Severity of COVID-19 infection in primary Sjögren's syndrome and the emerging evidence of COVID-19-induced xerostomia

F. Chowdhury[1,2], S. Grigoriadou[1,3], M. Bombardieri[1]

[1]Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Queen Mary University of London;
[2]Department of Oral Immunobiology and Regenerative Medicine, Institute of Dentistry, Queen Mary University of London;
[3]Department of Immunology, Royal London Hospital, Barts Health NHS Trust, London, UK.

Farzana Chowdhury, MSc
Sofia Grigoriadou, MD, PhD
Michele Bombardieri, MD, PhD

Please address correspondence to:
Michele Bombardieri,
Centre for Experimental Medicine and Rheumatology,
William Harvey Research Institute,
Queen Mary University of London,
Charterhouse Square,
EC1M 6BQ London, United Kingdom.
E-mail: m.bombardieri@qmul.ac.uk

Received on September 12, 2021; accepted in revised form on November 30, 2021.

Clin Exp Rheumatol 2021; 39 (Suppl. 133): S215-S222.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2021.

Key words: primary Sjögren's syndrome, COVID-19, autoimmune rheumatic disease, xerostomia, SARS-CoV-2 pathogenesis

Competing interests: none declared.

ABSTRACT

Since the beginning of the COVID-19 disease pandemic caused by the new coronavirus SARS-CoV-2, the disease has claimed over 205M cases (205,338,159) and 4,333,094 deaths (WHO dashboard – accessed 15/08/2021). In addition to the overwhelming impact on healthcare systems treating acutely ill patients, the pandemic has had an impact on all other aspects of health care delivery, including the management of chronic diseases, the risk that is posed in patients with chronic conditions and the risk of the infection itself in those with chronic conditions. Autoimmune rheumatic diseases (ARDs), including primary Sjögren's syndrome (pSS), characterised by immune dysregulation affecting several organs in variable severity, have been of particular interest given the accelerated phase of the immune response in the course of SARS-CoV-2 infection leading to the acute inflammatory response and respiratory distress syndrome or multi-organ failure.

On the other hand, the effect of immunosuppressive drug therapies can represent a double edge sword on the course of the disease, either by increased susceptibility to and severity of the infection, or by preventing the accelerated inflammatory response induced by the infection. Additionally, the long-term impact of SARS-CoV-2 infection on the host immune system has led to the onset of novel complex clinical manifestations, comprised under the large umbrella of "long-COVID", which we are only starting to understand. In this review we focus on two interrelated aspects: i) the impact of COVID-19 on patients with pSS and ii) the emerging evidence of long-term xerostomia after SARS-CoV-2 infection.

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune rheumatic disease (ARD) characterised by an inflammatory process that predominantly affects the exocrine glands (lacrimal and salivary), resulting in progressive loss of function and the manifestation of sicca symptoms, *i.e.* dry mouth and dry eyes. Other organs such as the musculoskeletal system, lungs and skin can be affected, while fatigue is almost a universal feature (1). A cardinal feature is also the development of circulating autoantibodies, such as those targeting the ribonucleoproteins Ro/SSA and La/SSB (*i.e.* anti-Ro/SSA and anti-La/SSB autoantibodies), and rheumatoid factor (RF). There is an increased risk of lymphoma associated with the mucosal lymphoid tissue (2, 3).

The impact of the pandemic in people with pSS, remains to be fully unveiled and is of particular interest given that this is a disease affecting predominantly the mucosa and the known mucosal association of SARS-CoV-2 infection.

Is pSS a risk factor for worse COVID-19 related outcomes?

As it became apparent early in the pandemic, the main predisposing factors associated with severe disease due to SARS-CoV-2 infection and worse prognosis are pre-existing cardiovascular disease, diabetes, hypertension, male gender, older age and obesity (4-7). Data addressing specifically the severity of COVID-19 infection in pSS is scarce with the majority of studies being conducted in the entire group of ARD, including pSS, rather than specific conditions, but it is possible to extrapolate some conclusions on pSS patients from observations in ARDs as a whole group of disorders.

In a cohort of patients from Northern Italy the incidence of confirmed COVID-19 infection in patients with rheumatic diseases treated with targeted synthetic or biologic DMARDS was similar to that expected in the general population (8). The majority of patients had rheumatoid arthritis (RA) and seronegative spondyloarthropathies, no fatalities reported, none of the patients required care in the intensive care unit or experienced severe complications.

The first report from Wuhan, the hotspot of infection in China, found increased rate of respiratory failure in patients with ARD whereas length of hospitalisation and mortality rate were not increased (9). A matched cohort study of hospitalised patients in a hot spot of the disease in the USA found those with ARD had similar rates of hospitalisation but higher need for mechanical ventilation (10); a similar study in Northern Italy did not confirm those results (11), neither did data on 29 patients with rheumatic diseases in Wuhan, China (12). A global registry of 600 patients with ARD (the majority from Northern America and Europe) found the presence of co-morbidities and use of prednisolone ≥10mg daily to be associated with worse outcomes whereas biologic drug monotherapy, driven mostly by anti-tumour necrosis alpha (TNF-α) therapy, was associated with lower risk of hospitalisation (13).

Pablos *et al*. reported on a study from 5 rheumatology centres in Spain (14), that connective tissue disease (CTD) but not inflammatory arthropathies was an independent risk factor for severe disease (*i.e*. ventilation or severe complications, ITU admission or death) in this study. A subanalysis within the CTD group did not show a disease preference but patient numbers were small. There were 13 pSS cases included in this study. Prior glucocorticoids use was associated with poorer outcomes, this was not the case for synthetic/conventional or biologic disease modifying anti-rheumatic drugs (DMARDS), age and male gender was also associated with poorer outcomes. Similar results were found by Freites-Nunez *et al*. in a single centre report from Spain comparing hospitalised versus non-hospitalised patients, glucocorticosteroids were not an independent risk factor in this study but the dose was not recorded. DMARDS were not associated with increased risk of hospitalisation (15). A more recent observational study from a Greek cohort of 77 patients reported age and lung disease (in the context of the underlying ARD) as independent risk factors for hospitalisation; prior treatment with corticosteroids, rituximab and mycophenolate mofetil were more frequent in those with serious illness or requiring hospitalisation (16). There were 8 patients with Sjögren's in this cohort. Consistent with these findings, in patients with inflammatory bowel disease glucocorticosteroids but not anti-TNF-α therapy was an independent risk factor for severe disease as well as increased age and comorbidities (17).

Altogether from all the studies that investigated the impact on COVID-19 pandemic in ARD, five included significant number (*i.e*. ≥3) of pSS cases with confirmed COVID-19 infection, totalling 38 cases (9, 13-16) there was no specific analysis for those cases, most likely due to small numbers of cases. There have been 6 studies where disease specific mortality has been reported, they include 66 patients with pSS, 5 deaths of pSS cases were recorded, indicating an average mortality rate of 7.57% (12, 15, 18-21). Table I summarises all the data available to date for pSS and COVID-19 infection, including some studies of ARD.

A handful of studies specifically focused on pSS; at the early stage of the pandemic, when PCR testing was not readily available, a monocentric survey of an Italian cohort of patients with pSS suggested mild symptoms in the majority of patients (22), the prevalence of the disease among pSS patients was in line with the general population (8%), 35.2% of patients were treated with hydroxychloroquine, corticosteroids or other immunosuppressive drug therapy. An observational survey from Italy on 102 patients with pSS reported increased disease activity and patient-reported symptoms during the first lockdown period of the pandemic, of this cohort 13% of patients experienced COVID-19-related symptoms but only 2% were PCR positive (23).

The largest study to date on pSS and SARS-CoV-2 infection comes from an international registry identifying patients with pSS and confirmed or highly likely SARS-CoV-2 infection, both hospitalised and primary care patients were included with a split of 49% and 51% respectively (21). The estimated infection rate (0.62%) was similar to that in the general population and baseline characteristics associated with more severe disease were also similar to those identified in the wider spectrum of ARD *i.e*. older age, male gender, chronic co-morbidities, there was no association between underlying therapies for pSS and hospitalisation. From this cohort, 5 patients required admission to intensive care unit and 4 patients died. The specified co-morbidities included cardiovascular disease, chronic kidney disease, obesity, neoplasia and chronic pulmonary disease. There were no specific details of chronic pulmonary disease (*i.e*. interstitial lung disease related to pSS or other), probably due to the small number of patients, which would have been of interest.

Does immunosuppressive drug therapy cause worse disease outcome in pSS?

Previous coronavirus outbreaks such as the severe acute respiratory syndrome in 2002 and the Middle Eastern respiratory syndrome in 2012 were not associated with increased fatality rates in immunocompromised individuals (chemotherapy or solid organ transplantation) (24, 25), in contrast with increased disease severity caused by other respiratory virus such as influenza (26).

The effect of corticosteroid therapy in the setting of ARD has already been discussed. While the use of immunosuppressive therapy/DMARDS in pSS is lower compared with other ARD, hydroxychloroquine is the drug of choice in the majority of patients, with other DMARDS such as methotrexate used less frequently. Rituximab, an anti-CD20 monoclonal antibody, has been used predominantly in clinical trials settings. Although initial *in vitro* studies suggested a promising role of hydroxychloroquine for the treatment of COVID-19 (27) the largest clinical trial

Table I. Outcome of SARS-CoV-2 infection in patients with autoimmune rheumatic disease and pSS.

Study	Cohort size (n=)	Mean age (yrs)	Baseline treatment	Prevalence of COVID-19-related symptoms	Hospitalisation on	Mortality in patients	Baseline co-morbidities
Zhao *et al*. (12)	1 (29[#])	61[#] (median)	HCQ 26.3% GC 24%	100%*[#]	100%*[‡]	0% (3.4%[#])	N/A
Nuñez *et al*. (15)	9 (123[#])	59.88[#]	[#]Antimalarials 21.95% [#]GC 49.59% Other N/A	100%*	55.5% (44%[#])	11.11% (22%[#])	[#]HTN (32.5%), DLD (21.95%), DM (13.82%), CD (18.7%), CLD (15.45%), CKD (4.88%), thyroid disease (13.8%), liver disease (5.69%), cancer (4.07%), depression (7.32%), venous thrombosis/lung embolism (2.44%)
Santos *et al*. (19)	1 (38[#])	75.3[#]	[#]HCQ 24% GC 58% Other 50%	100%*[#]	100%*[‡]	0% (26%[#])	[#]HTN (60%), DLD (55%), DM (32%), CD (50%), ILD (32%)
Huang *et al*. (20)	2 (17[#])	64[#] (median)	[#]HCQ 25% GC 37.5% Other 25%	100%*[#]	100%*[#‡]	0% (5.90%[#])	Hypertension (35.5%), Diabetes (0%), Cerebrovascular disease (5.9%), CKD (11.8%), Infectious disease (5.9%)
Brito-Zerón *et al*. (21)	51	60	HCQ 37.3% GC 17.6% Other 21.5%	0.62%	49%	7.8%	CD (15.7%), CLD (33.3%), Obesity (13.7%), other chronic diseases (7.8%)
Giardina *et al*. (22)	150	62	HCQ 18% GC 8.6% Other 8.6%	4%	0%	0%	70% overall (HTN, CD, DM, DLD, lung disease)
Carubbi *et al*. (23)	102	N/A	HCQ 38.2% GC N/A Other N/A	13%	0.9%	N/A	N/A

CD: cardiovascular disease; COPD: chronic obstructive pulmonary disorder; ILD: interstitial lung disease; CLD: chronic lung disease; SG: salivary gland; HTN: hypertension; CAD: coronary artery disease; DM: diabetes mellitus; DLD: dyslipidaemia; CKD: chronic kidney disease; HCQ: hydroxychloroquine; GC: glucocorticoids; Other: refers to other DMARDS.
*Study included only confirmed cases.
[#]Refers to entire ARD cohort rather than pSS.
[‡]Study looked only at hospitalised patients.

on COVID-19 treatment failed to demonstrate any benefit, at least for hospitalised patients (28).

A large global registry for patients with ARD found that previous treatment with rituximab increased the odds ratio of death by 4.04 (as opposed to methotrexate therapy) for all patient groups, including inflammatory arthropathies and CTDs (29). Although patients with pSS were included in this study, they were captured as part of a generic CTD group and no specific information as to their treatment was provided. The authors speculate that this may well be due to the effect of rituximab in depleting B cells and the development of anti-SARS-CoV-2 antibodies, the latter have been shown to play a crucial role in the development of immunity against SARS-CoV-2 (30). No data on specific timing of rituximab infusions in relation to infection was available, this would have been of interest as the further away rituximab infusion is from infection, the better chances of B cell recovery and antibody production is expected. A single centre retrospective study from Spain looked specifically in ARD cases treated with rituximab, including 2 cases of pSS, and reported high rate of severe COVID-19 infection (61.5%) leading to hospitalisation and death, this was a descriptive study with no control group (31).

The largest study to date on pSS and COVID-19 did not show any associations between baseline therapies and disease severity, the majority of patients were not on treatment (58.8%), the rest of the patients were on unspecified immunosuppressive drug therapy (27.4%) or on hydroxychloroquine (11.7%) (21).

Will the COVID-19 pandemic cause increased prevalence of autoimmune diseases such as pSS?

The concept of increased autoimmunity following viral infections is well known and in the case of pSS it has been linked with Epstein Barr virus (32), Cytomegalovirus, Coxsackie and other viruses (33). In pSS, viral infection of epithelial cells leads to the production of pro-inflammatory cytokines and an aberrant immune response via Toll-like receptors (TLRs) characterised by type I interferons (IFN) signature. This is typical of a viral infection resulting in the restriction of viral replication and spread.

In contrast, infection with SARS-CoV-2 also leads to excessive production of pro-inflammatory cytokines but the production of type I IFN is often blunted (33). Severe infection is associated with reduced type I IFN production and activity and excessive viral load in the blood (34). Neutralising antibodies against type I IFN in severe life-threatening cases have been described (35).

The concept of "molecular mimicry" has also been demonstrated with the proof of sequence homology between SARS-CoV-2 spike and nuclear pro-

teins and human peptides (36), (37). In addition, the production of a variety of autoantibodies during acute infection with SARS-CoV-2 has been established including anti-Ro/SSA antibodies, anti-neutrophil cytoplasmic antibodies (ANCA) and anti-cyclic peptide containing citrulline (CCP) antibodies (38, 39). The first report of pSS-specific antibodies anti-SSA/Ro in COVID-19 infected patients occurred in two individuals with acute respiratory distress syndrome and no history of autoimmune diseases. Tests for serum antibodies revealed high levels of anti-SSA/Ro (203-442 U/mL) (39) high levels of which in COVID-19 infection was suggested by authors to be a surrogate marker of pneumonia severity and poorer prognosis. Since levels of serum autoantibodies were not tested before the onset of symptoms in these patients, it is unclear whether breach of tolerance to SSA/Ro preceded acute respiratory distress syndrome following COVID-19 infection in these patients (39). Furthermore, there is prolonged T cell stimulation, T cell senescence and exhaustion as well as skewing towards a Th17 phenotype in patients with COVID-19 pneumonia, both associated with ARD, providing further support of the potential autoimmunity caused by COVID-19.

Probably above all the most intriguing fact is the capacity of SARS-CoV-2 to infect salivary glands (SGs) as the expression of ACE2, the receptor that the virus uses to enter epithelial cells, is more abundant in the minor SGs than the lung epithelium (40) generating therefore a potential reservoir for chronic infection as will be discussed later. pSS has been described as an autoimmune epithelitis, following a viral insult and tissue damage, the glandular epithelial cells can function as antigen presenting cells contributing to autoantigen presentation, breach of tolerance and eventually production of autoantibodies (41). Therefore, chronic or acute infection of SGs with SARS-CoV-2 can potentially provide the ideal environment for initiation of autoimmunity.

A recent report from Brazil indicating an increased incidence of pSS during the pandemic is in support of this hypothesis (42). It will therefore be of great interest the effect of COVID-19 and the incidence of autoimmune sialadenitis on the longer term. Only time will show whether indeed the pandemic will cause an increase in cases of pSS-like disease.

We will now review specifically the effect of SARS-CoV-2 infection on the SGs and the associated sicca symptoms.

Prevalence of COVID-19-induced xerostomia

In the early stages of the SARS-CoV-2 pandemic, symptoms related to the oral cavity quickly became common in infected patients, including COVID-19-induced anosmia (loss of smell), dysgeusia (alteration in the perception of taste) and xerostomia (dry mouth) (43). Existing literature on the latter is sparse, however, originating mainly from individual case reports or surveys, which have been summarised in Table II (44). An early study characterising symptoms in 60 COVID-19-infected patients from January 27th 2020 to February 11th 2020 in Shenzhen, China, observed dry mouth in 29% of the cohort (45). The first study to mention xerostomia in COVID-19 surveyed 108 patients in Wuhan, China, the site at which the virus first emerged, from 28th February 2020 – 4th March 2020. Of the 14 oral-related symptoms, 47.2% suffered dysgeusia/amblygeusia and 46.3% experienced dry mouth (46). As the virus spread worldwide, similar levels of oral manifestations became apparent in Middle Eastern cohorts, including in ambulatory, non-hospitalised COVID-19 patients between 25th March 2020 and 15th April 2020. In a cohort of 140 patients, 56% reported xerostomia, which correlated significantly with reports of burning mouth (p=0.002) and dysgeusia (p=0.009) (taste being the main stimulator of saliva production) (47). European studies showed a similar prevalence of xerostomia: 32% in a self-reporting survey using the Xerostomia Inventory score (48), 30% in a follow-up study of 122 hospitalised patients (43), and 45.9% in a third cohort with a median dryness score of 5 (range 3–8) (49). While these studies had small sample sizes which used self-assessments to report the severity of xerostomia experienced, a preponderance of dry mouth was observed in females compared to males (44, 46, 47).

Clinical presentation of COVID-19-induced xerostomia

While fever, cough and fatigue are among some of the most common symptoms of COVID-19 infection (50), a large proportion presented with asymptomatic or more mild symptoms, contributing to the rapid spread of disease. Chemosensory symptoms (gustatory and olfactory) and xerostomia are thought of as mild symptoms, appearing early on in infection and lasting over a long period of time (49,51). In an effort to recognise early COVID-19 infections which do not typically present with respiratory tract-specific symptoms, Luo *et al*., found a high correlation between dry mouth and bitter taste in a pairwise analysis of 60 infected patients (45). This can be explained by the fact that reduced salivary flow damages gustatory papillae, altering the perception of taste (52). 60% of patients in one cohort experienced dry mouth as a prodromal symptom, 3-4 days prior to other COVID-19 symptoms (fever, shortness of breath, fatigue etc) and assessed using visual analogue scales (VAS, 0 = absence of dry mouth, 10 = most severe case of dry mouth) (53). In a study by Fantozzi *et al*., prior to any COVID-19 diagnosis, 74.5% of the cohort associated xerostomia as one of the first symptoms of infection, with a median onset time of 7 days (range: 4–7.8). Other symptoms related to hyposalivation were difficulties in swallowing and needing to sip liquids to aid the swallowing process (27.5% and 37.3% respectively of the dry mouth cohort) (49), burning sensation (p=0.002) and change in taste (p=0.009) (47). In fact, the odds ratio of developing dry mouth with COVID-19 in one study was 5.13 (95% CI 2.9220–10.9009, p<0.001) (44).

Xerostomia impedes the flushing function of saliva, increasing the chances of retrograde infection in ductal orifices. It also has a role in the formation of sialolithiasis (SG stones), which have been reported in one COVID-19 case

Table II. Prevalence of xerostomia and other oral manifestations during SARS-CoV-2 infection.

Study	Cohort size (n=)	Mean age (yrs)	Xerostomia	Dysgeusia	Other oral manifestations
Gherlone *et al*. (43)	122	62.5 (median)	30%	17%	Masticatory muscle weakness (19%)
Luo *et al*. (45)	60	43.56±2.70 (males) 50.04±1.75 (females)	29.1%	Bitter taste 12.73%	Cough (68.33%)
Chen *et al*. (46)	108	52.0	46.3%	47.2%	Inflammation of mouth (11.1%), enlargement of lymph nodes in submandibular regions (0.9%), cough (67.7%)
Biadsee *et al*. (47)	128	36.25	56%	32.8%	Change in sensation of tongue (15.6%), plaque-like changes in tongue (7.03%), cough (94%), sore throat (34%), swelling in palate (3.13%), swelling of tongue (3.13%), swelling of gums (1.56%).
Freni *et al*. (48)	50	37.7 ± 17.9	32%	70%	Cough (60%)
Fantozzi *et al*. (49)	111	57 (median)	45.9%	59.5%	Difficulties in swallowing (39.2%), difficulties in swallowing dry foods (27.5%), need to sip liquids to help swallowing (37.3%)

due to the deposition of inorganic salts on ductal walls (54). Obstruction of the Stensen duct was suggested to be the cause of parotid gland enlargement by Lechien *et al*., who noted parotitis-like symptoms at the onset of disease in two COVID-19 patients and during the disease course in another. It is unclear whether the parotid gland enlargement in these cases blocks the duct to cause xerostomia, however hyposecretion and inflammation of the tissue were observed, albeit by subjective means (55). SG ectasia (swelling) occurred in a cohort of patients who experienced more severe COVID-19 infection and were significantly older (63.6 years) compared to those without (61.7 years). Upon hospitalisation they presented with higher serum levels of the systemic inflammation marker C-reactive protein (CRP) (89.2 mg/dL) and the necrosis marker lactate dehydrogenase (LDH) (424 U/L), together with lower absolute lymphocytic counts (0.8 $x10^9$/L). Univariable logistic regression analysis confirmed increasing CRP and LDH is associated with a greater risk of developing SG ectasia while the underlying disorder diabetes mellitus could independently predict dry mouth (43).

Upon the resolution of infection, dry mouth persisted in 30% of patients 3 months after hospital discharge while 83.6% had other abnormalities in the mouth, despite never experiencing oral disorders prior to COVID-19 (43). On the other hand, another cohort of hospitalised COVID-19 patients with no previous complaints of dry mouth showed that following antiviral drug therapy, hydroxychloroquine, antibiotics or oxygen supplementation, xerostomia was resolved in 80% of cases within 2–13 days, lasting three weeks in two patients albeit at lower severities (VAS = 1-2) (53). 15 days after a negative RT-PCR test, dry mouth decreased significantly in 15 patients in a separate cohort ($p<0.001$) and persisting only in 1 patient (48). It should be noted however that invasive clinical practices, particularly in intensive care units such as intubation, assisted external ventilation and tracheostomy, may also contribute to complaints of dry mouth and exacerbation of existing oral injuries (44, 56, 57).

Pathophysiological mechanisms of COVID-19-induced xerostomia

Dry mouth is a multifactorial phenomenon associated with autoimmune, endocrine, neurological and iatrogenic aetiologies (44). It has been suggested that the xerostomia experienced by COVID-19 infected patients could be due to the neuroinvasive and neurotropic properties of SARS-CoV-2. Evidence of coronavirus directly invading the central nervous system first appeared in a case report of a SARS patient in 2005 who presented with progressive neurological sequelae of dysphoria and deliria (58), which was followed by more cases (59-61). This is of relevance, since the parotid and submandibular SGs receive both sympathetic and parasympathetic innervation, providing a possible mechanism in the pathophysiology of COVID-19-induced xerostomia (48).

The barrier mucosa of the oral cavity may also provide alternative entry sites for COVID-19 infection due to the abundant expression of angiotensin converting enzyme II (ACE2). The cell surface protein is the likely receptor for SARS-CoV-2 spike (S) protein (62) to mediate cellular adsorption (62,63), therefore any cell which harbours it can be exploited to host and facilitate viral replication. Viral S glycoprotein is then cleaved by host protein transmembrane protease serine 2 (TMPRSS2), facilitating viral activation and pathogenesis (64). Liu *et al*., were the first to show ACE2 expression in SG epithelium in non-human primates and that SGs were early targets of SARS-CoV infection (65). More recently, other studies have confirmed an abundant expression of ACE2 in squamous epithelial cells of the dorsal tongue (66, 67), followed by gingiva, buccal tissue (66) as well as TMPRSS2 in taste bud cells, and submandibular SGs (67).

Publicly available datasets in GTEx-portal (https://gtexportal.org) have shown ACE2 protein is expressed at 1.8 pTP (protein coding transcripts per million) in the SGs, ranking 10th amongst all organs and at higher levels than in the lungs (54). Supporting findings from other studies (67), the most recent human single cell atlas on minor SGs and gingiva revealed that

they co-express ACE2 and TMPRSS2 in epithelial clusters, namely in the ducts, mucous acini and myoepithelial structures. SARS-CoV-2 was detected in 57% of SGs (as S-positive cells), with a trend towards higher loads in minor SGs compared to paired parotid SGs (68). Acinar cells were not ACE2-positive in a study by Usami *et al*., while it was expressed abundantly in interlobular excretory ducts (69). Furthermore, Song *et al*. reported high expression of TMPRSS2 in ductal and acinar cells of parotid SGs and in the ductal and myoepthelial cells of the submandibular glands. They also observed positive correlation between ACE2 and TMPRSS2 expression levels in most organs, including the SGs (R=0.35, p=0.01). Since the receptor of SARS-CoV-2 is expressed superficially on SGs, the virus could use them to enter host cells (70).

Using autopsied minor SGs, fluorescence in-situ hybridisation demonstrated functional evidence of SARS-CoV-2 replication in sense protein-positive cells (68), potentially acting as a reservoir for the virus. Gene ontology analyses on healthy SGs revealed an upregulation of ribosomal pathways with ACE2 and TMPRSS2 expression, which is required to mediate more functional processes such as viral RNA and protein synthesis (70). Histological features of SARS-CoV-2-infected minor SGs include focal lymphocytic sialadenitis (FLS), cellular atrophy, fibrosis and ductal rupture. Immune cell phenotyping in the same glands showed a high proportion of IgA+ plasma cells (as expected at mucosal sites) and tissue-resident cytotoxic T-cells (68) to provide a rapid response to foreign antigen (71). Enriched B-cell infiltration was observed in cases of FLS (68) and is reminiscent of the hallmark lesions observed in the SGs of pSS patients (72). These inflammatory features may be evidence of glandular repair, in response to SARS-CoV-2 antigen. Wang *et al*. suggest that hyposalivation could be a result of fibrous repair of damaged acinar cells, while hyperplastic fibrous scars could lead to stenosis (or narrowing) of SG ducts (54). In the same vein, viruses are believed to be a co-factor of SG inflammation in pSS patients, due to antigen-driven immune cell recruitment, which leads to glandular damage, dysfunction, and symptoms of dry mouth (73). Other diseases in which viruses have been implicated in autoimmunity and autoinflammation are influenza viruses for acute disseminated encephalomyelitis and herpesvirus for systemic lupus erythematosus, rheumatoid arthritis and adult-onset Still's disease (74).

As well as the SGs, saliva may act as a potential reservoir of SARS-CoV-2 (75, 76), hence understanding its potential role in transmission of the disease would be vital in limiting the outbreak. Epidemiological studies confirmed transmission of COVID-19 can occur not only in pre-symptomatic patients, but those that are asymptomatic too (68,77) and whilst development of COVID-19 could occur through both direct transmission (cough, sneeze and droplet inhalation) and contact transmission (contact with oral, nasal and eye mucous membranes) (78), there is mounting evidence to suggest that it may pass from person-to-person through saliva. SARS-CoV-2 can be detected in saliva at a median of 2 days post hospitalisation (79), and before the appearance of lung lesions (76), which could be a valuable non-invasive specimen for early diagnosis of COVID-19. Full length salivary TMPRSS2 protein was observed in another study (67), while anti-S-IgG and anti-RBD-IgG antibodies remained stable in saliva throughout a three-month period in COVID-19 patients, which correlated with serum levels of these antibodies too (80). Salivary composition analysis revealed the presence of protease inhibitors such as plasminogen activator inhibitory type 1 (67), which may inhibit TMPRSS2 activity (46, 81) and hence viral entry. Chen *et al*., suggest the presence of such proteins could be an indicator of COVID-19 infection (46) while others suggest it could predict disease severity since the viral detection rate was high (75%) amongst critically ill patients (79). High salivary viral loads in most severe cases could be explained by a weakened immune response unable to clear the pathogen (79). Considering this, Bourgonje *et al*. demonstrated that ACE2 expression in organs is strongly associated with clinical manifestations of COVID-19 (82). It is important to remember, however, that viral shedding in saliva could arise not only from SGs, but also from the nasopharynx, hence the source of salivary SARS-CoV-2 should be better characterised (79). Given each cough generates 3000 saliva droplets and each sneeze equivalent to 40,000 saliva droplets, saliva presents a stable and far-reaching mechanism of viral shedding (83).

These studies indicate the susceptibility that the ACE2-expressing oral cavity has to SARS-CoV-2 infection, providing a mechanism for viral entry in early disease stages when patients present with dry mouth. More specifically, SG epithelia have been shown to mediate SARS-CoV-2 entry, replication, immune cell activation and viral transmission, all of which may be involved in the pathogenesis of COVID-19 induced xerostomia. However, the clinical stages of COVID-19 infection are still unclear, and whether this is an infection affecting the oral cavity first, spreading to the nasal cavity or vice versa remains to be seen. Larger studies focused on xerostomia are required to better understand this, using objective methods of investigation to supplement questionnaires assessing dry mouth. Deeper understanding of COVID-19-induced xerostomia would allow us to understand whether it is direct cause of infection or whether it is a manifestation of symptoms in a patient with a compromised immune response. This information could play an active role in the diagnosis of SARS-CoV-2 infection or predict the disease prognosis.

References

1. FOX RI: Sjögren's syndrome. *Lancet* 2005 366: 321-31.
2. NOCTURNE G, MARIETTE X: Sjögren syndrome-associated lymphomas: An update on pathogenesis and management. *Br J Haematol* 2015; 168: 317-27.
3. MANFRÈ V, CAFARO G, RICCUCCI I *et al*.: One year in review 2020: comorbidities, diagnosis and treatment of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2021; 38 (Suppl. 126): S10-22.
4. ZHOU F, YU T, DU R *et al*.: Clinical course

and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; 395: 1054-62.
5. CUMMINGS MJ, BALDWIN MR, ABRAMS D *et al*.: Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet* 2020; 395: 1763-70.
6. LIANG W, LIANG H, OU L *et al*.: Development and validation of a clinical risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19. *JAMA Intern Med* 2020;180: 1081-9.
7. WILLIAMSON EJ, WALKER AJ, BHASKARAN K *et al*.: Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020; 584: 430-6.
8. FAVALLI EG, MONTI S, INGEGNOLI F, BALDUZZI S, CAPORALI R, MONTECUCCO C: Incidence of COVID-19 in patients with rheumatic diseases treated with targeted immunosuppressive drugs: what can we learn from observational data? *Arthritis Rheumatol* 2020; 72: 1600-6.
9. YE C, CAI S, SHEN G *et al*.: Clinical features of rheumatic patients infected with COVID-19 in Wuhan, China. *Ann Rheum Dis* 2020; 79: 1007-13.
10. D'SILVA KM, SERLING-BOYD N, WALLWORK R *et al*.: Clinical characteristics and outcomes of patients with coronavirus disease 2019 (COVID-19) and rheumatic disease: A comparative cohort study from a US hot spot. *Ann Rheum Dis* 2020; 79: 1156-62.
11. FREDI M, CAVAZZANA I, MOSCHETTI L *et al*.: COVID-19 in patients with rheumatic diseases in northern Italy: a single-centre observational and case-control study. *Lancet Rheumatol* 2020; 2: 549-56.
12. ZHAO J, PANG R, WU J *et al*.: Clinical characteristics and outcomes of patients with COVID-19 and rheumatic disease in China hot spot' versus in US hot spot similarities and differences. *Ann Rheum Dis* 2021; 80: e63.
13. GIANFRANCESCO M, HYRICH K, AL-ADELY *et al*.: Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry On behalf of the COViD-19 Global Rheumatology alliance. *Ann Rheum Dis* 2020; 79: 859-66.
14. PABLOS JL, GALINDO M, CARMONA L *et al*.: Clinical outcomes of hospitalised patients with COVID-19 and chronic inflammatory and autoimmune rheumatic diseases: a multicentric matched cohort study. *Ann Rheum Dis* 2020; 79: 1544-9.
15. NUÑEZ F: Risk factors for hospital admissions related to COVID-19 in patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis* 2020; 79: 1393-9.
16. BAKASIS AD, MAVRAGANI CP, BOKI KA *et al*.: COVID-19 infection among autoimmune rheumatic disease patients: Data from an observational study and literature review. *J Autoimmun* 2021; 123: 102687.
17. BRENNER EJ, UNGARO RC, GEARRY RB *et al*.: Corticosteroids, but not TNF antagonists, are associated with adverse COVID-19 outcomes in patients with inflammatory bowel diseases: results from an International Registry. *Gastroenterology* 2020; 159: 481-91.
18. DURET PM, SPIELMANN L, MESSER L: Response to: 'Correspondence on recovery from COVID-19 in a patient with spondyloarthritis treated with TNF-alpha inhibitor etanercept. A report on a COVID-19 patient with psoriatic arthritis receiving ustekinumab' by Messina *et al*. *Ann Rheum Dis* 2021; 80: e80.
19. SANTOS CS, MORALES CM, ÁLVAREZ ED, CASTRO CÁ, ROBLES AL, SANDOVAL TP: Determinants of COVID-19 disease severity in patients with underlying rheumatic disease. *Clin Rheumatol* 2020; 39: 2789-96.
20. HUANG Y, CHEN Z, WANG Y *et al*.: Clinical characteristics of 17 patients with COVID-19 and systemic autoimmune diseases: a retrospective study. *Ann Rheum Dis* 2020; 79: 1163-9.
21. BRITO-ZERÓN P, MELCHOR S, LE SEROR R *et al*.: SARS-CoV-2 infection in patients with primary Sjögren's syndrome: characterization and outcomes of 51 patients. *Rheumatology* 2021; 60: 2946-57.
22. GIARDINA F, IZZO R, GATTAMELATA A, COLAFRANCESCO S, CONTI F, PRIORI R: COVID-19 in Italian Sjögren's syndrome patients: a monocentric study. *Rheumatol Int* 2021; 41: 235-6.
23. CARUBBI F, ALUNNO A, FERRI C *et al*.: The impact of SARS-CoV-2 outbreak on primary Sjögren's syndrome: an Italian experience. *Front Med* 2020; 7: 608728.
24. D'ANTIGA L: Coronaviruses and immunosuppressed patients: the facts during the third epidemic. *Liver Transpl* 2020; 26: 832-4.
25. HUI DS, AZHAR EI, KIM Y-J, MEMISH ZA, OH M, ZUMLA A: Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. *Lancet Infect Dis* 2018; 18: e217-27.
26. MEMOLI MJ, ATHOTA R, REED S *et al*.: The natural history of influenza infection in the severely immunocompromised vs non-immunocompromised hosts. *Clin Infect Dis* 2014; 58: 214-24.
27. YAO X, YE F, ZHANG M *et al*.: *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2020; 71: 732-9.
28. HORBY PW, MAFHAM M, LINSELL L *et al*.: Effect of hydroxychloroquine in hospitalized patients with COVID-19: Preliminary results from a multi-centre, randomized, controlled trial. *medRxiv* 2020.
29. STRANGFELD A, SCHÄFER M, GIANFRANCESCO MA *et al*.: Factors associated with COVID-19-related death in people with rheumatic diseases: Results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis* 2021; 80: 930-42.
30. ROBBIANI DF, GAEBLER C, MUECKSCH F *et al*.: Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 2020; 584: 437-42.
31. LOARCE-MARTOS J, GARCÍA-FERNÁNDEZ A, LÓPEZ-GUTIÉRREZ F *et al*.: High rates of severe disease and death due to SARS-CoV-2 infection in rheumatic disease patients treated with rituximab: a descriptive study. *Rheumatol Int* 2020; 40: 2015-21.
32. LUCCHESI D, PITZALIS C, BOMBARDIERI M: EBV and other viruses as triggers of tertiary lymphoid structures in primary Sjögren's syndrome. *Expert Rev Clin Immunol* 2014; 10: 445-55.
33. TZIOUFAS AG, KAPSOGEORGOU EK, MOUTSOPOULOS HM: Pathogenesis of Sjögren's syndrome: What we know and what we should learn. *J Autoimmun* 2012; 39: 4-8.
34. HADJADJ J, YATIM N, BARNABEI L *et al*.: Impaired type I interferon activity and exacerbated inflammatory responses in severe Covid-19 patients. *medRxiv* 2020 Apr 23.
35. BASTARD P, ROSEN LB, ZHANG Q *et al*.: Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020; 370: eabd4585.
36. VOJDANI A, KHARRAZIAN D: Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases. *Clin Immunol* 2020; 217: 108480.
37. KANDUC D, SHOENFELD Y: Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine. *Immunol Res* 2020; 68: 310-3.
38. GAO Z, ZHANG H, LIU C, DONG K: Autoantibodies in COVID-19: frequency and function. *Autoimmun Rev* 2021; 20: 102754.
39. FUJII H, TSUJI T, YUBA T *et al*.: High levels of anti-SSA/Ro antibodies in COVID-19 patients with severe respiratory failure: a case-based review: High levels of anti-SSA/Ro antibodies in COVID-19. *Clin Rheumatol* 2020; 39: 3171-5.
40. XU J, LI Y, GAN F, DU Y, YAO Y: Salivary glands: potential reservoirs for COVID-19 asymptomatic infection. *J Dent Res* 2020; 99: 989.
41. NOCTURNE G, MARIETTE X: Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2013; 9: 544-56.
42. MARTELLI JÚNIOR H, GUEIROS LA, DE LUCENA EG, COLETTA RD: Increase in the number of Sjögren's syndrome cases in Brazil in the COVID-19 Era. *Oral Dis* 2021 May 27 [Online ahead of print].
43. GHERLONE EF, POLIZZI E, TETÈ G *et al*.: Frequent and persistent salivary gland ectasia and oral disease after COVID-19. *J Dent Res* 2021; 100: 464-71.
44. KATZ J: Prevalence of dry mouth in COVID-19 patients with and without Sicca syndrome in a large hospital center. *Ir J Med Sci* 2021; 190: 1639-41.
45. LUO Y, WU J, LU J *et al*.: Investigation of COVID-19-related symptoms based on factor analysis. *Ann Palliat Med* 2020; 9: 1851-8.
46. CHEN L, ZHAO J, PENG J *et al*.: Detection of 2019-nCoV in saliva and characterization of oral symptoms in COVID-19 patients. *Cell Prolif* 2020; 53: e12923.
47. BIADSEE A, BIADSEE A, KASSEM F, DAGAN O, MASARWA S, ORMIANER Z: Olfactory and oral manifestations of COVID-19: sex-related symptoms – a potential pathway to early diagnosis. *Otolaryngol Head Neck Surg* 2020; 163: 722-8.

48. FRENI F, MEDURI A, GAZIA F *et al*.: Symptomatology in head and neck district in coronavirus disease (COVID-19): A possible neuroinvasive action of SARS-CoV-2. *Am J Otolaryngol* 2020; 41: 102612.
49. FANTOZZI PJ, PAMPENA E, DI VANNA D *et al*.: Xerostomia, gustatory and olfactory dysfunctions in patients with COVID-19. *Am J Otolaryngol* 2020; 41: 102721.
50. HUANG C, WANG Y, LI X *et al*.: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497-506.
51. GIACOMELLI A, PEZZATI L, CONTI F *et al*.: Self-reported olfactory and taste disorders in patients with severe acute respiratory coronavirus 2 infection: A cross-sectional study. *Clin Infect Dis* 2020; 71: 889-90.
52. YAGI T, ASAKAWA A, UEDA H, IKEDA S, MIYAWAKI S, INUI A: The role of zinc in the treatment of taste disorders. *Recent Pat Food Nutr Agric* 2013; 5: 44-51.
53. FATHI Y, HOSEINI EG, ATOOF F, MOTTAGHI R: Xerostomia (dry mouth) in patients with COVID-19: a case series. *Future Virol* 2021; 10.2217.
54. WANG C, WU H, DING X *et al*.: Does infection of 2019 novel coronavirus cause acute and/or chronic sialadenitis? *Med Hypotheses* 2020;140: 109789.
55. LECHIEN JR, CHETRIT A, CHEKKOURY-IDRISSI Y *et al*.: Parotitis-Like symptoms associated with COVID-19, France, March-April 2020. *Emerg Infect Dis* 2020; 26: 2270-1.
56. ZANGRILLO A, BERETTA L, SCANDROGLIO AM *et al*.: Characteristics, treatment, outcomes and cause of death of invasively ventilated patients with COVID-19 ARDS in Milan, Italy. *Crit Care Resusc* 2020; 22: 200-11.
57. WU C, CHEN X, CAI Y *et al*.: Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Int Med* 2020; 180: 934-43.
58. XU J, ZHONG S, LIU J *et al*.: Detection of severe acute respiratory syndrome coronavirus in the brain: Potential role of the chemokine mig in pathogenesis. *Clin Infect Dis* 2005; 41; 1089-96.
59. CHENG SKW, TSANG JSK, KU KH, WONG CW, NG YK: Psychiatric complications in patients with severe acute respiratory syndrome (SARS) during the acute treatment phase: A series of 10 cases. *Br J Psychiatry* 2004; 184: 359-60.
60. DING Y, HE L, ZHANG Q *et al*.: Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: Implications for pathogenesis virus transmission pathways. *J Pathol* 2004; 203: 622-30.
61. GU J, GONG E, ZHANG B *et al*.: Multiple organ infection and the pathogenesis of SARS. *J Exp Med* 2005; 202: 415-24.
62. ZHOU P, YANG X, WANG XG *et al*.: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270-3.
63. KUPFERSCHMIDT K, COHEN J: Race to find COVID-19 treatments accelerates. *Science* 2020; 367: 1412-3.
64. HOFFMANN M, KLEINE-WEBER H, SCHROEDER S *et al*.: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; 181: 271-80.
65. LIU L, WEI Q, ALVAREZ X *et al*.: Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. *J Virol* 2011; 85: 4025-30.
66. XU H, ZHONG L, DENG J *et al*.: High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci* 2020; 12: 8.
67. SAKAGUCHI W, KUBOTA N, SHIMIZU T *et al*.: Existence of SARS-CoV-2 entry molecules in the oral cavity. *Int J Mol Sci* 2020; 21: 6000.
68. HUANG N, PÉREZ P, KATO T *et al*.: SARS-CoV-2 infection of the oral cavity and saliva. *Nat Med* 2021; 27: 892-903.
69. USAMI Y, HIROSE K, OKUMURA M, TOYOSAWA S, SAKAI T: Brief communication: Immunohistochemical detection of ACE2 in human salivary gland. *Oral Sci Int* 2021; 18 (2).
70. SONG J, LI Y, HUANG X *et al*.: Systematic analysis of ACE2 and TMPRSS2 expression in salivary glands reveals underlying transmission mechanism caused by SARS-CoV-2. *J Med Virol* 2020; 92: 2556-66.
71. KARLSSON AC, HUMBERT M, BUGGERT M: The known unknowns of T cell immunity to COVID-19. *Sci Immunol* 2020; 5: eabe8063.
72. BARONE F, CAMPOS J, BOWMAN S, FISHER BA: The value of histopathological examination of salivary gland biopsies in diagnosis, prognosis and treatment of Sjögren's syndrome. *Swiss Med Wkly* 2015; 145: w14168.
73. FOX RI, FOX CM: Sjögren's syndrome: infections that may play a role in pathogenesis, mimic the disease, or complicate the patient's course. *Indian J Rheumatol* 2011; 6: 13-25.
74. RODRÍGUEZ Y, NOVELLI L, ROJAS M *et al*.: Autoinflammatory and autoimmune conditions at the crossroad of COVID-19. *J Autoimmun* 2020; 114: 102506.
75. XU J, LI Y, GAN F, DU Y, YAO Y: Salivary Glands: Potential Reservoirs for COVID-19 Asymptomatic Infection. *J Dental Res* 2020; 99: 989.
76. XU R, CUI B, DUAN X, ZHANG P, ZHOU X, YUAN Q: Saliva: potential diagnostic value and transmission of 2019-nCoV. *Int J Oral Sci* 2020; 12: 11.
77. ROTHE C, SCHUNK M, SOTHMANN P *et al*.: Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. *N Engl J Med* 2020; 382: 970-1.
78. PENG X, XU X, LI Y, CHENG L, ZHOU X, REN B: Transmission routes of 2019-nCoV and controls in dental practice. *Int J Oral Sci* 2020; 12: 9.
79. TO KK, TSANG OT, YIP CC *et al*.: Consistent detection of 2019 novel Coronavirus in saliva. *Clin Infect Dis* 2020; 71: 841-3.
80. ISHO B, ABE KT, ZUO M *et al*.: Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol 2020; 5: eabe5511.
81. SHEN LW, MAO HJ, WU YL, TANAKA Y, ZHANG W: TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie* 2017; 142: 1-10.
82. BOURGONJE AR, ABDULLE AE, TIMENS W *et al*.: Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol* 2020; 251: 228-48.
83. BAGHIZADEH FINI M: Oral saliva and COVID-19. *Oral Oncol* 2020; 108: 104821.