed positivity for CD34 (endothelial cells), CD3 (lymphocytic infiltrate) and CD8 (lymphocytic infiltrate). In addition, HHV-1, HHV-2, CMV, *Treponema pallidum*, and EBV determined by *in situ* hybridization were negative. Another study (Soares et al., 2021) reported positivity for SARS-CoV-2 spike protein. HSV-1, HSV-2, human herpesvirus-3, and CMV were negative. The complete immunohistochemical description of each of the cases included in this systematic review is summarized in Supplementary Table 2.

In Fig. 2, we illustrated some histopathological findings in a 47-year-old male patient with an ulcer in the palate (from the authors' files). In the lamina propria, an intense inflammatory infiltrate admixed with some microvessel thrombi was observed. Also, the spike protein of the SARS-CoV-2 was positive in the lymphocytes, macrophages and other mononuclear inflammatory cells and in the endothelium.

3.6. Critical appraisal of the included studies

A critical appraisal of the reported cases demonstrated that all articles provided a clear description of the patient's demographic characteristics and details of their current clinical conditions. The diagnostic tests or assessment methods employed, the interventions and treatment procedure, the postintervention clinical condition and clear information about the patient's medical history and timeline were clearly described in all articles. Only one article identified or described adverse events or unanticipated events. All articles provided lessons. The critical appraisal of the studies is presented in Supplementary Table 3.

4. Discussion

Since the onset of the COVID-19 pandemic, case reports and letters to the editor have been published documenting individuals with oral manifestations after SARS-CoV-2 infection. However, only a few of them have explored their morphological features (Ansari et al., 2021; Cruz Tapia et al., 2020; Soares et al., 2020, 2021). The present LSR evaluated the available evidence related to the molecular characterization of

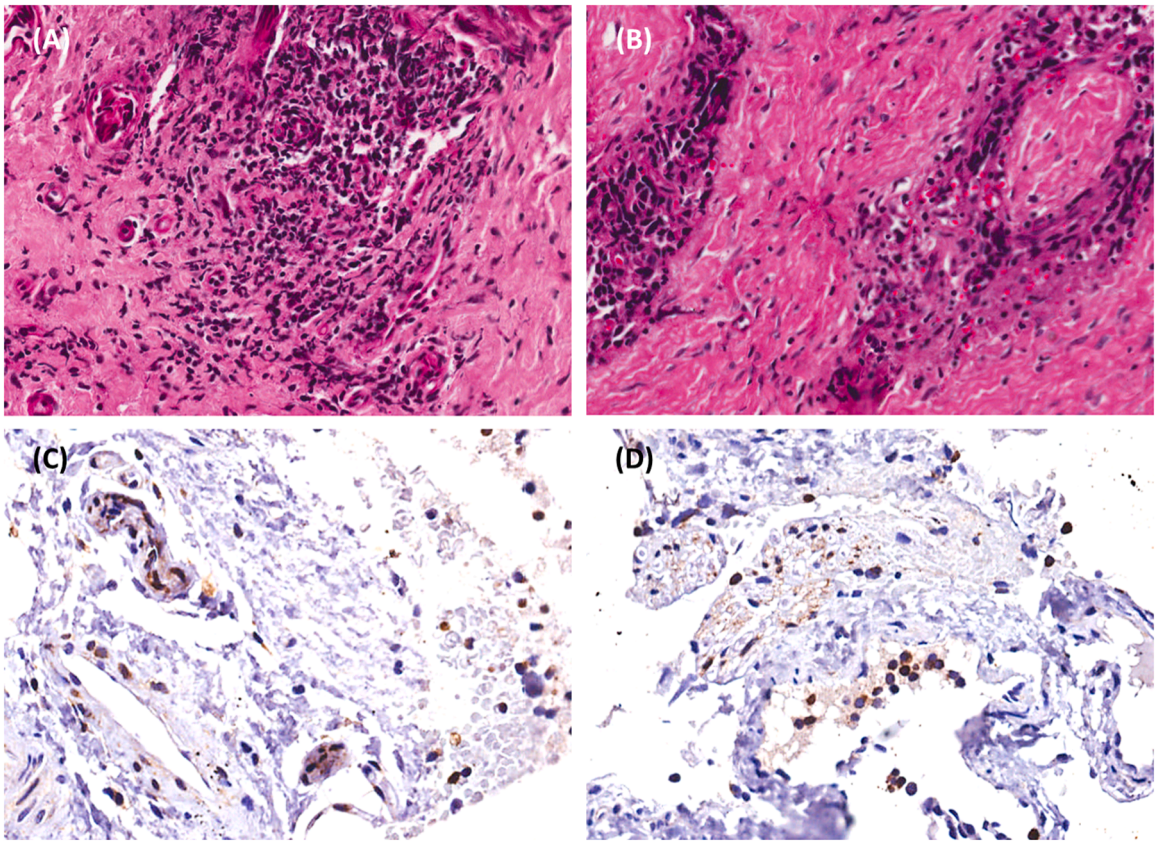

Fig. 2. Histopathologic and immunohistochemical findings in an ulcerated lesion in the hard palate of a patient with COVID-19. **(A, B)** Intense chronic inflammatory infiltrate, and some microvessel thrombi composed by fibrin and necrotic endothelial cells. **(C, D)** Immunohistochemical detection of spike protein (surface protein of the SARS-CoV-2), endothelium and also inflammatory cells were positive for this marker.

OLICs. Overall, white female individuals in their fifth decade of life were the most affected. According to previous studies (Santos et al., 2021a, 2021b), approximately 45% of individuals with COVID-19 eventually present some oral features. The same authors observed that females represented almost 57% of the sample. Similarly, Nuno-Gonzalez, Martin-Carrillo, Magaletsky, Martin Rios, Herranz Mañas, and Artigas Almazan (2021) also found a slight female preference. In contrast, some epidemiological studies reported that males had significantly higher disease severity than females (Gebhard, Regitz-Zagrosek, Neuhauser, Morgan, & Klein, 2020; Wu & McGoogan, 2020). Recent studies suggest that sex hormones may influence the viral infectivity process (Mohamed, Moulin, & Schiöth, 2021). However, the pathophysiology of this difference has not yet been established and it was observed that genes encoding entry factors for SARS-CoV-2 appeared similar in epithelial cells from males and females (Huang, P é rez, Kato, Mikami, Okuda & Gilmore, 2021; Marchesan, Warner, & Byrd, 2021).

The most common general clinical features observed in cases analyzed in this LSR were fever, dry cough, and shortness of breath. Although variable - especially with the patient's immune system and age, these features are also reported as main general clinical manifestations in recent epidemiological studies of individuals infected by COVID-19 (Guan, Ni, Hu, Liang, Ou & He, 2020; Hu, Guo, Zhou, & Shi, 2021; Tu, Tu, Gao, Shao, & Sheng, 2020). The current gold standard for diagnosing COVID-19 is based on a molecular test using real time (RT)-PCR, aiming to detect the RNA of the virus in respiratory samples, such as nasopharyngeal swabs or bronchial aspirate (Böger et al., 2021). However, in some cases, serological tests are useful to discard opportunistic infections, such as HSV infection. Currently, there is no specific antiviral treatment recommended for COVID-19, but some vaccines are already available. For mildly to moderately ill patients, active symptomatic support remains the key for management, such as maintaining hydration and nutrition and controlling fever and cough, as observed in most of the present cases. Nonetheless, for patients with severe infection or those with respiratory failure, oxygen inhalation through a mask, high nasal oxygen flow inhalation, noninvasive ventilation, or mechanical ventilation is needed (Hu et al., 2021).

Regarding the oral lesions manifested in the patients included in this study, palate was the most common site, and ulcer was the most common manifestation. Interestingly, other viral infections also generally occur more often in the hard palate (i.e., keratinized mucosa) (Reichart, 2003; Arduino, Campolongo, Scinnameo, Conrotto, Gambino & Cabras, 2018). In recent systematic reviews (Santos et al., 2021a, 2021b) of oral manifestations in COVID-19 patients, authors reported that oral lesions exhibited miscellaneous clinical aspects, such as ulcers, blisters, macules, and plaques, varying in quantity, color appearance, and anatomical localization. Most patients had oral mucosal injury during the hospitalization period, supporting the hypothesis of coinfections, immunity impairment, or adverse reactions from medications for COVID-19 treatment. In this sense, it is important to consider whether these oral lesions are directly related or secondary to SARS-CoV-2 infection (Santos et al., 2021a, 2021b). It has been reported that angiotensin-converting enzyme 2 (ACE2) is the main host cell receptor for the novel coronavirus, therefore presenting a key role in the entry of the virus into cells (Zou et al., 2020). Considering the oral mucosa tissues as a possible route for SARS-CoV-2 infection, Xu et al. (2020) showed that the expression of ACE2 is highly enriched in the epithelial cells of the tongue, which could then be considered a possible route for coronavirus infection through oral cavity tissues.

The attachment of the SARS-CoV-2 virus to ACE2 on target cells resembles that of other coronavirus infections (Jackson, Farzan, Chen, & Choe, 2021; Peiris, Mesa, Aysola, Manivel, Toledo & Borges-Sa, 2021). Although ACE2 is more commonly present in respiratory tissues, it could also be found in several other organs, which could explain the oral and some extrapulmonary manifestations. In this context, a recent systematic review (Peiris et al., 2021) reported the pathological findings and the presence of SARS-CoV-2 in the organs of patients with COVID-19. The authors reported that viral particles suggestive of SARS-CoV-2 were demonstrated in the trachea, lung, liver, colon, kidney, and central nervous system using electron microscopy, immunohistochemistry or immunofluorescence, and in the skin, pancreas, heart, saphenous vein, tonsils, testes, retina, pleural effusion, and placenta through PCR. In addition to ACE2, studies concerning SARS-CoV-2 host entry factors have also focused on the TMPRSS protease family (TMPRSS2, TMPRRS4, and TMPRSS11D) and cathepsins (CTSB and CSTL) (Singh, Bansal, & Fescotte, 2020).

The histopathological features reported in the few cases included in the present systematic review showed mainly nonspecific findings. Although not pathognomonic, it is important to discuss the main reported histopathological features observed in the cases evaluated in this study, namely, vacuolization of epithelial cells/tissue edema, infiltration of inflammatory cells and thrombi/microvascular thrombosis. First, it could be suggested that the occurrence of vacuolization in the epithelial cells reported in all included cases could be an alteration related to a specific viral infection in these cells. Some morphological epithelial alterations, called koilocytosis and Tzanck cells, are pathognomonic of other types of viral infections different from COVID-19 once these organisms are epidermotropic. Koilocytes are epithelial cells presenting hyperchromatic nuclei acentrically displaced by perinuclear vacuoles occurring in some epithelial cells infected by human papillomavirus (Hajdu, 2006; Krawczyk et al., 2008). Herpesvirus-infected cells may present intraepidermal vesicles associated with intercellular edema and acantholytic cells (Yamamoto & Aoyama, 2021). Although these morphological alterations may relate to viral origin, it is not specific for COVID-19 infection and only one study included in this systematic review analyzed the presence of the SARS-CoV-2 protein in the keratinocytes of the lesions (Soares et al., 2021). The other reports did not use any molecular method to verify the presence of coronavirus in the lesions, which did not allow us to confirm whether these epithelial morphological alterations could be etiologically related to SARS-CoV-2 infection. A recent study concluded that *in situ* hybridization for SARS-CoV-2 is more specific, easier to analyze and the interpretation is associated with an improved interobserver agreement when compared to immunohistochemistry in human tissues, probably being the best assay to investigate the presence of the virus (Massoth et al., 2021). It is worthwhile to mention that the immunohistochemical panel used in some of the presented cases in this LSR excluded the possibility of originating from other viral and bacterial infections, which could reinforce COVID-19-related involvement in the development of these lesions. For example, based on the included studies in this LSR, the oral lesions were negative for HHV-1, HHV-2, HHV-3, CMV, and *Treponema pallidum.* Nevertheless, in this sense, it is important to mention recent robust evidence that demonstrates the rich expression of SARS-CoV-2 viral entry factors in the salivary glands and epithelia of the oral mucosa, which are also sites for SARS-CoV-2 infection and replication, suggesting that the oral cavity is a hot spot for COVID-19 infection (Huang et a., 2020; Huang, P é rez, Kato, Mikami, Okuda & Gilmore, 2021; Marchesan et al., 2021).

The inflammatory reaction following COVID-19 infection may also induce hemodynamic/clotting disorders and cutaneous changes suggestive of thrombotic microangiopathy (Liu, Blet, Smyth, & Li, 2020). Accordingly, inflammatory cell infiltration and thrombi formation were reported in five and three cases included in this study, respectively. These two important reported mesenchymal features could be related to the inflammatory systemic condition and to the vascular/thrombotic events occurring in patients infected by COVID-19. Ackermann, Verleden, Kuehnel, Haverich, Welte, and Laenger (2020) documented that the histological pattern of the lungs of patients who died from COVID-19 presented diffuse alveolar damage with perivascular T-cell infiltration and severe endothelial injury. Additionally, the pulmonary vessels in those patients showed widespread thrombosis with microangiopathy, with alveolar capillary microthrombi being 9 times more prevalent in patients with COVID-19 than in patients with influenza. Furthermore,

Menter, Haslbauer, Nienhold, Savic, Hopfer, and Deigendesch (2020) reported that the predominant histopathological findings in the lungs of autopsied patients who died from COVID-19 were capillary congestion, microthrombi and moderate intra-alveolar fibrin exudation, and Varga et al. (2020) observed endothelial damage in the kidneys and intestines of patients with COVID-19. Therefore, although the pathophysiology of COVID-19 has not yet been well elucidated, there is evidence of virus-induced vascular dysfunction in infected individuals.

The results of the present systematic review have limitations that should be addressed. First, only a few cases of OLICs with associated histopathological reports have been reported to date, and most of them are letters to the editor that provide mostly objective information. Currently, most of the manifestations of COVID-19 have been studied in organs that present more severe complications, and studies on oral lesions are still sparse. In addition, some authors have described an alarming impact of the COVID-19 pandemic on overall general outpatient visits in oral medicine centers and on the rate of oral biopsies (Alves et al., 2021; Gomes, Schuch, Tarquinio, Etges, & Vasconcelos, 2020; Marques et al., 2020). It is possible that this situation could contribute to the underestimation of the real frequency of these lesions. Finally, it is becoming increasingly difficult to publish case reports – even those related to pandemic scenarios.

5. Conclusion

In summary, as few cases thus far have had the histopathological features of OLICs and all of them had mostly nonspecific characteristics, it is unfeasible to determine the pathophysiology of these lesions. It is suggested that histopathology alone might not answer the relevant clinical question about the etiology of OLICs. Future studies evaluating the molecular profile of OLICs are needed to better characterize these lesions. It is important that oral medicine providers share OLIC cases with different medical specialties and oral and maxillofacial pathologists to establish a better understanding of this new condition. Finally, the clinicians should be aware of intra and extraoral manifestations of COVID-19 - especially in scenario with new variants.

Ethics approval statement

Not applicable.

Funding source declaration

Partial funding of Sectorial Commission for Scientific Research (CSIC) 2018, University of the Republic, Uruguay.

CRediT authorship contribution statement

Felipe Silveira: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Ana Carolina Vasconcelos**: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Supervision. **André Mello:** Conceptualization, Investigation, Writing – review & editing. **Laura Fonseca:** Conceptualization, Investigation, Writing – review & editing**. Luíse Ferreira:** Conceptualization, Investigation, Writing – review & editing. **Ciro Soares:** Data curation, Investigation, Resources, Writing – review & editing. **Alan Santos-Silva:** Data curation, Investigation, Resources, Writing – review & editing. **Alexandre Sales**: Resources, Visualization, Writing – review & editing. **Laura Kirschnick:** Investigation, Visualization, Writing – review & writing. **Manoela Martins:** Investigation, Visualization, Writing – review & writing. **Lauren Schuch:** Investigation, Visualization, Writing – review & writing. **Alcides Arruda:** Investigation, Visualization, Writing – review & writing. **Ronell Bologna-Molina:** Investigation, Visualization, Writing – review & writing.

Declarations of interest

None.

Author agreement

All authors have seen and approved the final version of the manuscript.

Data sharing

Data available in article supplementary material.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001), Brazil. L.B.K., L.F.S., and L. S.F. are the recipients of fellowships. We also acknowledge the Brazilian National Council for Scientific and Technological Development (CNPq). M.D.M and A.R.S.S. are research fellows of CNPq.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2022.105374.

References

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *The New England Journal of Medicine, 383*(2), 120–128. https://doi.org/10.1056/NEJMoa2015432

Alves, F. A., Saunders, D., Sandhu, S., Xu, Y., de Mendonça, N. F., & Treister, N. S. (2021). Implication of COVID-19 in oral oncology practices in Brazil, Canada, and the United States. *Oral Diseases, 27*, 793–795. https://doi.org/10.1111/odi.13493

Ansari, R., Gheitani, M., Heidari, F., & Heidari, F. (2021). Oral cavity lesions as a manifestation of the novel virus (COVID-19). *Oral Diseases, 27*, 771–772. https://doi.org/10.1111/odi.13465

Arduino, P. G., Campolongo, M. G., Sciannameo, V., Conrotto, D., Gambino, A., Cabras, M., et al. (2018). Randomized, placebo-controlled, double-blind trial of clobetasol propionate 0.05% in the treatment of oral lichen planus. *Oral Diseases, 24*(5), 772–777. https://doi.org/10.1111/odi.12821

Böger, B., Fachi, M. M., Vilhena, R. O., Cobre, A. F., Tonin, F. S., & Pontarolo, R. (2021). Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *American Journal of Infection Control, 49*(1), 21–29. https://doi.org/10.1016/j.ajic.2020.07.011

Bourgonje, A. R., Abdulle, A. E., Timens, W., Hillebrands, J. L., Navis, G. J., Gordijn, S. J., … van Goor, H. (2020). Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *The Journal of Pathology, 251*(3), 228–248. https://doi.org/10.1002/path.5471

Cruz Tapia, R. O., Peraza Labrador, A. J., Guimaraes, D. M., & Matos Valdez, L. H. (2020). Oral mucosal lesions in patients with SARS-CoV-2 infection. Report of four cases. Are they a true sign of COVID-19 disease? *Special care in Dentistry: Official Publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry, 40*(6), 555–560. https://doi.org/10.1111/scd.12520

Elliott, J. H., Synnot, A., Turner, T., Simmonds, M., Akl, E. A., McDonald, S., et al. (2017). Living systematic review: 1. Introduction-the why, what, when, and how. *Journal of Clinical Epidemiology, 91*, 23–30. https://doi.org/10.1016/j.jclinepi.2017.08.010

Gagnier, J. J., Kienle, G., Altman, D. G., Moher, D., Sox, H., Riley, D., & Group, C. A. R. E. (2013). The CARE guidelines: consensus-based clinical case report guideline development. *Journal of Dietary supplements, 10*(4), 381–390. https://doi.org/10.3109/19390211.2013.830679

Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H. K., Morgan, R., & Klein, S. L. (2020). Impact of sex and gender on COVID-19 outcomes in Europe. *Biology of sex differences, 11*(1), 29. https://doi.org/10.1186/s13293-020-00304-9

Gomes, A., Schuch, L. F., Tarquinio, S., Etges, A., & Vasconcelos, A. (2020). Reduced demand for oral diagnosis during COVID-19: a Brazilian center experience, 10.1111/odi.13547. Advance online publication *Oral Diseases*. https://doi.org/10.1111/odi.13547.

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *The New England Journal of Medicine, 382*(18), 1708–1720. https://doi.org/10.1056/NEJMoa2002032

Hajdu, S. I. (2006). The link between koilocytes and human papillomaviruses. *Annals of Clinical and laboratory Science, 36*(4), 485–487.

Hanff, T. C., Mohareb, A. M., Giri, J., Cohen, J. B., & Chirinos, J. A. (2020). Thrombosis in COVID-19. *American Journal of Hematology, 95*(12), 1578–1589. https://doi.org/10.1002/ajh.25982

Hu, B., Guo, H., Zhou, P., & Shi, Z. L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology, 19*(3), 141–154. https://doi.org/10.1038/s41579-020-00459-7

Huang, N., Perez, P., Kato, T., Mikami, Y., Okuda, K., Gilmore, R. C., et al. (2020). Integrated single-cell atlases reveal an oral SARS-CoV-2 infection and transmission axis. *medRxiv: the Preprint Server for Health Sciences*. https://doi.org/10.1101/2020.10.26.20219089

Huang, N., Pérez, P., Kato, T., Mikami, Y., Okuda, K., Gilmore, R. C., et al. (2021). SARS-CoV-2 infection of the oral cavity and saliva. *Nature Medicine, 27*(5), 892–903. https://doi.org/10.1038/s41591-021-01296-8

Iranmanesh, B., Khalili, M., Amiri, R., Zartab, H., & Aflatoonian, M. (2021). Oral manifestations of COVID-19 disease: a review article. *Dermatologic Therapy, 34*(1), Article e14578. https://doi.org/10.1111/dth.14578

Jackson, C. B., Farzan, M., Chen, B., & Choe, H. (2021). Mechanisms of SARS-CoV-2 entry into cells (Advance online publication) *Nature Reviews Molecular Cell Biology*, 1–18. https://doi.org/10.1038/s41580-021-00418-x.

Krawczyk, E., Suprynowicz, F. A., Liu, X., Dai, Y., Hartmann, D. P., Hanover, J., & Schlegel, R. (2008). Koilocytosis: a cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. *The American Journal of Pathology, 173*(3), 682–688. https://doi.org/10.2353/ajpath.2008.080280

Liu, P. P., Blet, A., Smyth, D., & Li, H. (2020). The science underlying COVID-19: implications for the cardiovascular system. *Circulation, 142*(1), 68–78. https://doi.org/10.1161/CIRCULATIONAHA.120.047549

Marchesan, J. T., Warner, B. M., & Byrd, K. M. (2021). The "oral" history of COVID-19: primary infection, salivary transmission, and post-acute implications. *Journal of periodontology, 92*(10), 1357–1367. https://doi.org/10.1002/JPER.21-0277

Marques, N. P., da Silveira, D., Martelli, P., Martelli, D., de Lucena, E., & Martelli-Júnior, H. (2020). Brazilian Oral Medicine and public health system: the enormous impact of the COVID-19 Era, 10.1111/odi.13677. Advance online publication *Oral Diseases*. https://doi.org/10.1111/odi.13677.

Massoth, L. R., Desai, N., Szabolcs, A., Harris, C. K., Neyaz, A., Crotty, R., … Deshpande, V. (2021). Comparison of RNA in situ hybridization and immunohistochemistry techniques for the detection and localization of SARS-CoV-2 in human tissues. *The American Journal of surgical Pathology, 45*(1), 14–24. https://doi.org/10.1097/PAS.0000000000001563

Menter, T., Haslbauer, J. D., Nienhold, R., Savic, S., Hopfer, H., Deigendesch, N., et al. (2020). Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology, 77*(2), 198–209. https://doi.org/10.1111/his.14134

Mohamed, M. S., Moulin, T. C., & Schiöth, H. B. (2021). Sex differences in COVID-19: the role of androgens in disease severity and progression. *Endocrine, 71*(1), 3–8. https://doi.org/10.1007/s12020-020-02536-6

Nuno-Gonzalez, A., Martin-Carrillo, P., Magaletsky, K., Martin Rios, M. D., Herranz Mañas, C., Artigas Almazan, J., et al. (2021). Prevalence of mucocutaneous manifestations in 666 patients with COVID-19 in a field hospital in Spain: oral and palmoplantar findings. *The British Journal of dermatology, 184*(1), 184–185. https://doi.org/10.1111/bjd.19564

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ (Clinical Research ed.), 372*, n71. https://doi.org/10.1136/bmj.n71

Peiris, S., Mesa, H., Aysola, A., Manivel, J., Toledo, J., Borges-Sa, M., et al. (2021). Pathological findings in organs and tissues of patients with COVID-19: a systematic review. *PloS One, 16*(4), Article e0250708. https://doi.org/10.1371/journal.pone.0250708

Reichart, P. A. (2003). Oral manifestations in HIV infection: fungal and bacterial infections, Kaposi's sarcoma. *Medical Microbiology and immunology, 192*(3), 165–169. https://doi.org/10.1007/s00430-002-0175-5

Santos, J. A., Normando, A., Carvalho da Silva, R. L., Acevedo, A. C., De Luca Canto, G., Sugaya, N., et al. (2021aaa). Oral manifestations in patients with COVID-19: a living systematic review. *Journal of dental Research, 100*(2), 141–154. https://doi.org/10.1177/0022034520957289

Santos, J. A., Normando, A., Carvalho da Silva, R. L., Acevedo, A. C., De Luca Canto, G., Sugaya, N., et al. (2021bbb). Oral manifestations in patients with COVID-19: a 6-month update. *Journal of dental Research, 100*(12), 1321–1329. https://doi.org/10.1177/00220345211029637

Singh, M., Bansal, V., & Feschotte, C. (2020). A single-cell RNA expression map of human coronavirus entry factors. *Cell Reports, 32*(12), Article 108175. https://doi.org/10.1016/j.celrep.2020.108175

Soares, C. D., Carvalho, R. A., Carvalho, K. A., Carvalho, M. G., & Almeida, O. P. (2020). Letter to Editor: Oral lesions in a patient with Covid-19. *Medicina oral, patologia oral York cirugia Bucal, 25*(4), e563–e564. https://doi.org/10.4317/medoral.24044

Soares, C. D., Mosqueda-Taylor, A., de Carvalho, M., & de Almeida, O. P. (2021). Oral vesiculobullous lesions as an early sign of COVID-19: immunohistochemical detection of SARS-CoV-2 spike protein. *The British Journal of dermatology, 184*(1), Article e6. https://doi.org/10.1111/bjd.19569

Tang, K., Wang, Y., Zhang, H., Zheng, Q., Fang, R., & Sun, Q. (2020). Cutaneous manifestations of the coronavirus disease 2019 (COVID-19): a brief review. *Dermatologic Therapy, 33*(4), Article e13528. https://doi.org/10.1111/dth.13528

Tu, H., Tu, S., Gao, S., Shao, A., & Sheng, J. (2020). Current epidemiological and clinical features of COVID-19; a global perspective from China. *The Journal of Infection, 81*(1), 1–9. https://doi.org/10.1016/j.jinf.2020.04.011

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet (London, England), 395*(10234), 1417–1418. https://doi.org/10.1016/S0140-6736(20)30937-5

World Health Organization. COVID-19 Weekly Epidemiological Update https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—20-july-2021. (Accessed 17 January 2022).

Wu, Z., & McGoogan, J. M. (2020). Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA, 323*(13), 1239–1242. https://doi.org/10.1001/jama.2020.2648

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine, 8*(4), 420–422. https://doi.org/10.1016/S2213-2600(20)30076-X

Yamamoto, T., & Aoyama, Y. (2021). Detection of multinucleated giant cells in differentiated keratinocytes with herpes simplex virus and varicella zoster virus infections by modified Tzanck smear method. *The Journal of dermatology, 48*(1), 21–27. https://doi.org/10.1111/1346-8138.15619

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., et al. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *The New England Journal of Medicine, 382*(12), 1177–1179. https://doi.org/10.1056/NEJMc2001737