Clinical Immunology 279 (2025) 110545

Contents lists available at ScienceDirect

Clinical Immunology

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

# High activity of human cytomegalovirus in patients with Sjögren's disease

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ARTICLE INFO

*Keywords:*
HCMV
Sjögren's disease
Immunohistochemistry
Serology
Autoimmunity

ABSTRACT

Sjögren's disease (SD) is a chronic autoimmune disease characterized by immune-mediated damage to salivary and lacrimal glands. This study aimed to explore the potential link between SD and human cytomegalovirus (HCMV), by analyzing presence of HCMV proteins in salivary gland tissue specimens and prevalence of HCMV-specific antibodies in serum samples from SD patients and controls. HCMV-immediate early (IE) and late proteins (LA and pp65) were highly abundant in tissue specimens from SD patients (88.9 %, 69.2 %, 45.8 %, respectively), and less abundant in patients with Sicca symptoms without SD (70.5 %, 20.0 %, 12.5 %, respectively). Samples in the SD group were also positive at higher scores for the HCMV proteins than Sicca symptom patients without SD. IgM prevalence was higher in SD patients than in healthy controls (32.1 % vs. 13.4 %, $P = 0.04$) and HCMV-IgG titers were higher ($P < 0.0001$). Understanding the potential role of HCMV in SD pathogenesis may contribute to advancements in disease prevention and treatment.

## 1. Introduction

Sjögren's disease (SD) is a chronic autoimmune condition marked by lymphocyte infiltration and damage to the salivary and lacrimal glands. When it occurs alone, it is termed "primary" (pSD), and when it is associated with other autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or systemic sclerosis (SSc), it is termed "secondary" [1]. Symptoms include dryness of the mouth, eyes, airways, digestive tract, and vagina, along with fatigue, musculoskeletal pain, and skin dryness [1]. Diagnosis is based on autoantibody profiles (e.g., anti-Ro/SSA, anti-La/SSB), measurements of ocular and oral dryness, and salivary gland biopsy findings [1].

Environmental factors that have been studied in the context of SD pathogenesis include various viruses, bacteria, vaccination, serum vitamin D levels, stress, smoking, alcohol, solvents, silica and silicones. The predominance of women with pSD suggests a role for female sex hormones in the disease [2]. Viral infections proposed to trigger SD include Epstein-Barr virus (EBV), Human T-lymphotropic virus type 1 (HTLV-1), hepatitis C virus (HCV), and coxsackie virus, all of which have been associated with SD in different studies, although causal relationships remain to be determined [2].

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus known for lifelong latency and immune evasion. Initially called "the salivary gland virus", HCMV targets salivary glands and can infect various cell types. After primary infection, HCMV establishes latency in cells of the myeloid lineage [3], and is reactivated upon differentiation of the myeloid progenitor cells to macrophages and dendritic cells [4]. HCMV seroprevalence in adults in Sweden is about 70–82 % [5]. While largely unnoticed in immunocompetent individuals, HCMV can cause severe disease in immunocompromised patients [6].

In order for HCMV to co-exist with its host, its evolution has led to the development of sophisticated immune evasion mechanisms [7]. At

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https://doi.org/10.1016/j.clim.2025.110545
Received 7 February 2025; Received in revised form 15 June 2025; Accepted 16 June 2025
Available online 18 June 2025

the same time, this virus can promote inflammatory processes, which are beneficial for the virus; if this occurs in patients this may maintain chronic inflammation and propagate autoimmune reactions [8]. HCMV infection is known to trigger the production of autoantibodies, mainly against endothelial cells and smooth muscle cells, although anti-nuclear, anti-phospholipid, and anti-CD13 autoantibodies are also common following infection [9–13].

HCMV-induced autoimmunity often involves molecular mimicry and bystander activation [14]. HCMV-induced molecular mimicry has been implicated in the development of SSc [15,16], RA, and SLE [17,18]. Bystander activation is non-antigen-specific and is caused by stimulation of autoreactive T-cells by presentation of self-antigens by APCs, leading to enhanced inflammation. HCMV has been suggested to contribute to bystander activation in RA [19–21], SLE and SSc [22] and SSc [23,24].

Although HCMV has strong tropism for salivary glands, and HCMV infection was shown to induce SD-like disease in a mouse model [25], only few studies have been dedicated to examine the role of HCMV in the pathogenesis of SD [2], and among them HCMV IgM and IgG prevalence studies have shown contrasting results [26,27].

As HCMV is a likely candidate for an environmental factor that could contribute to triggering autoimmunity and maintaining chronic inflammation in SD, this study aimed to examine the seroprevalence of HCMV in Swedish SD patients, and to investigate whether HCMV antigens are present in salivary gland biopsies from SD patients.

## 2. Material and methods

### 2.1. Patient samples

This retrospective study examined collected paraffin-embedded salivary gland tissue sections from 28 patients and blood serum samples from 28 patients diagnosed with Sjögren's disease (SD) at our hospital over a specified period. All participants received treatment at the Rheumatology Department at Karolinska University Hospital in Stockholm, Sweden (Table 1). Anti-SSA and -SSB autoantibodies were detected in 24 out of 28 (85.7 %) and 18 out of 28 (64.3 %) SD patients, respectively (Table 1, Supplementary Table 1).

Among these samples 22 were paired with available samples from both tissue specimens and serum and included seven serum samples with a ten-year follow-up (Supplementary Fig. 1). These samples were separately analyzed. Clinical and laboratory data, along with patient characteristics are summarized in Table 1. Immunohistochemistry staining (IHC) was performed on 28 salivary gland biopsies to detect human cytomegalovirus (HCMV) proteins; immediate early (IE), late (LA) and pp65, and 22 paired serum samples were examined for presence of HCMV IgG and IgM antibodies with commercial kits.

Salivary gland tissue sections ($n = 17$) and serum samples ($n = 6$) from patients with sicca symptoms but not fulfilling a diagnosis of SD were used as controls. Additionally, serum samples from 50 healthy blood donors collected during year 2020 (50 % female, median age 46.1 years, interval 18–83 years old) and 17 age-matched healthy females, obtained from biobanks at Karolinska University Hospital Huddinge and recruited at Karolinska University Hospital Solna, respectively, were included.

**Table 1**
Demographic and clinical characteristics.

| | SD patients | Sicca controls | Healthy controls |
|---|---|---|---|
| | n = 28 | n = 17 | n = 17 |
| Age at biopsy/study entry, years (mean ± SD) | 51.7 ± 14.57 | 49.8 ± 15.0 | 49.5.3 ± 8.2 |
| SSA positive, n (%) | 24 (85.7) | 0 | n.a. |
| Ro52, n (%)* | 14 (73.7) | 0 | |
| Ro60, n (%)* | 13 (68.4) | 0 | |
| SSB positive, n (%) | 18 (64.3) | 0 | n.a. |
| Focus Score (mean ± SD) | 6.01 ± 3.36 | 0 | n.a. |
| Biopsy with Focus Score ≥ 1, n (%) | 27 (96.4) | 0 | |
| Chisholm Mason score 4, n (%) | 21 (75) | n.a. | n.a. |
| Unstimulated salivation rate, ml/15 min, median (95 % CI) | 0.65 (0.30–1.70) | 1.00 (0.00–1.00) | |
| Unstimulated salivation positive, n (%) | 19 (67.9) | 13 (76.5) | |
| Stimulated salivation rate, ml/5 min, median (95 % CI) | 3.00 (0.00–13.0) | 4.00 (1.50–7.00) | |
| Schirmer test left, mm/5 min, median (95 % CI) | 3.50 (0.00–8.00) | 24.0 (15.0–30.0) | |
| Schirmer test right, mm/5 min, median (95 % CI) | 3.00 (0.00–5.00) | 15 (15.0–19.0) | |
| Schirmer test positive, n (%) | 21 (75) | 2 (11.8) | |

SD, Sjögren's disease; n.a., not applicable.
* $n = 19$ (Data on Ro52 and Ro60 autoantibodies available for 19 patients. Subtype not specified for the remaining SSA positive cases).

### 2.2. Immunohistochemical staining

Salivary gland tissue biopsies were cut into 5 μm sections, mounted on SuperFrost Plus slides (ThermoFisher, Cat. No. 12312148), and stained using the immunohistochemical technique as described [28]. Primary antibodies used included HCMV-IE (Merck KGaA, 1:400), pp65 (Leica Biosystem, 1:100), and HCMV-LA (Merck Millipore, 1:300). An isotype control (BIOCARE, Cat. No. NC498) served as a negative control. Positive signals were detected with ImmPRESS™ Anti-Mouse IgG Peroxidase (Cat. No. MP-7402) and DAB Chromogen Kit (Cat. No. DB801). The slides were counterstained with ready-to-use Hematoxylin (Histolab). The sections were scanned using a Hamamatsu NanoZoomer-XR Digital slide scanner (Hamamatsu Photonics K.K.) and evaluated independently by AR, MP, and XX. Slides were scored based on the percentage of HCMV-positive cells: 0: Negative, 1 (+): ≤25 %, 2: >25–50 %, 3: >50–75 %, and 4: >75 % [38,39].

### 2.3. Quantitative HCMV serology

HCMV-IgG and -IgM in blood serum were detected by ELISA using Human Anti-Cytomegalovirus IgG and IgM Kits (Abcam, Cat. Nos. ab108724 and ab108725) following the manufacturer's protocol. The cutoff for positive results was an optical density (OD) of 0.9.

### 2.4. Statistical analysis

Non-parametric one-way ANOVA followed by Dunn's test was used for multiple comparisons of antibody levels. Fisher's exact test evaluated HCMV-IgM prevalence between patients and controls. The Mann-Whitney $U$ test compared ordinal data between two groups. Correlation analysis was also conducted. All tests were two-sided, with $P \leq 0.05$ indicating significance. Statistical analysis was performed using GraphPad Prism (version 10, GraphPad Software, Inc.; Dotmatics).

## 3. Results

### 3.1. Frequent detection of high abundance of HCMV protein expression in the salivary gland of patients with SD

Paraffin embedded salivary gland tissue sections obtained from 28 patients with SD and 17 patients with sicca symptoms were examined by IHC for detection of different HCMV proteins (Fig. 1). Some tissues were lost during the staining procedure. Detailed results are presented in Supplementary Tables 1 and 2. Overall, HCMV proteins were abundantly detected in the tissue specimens, mainly in the epithelial cells, inflammatory cells and blood vessels within the tissues.

In patients with SD, the presence of HCMV-IE was observed in 88.9 % (24 out of 27) of the available tissues. Scores 1 and 2 were each detected in 3.7 % ($n = 1$), score 3 in 18.5 % ($n = 5$), and score 4 in 62.9 % ($n = 17$)

in the examined tissue specimens. HCMV-LA was positive in 69.2 % (18 out of 26) available samples, and detected at score 1 in 46.2 % ($n = 12$), score 2 and 3 each in 11.5 % ($n = 3$), and 0 % at score 4. HCMV-pp65 was found in 45.8 % (11 out of 24) of available samples, and score 1 was found in 37.5 % ($n = 9$) of cases, score 2 in 0 %, and scores 3 and 4 each in 4.2 % ($n = 1$) of the examined tissue specimens (Supplementary Table 1).

In patients with sicca symptoms, the immunohistochemical analysis revealed HCMV IE expression in 70.5 % (12 out of 17) of the tissue specimens at varying degrees of positivity; scores 1, 2, 3, and 4 were assigned in 17.6 % (n = 3), 5.9 % (n = 1), 17.6 % (n = 3), and 29.4 % ($n = 5$) of tissue sections. HCMV-LA was detected in 20.0 % (3 out of 15) of available samples. Of these scores 1, 2, and 4 were assigned in 6.7 % (n = 1) each, and 0 % for score 3 of the tissue specimens, respectively. HCMV-pp65 was only detected in 12.5 % (2 out of 16) of the available samples, all at score 1, while no samples had scores 2, 3, or 4 (Supplementary Table 2).

Significantly more samples in the SD group were positive at a higher score (score 2–4) for HCMV-IE ($P = 0.016$), HCMV-LA (P = 0.01) and HCMV-pp65 proteins ($P = 0.032$) as compared with the sicca symptoms group (Fig. 2).

### 3.2. High prevalence of HCMV-IgM and high titers of HCMV-IgG are found in patients with SD

The prevalence of HCMV-IgG and -IgM seropositivity among patients with histologically confirmed SD ($n = 28$) was 75 % ($n = 21$) and 32.1 % ($n = 9$), respectively (Fig. 3, supplementary Table 1). One SD patient was HCMV-IgM positive and negative for HCMV-IgG, indicating a primary HCMV infection. HCMV-IgG prevalence in patients with sicca symptoms with negative SD histology ($n = 6$) was 83.3 % ($n = 5$) and only one patient was HCMV-IgM positive (Supplementary Table 2). Among the 67 healthy controls in total analyzed for HCMV serology, 62.7 % ($n = 42$) tested positive for HCMV IgG, and 13.4 % (n = 9) were IgM positive. However, the prevalence of HCMV-IgM was higher in patients with SD compared to healthy controls ($P = 0.04$). No difference was found between patients with SD and sicca symptoms ($P = 0.64$). HCMV-IgG titers were significantly higher in patients with SD, compared to healthy controls ($P < 0.0001$), but not patients with sicca ($P = 0.60$, Fig. 3A).

The prevalence of HCMV-IgG and IgM was 64.7 % ($n = 11$) and 41.2 % ($n = 7$), respectively, in the 17 age-matched female healthy controls. The HCMV-IgG levels were significantly higher in SD patients compared to the age-matched healthy controls ($P = 0.009$, Fig. 3C). Among these healthy controls, five out of 17 had primary HCMV infection (IgM positive and IgG negative), which is unusual.

HCMV-serological analyses showed significantly higher HCMV-IgG levels and a higher prevalence of IgM in SD patients compared to 50 healthy blood donors (50 % female). ($P < 0.0001$ and $P = 0.0012$, respectively, Supplementary Fig. 2).

### 3.3. A few patients with SD had HCMV-IgM positivity at 10-year follow-up

Paired serum samples, collected at the time of diagnosis and at 10-year follow-up, were available from seven SD patients. At diagnosis, three samples were positive for both HCMV-IgM and -IgG and, notably, two of them remained HCMV-IgM positive at the 10-year follow-up.

### 3.4. Detection of HCMV-IgG and -IgM antibodies in SD patients with high levels of HCMV-IE in their salivary gland tissues

Paired salivary gland tissue sections and blood serum samples were

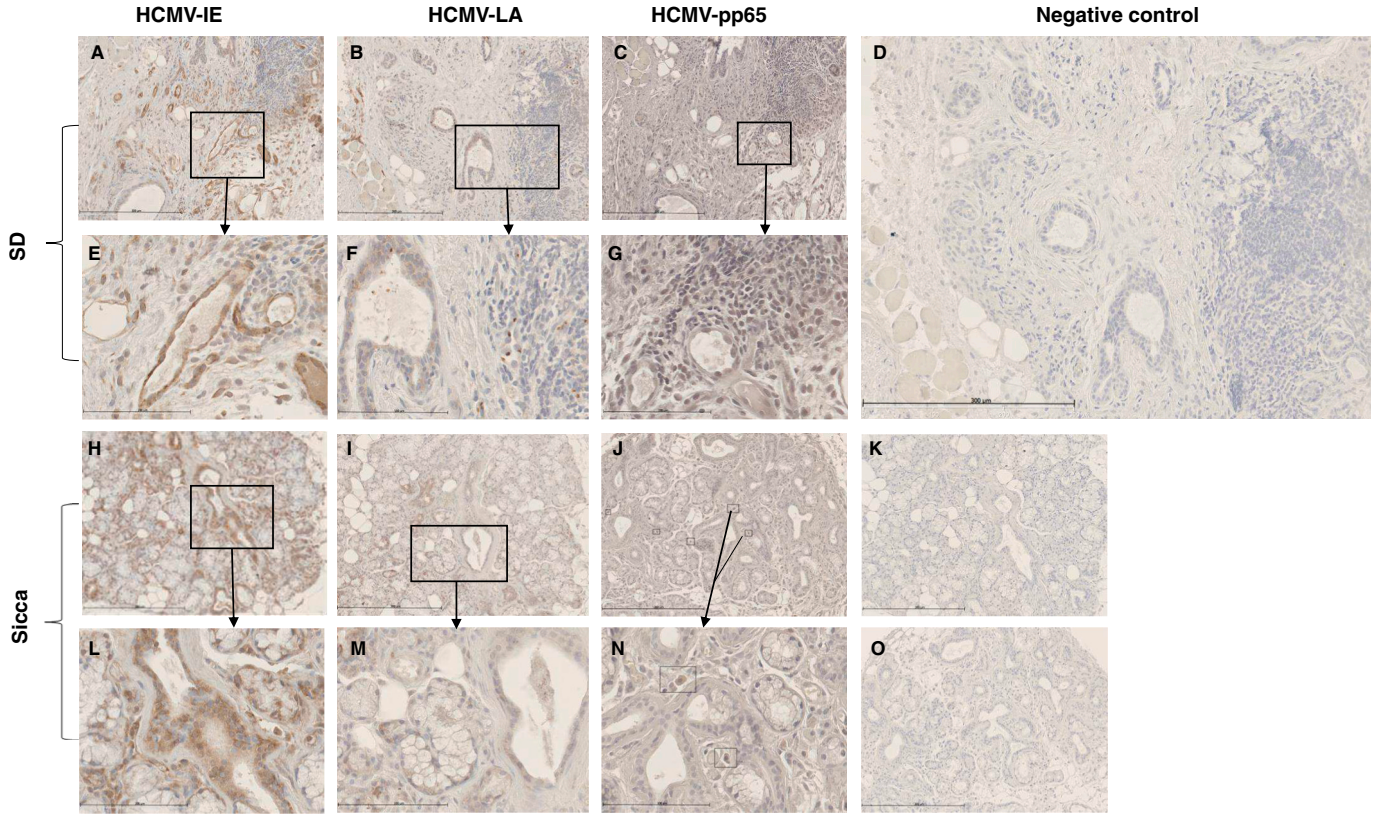


**Fig. 1.** Detection of HCMV-IE, LA and PP65 proteins in salivary tissue glands from patients with Sjögren's disease (SD) and sicca symptoms. Expression of viral proteins in the vessel wall and inflammatory cells within the salivary glands tissue obtained from SD patients (A; HCMV-IE, B; HCMV-LA and C; HCMV-pp65), (D) Negative control. Detection of HCMV-IE and LA proteins (H; HCMV-IE, I; HCMV-LA) in the epithelia and inflammatory cells, HCMV-pp65 (J) in the inflammatory cells within the salivary glands from sicca symptom patients, (K and O) corresponding negative controls.

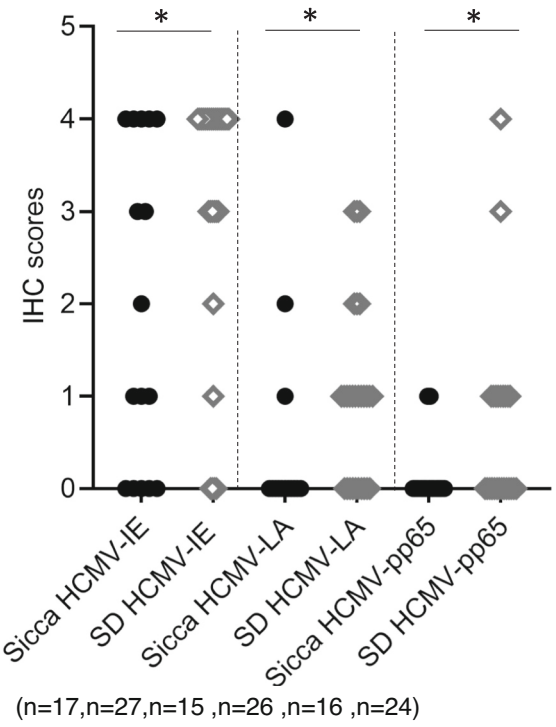


**Fig. 2.** Expression of HCMV proteins is more prevalent in salivary gland tissues from Sjögren's disease (SD) patients compared with patients with sicca symptoms. Higher scores of HCMV proteins were found in tissue sections from SD patients compared to sicca symptom patients. Significantly more samples in the SD group were positive with higher score (scores 2–4) for HCMV-IE ($P = 0.016$), HCMV-LA (P = 0.01) and HCMV-pp65 ($P = 0.03$) proteins than patients with sicca symptoms only.

available from 22 patients with SD (Supplementary Table 1) and from 6 patients with sicca symptoms (Supplementary Table 2). Among the patients with SD, HCMV-IgG was detected in 19 out of 22 (86.3 %) of serum samples and HCMV-IgM was found in 9 out of 22 (40.9 %) patients. One of these nine patients had primary HCMV infection (HCMV-IgM positive and -IgG negative), and eight patients were positive for both HCMV-IgG and -IgM, indicating either a reactivation of the virus from latency or a new primary infection in a seropositive person. Among the 22 patients with paired tissue and blood samples, 14 had HCMV-IE at score 4 (>75 % positive cells) in the biopsies. A majority of these were HCMV-IgM (57 %; $n = 8$) and -IgG (92.8 %; $n = 13$) positive.

In patients with sicca symptoms, HCMV-IgG and IgM were detected in five out of six (83.3 %) and in one of six (16.7 %) available serum samples, respectively. The paired biopsy of the sole IgM positive sample had a HCMV-IE score of 3.

One patient with SD and one patient with sicca symptoms were HCMV-IgM and -IgG negative but HCMV-IE positive (score 3, and 1, respectively) in their salivary gland tissues.

In SD patients, we analyzed whether HCMV levels or expression in salivary glands correlated with any of the recorded clinical parameters (Table 1), but observed no significant correlations (data not shown). However, HCMV-IgM positivity correlated with HCMV-LA expression levels in salivary glands ($r = 0.55, p < 0.02$) indicating persistent HCMV-LA protein expression may contribute to continuous IgM production to this antigen. Moreover, anti-SSA and -SSB autoantibodies were detected in 24 out of 28 (85.7 %) and 18 out of 28 (64.3 %) SD patients and no correlation was found between HCMV presence in salivary glands with autoantibody these autoantibodies. In the 24 SD patients with anti-SSA autoantibodies, 15 were highly positive for HCMV-IE (score 4), but only one highly positive for HCMV-pp65 (score 3) (Supplementary Table 1).

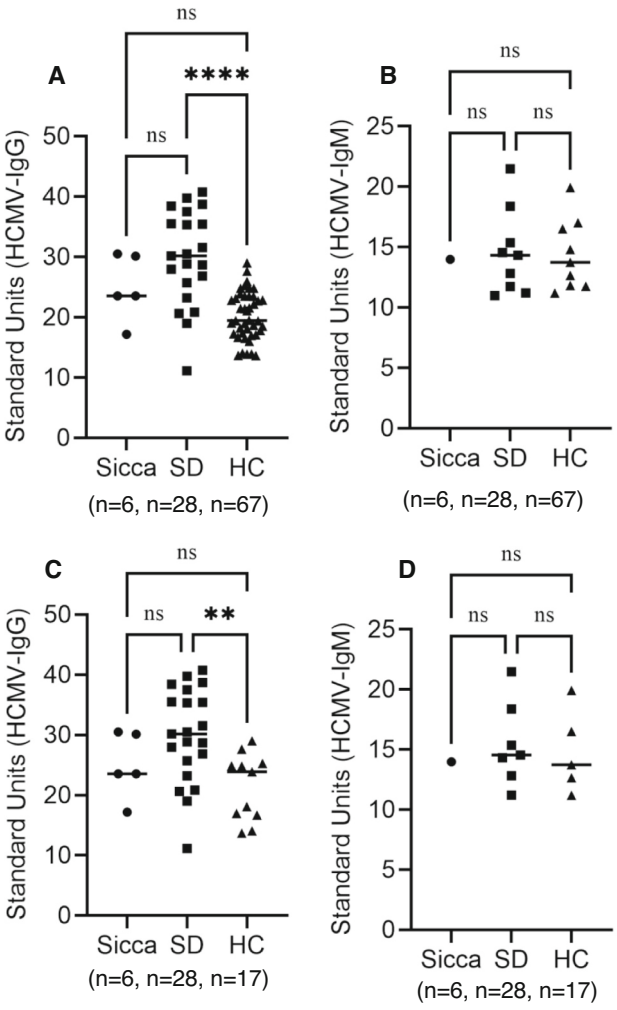


**Fig. 3.** High level of HCMV antibodies in patients with Sjögren's disease (SD). HCMV-IgG positive SD patients had significantly higher HCMV-IgG levels than healthy controls ($P < 0.0001$) (A). HCMV-IgG levels were higher in SD but not in sicca patients compared to the age-matched controls ($P = 0.009$) (C). No significant differences in IgM titers were observed across groups (B,D).

## 4. Discussion

In this retrospective study, we analyzed the presence of HCMV in SD through analysis of salivary gland biopsies and serum samples. Our findings reveal significant differences in HCMV protein prevalence between SD patients and those with sicca symptoms without SD.

Notably, HCMV proteins showed markedly higher detection rates in SD patients. High levels of HCMV-IE protein (>75 % of the cells in the biopsy, score 4) was found in 62.9 % of SD patients, compared to 29.4 % of sicca symptom patients, while HCMV-LA protein was present in 69.2 % versus 20 % of sicca controls. The immunogenic pp65 protein was detected in 45.8 % of SD patient samples, contrasting with only 12.5 % in sicca controls. HCMV-IE proteins can be present in non-permissive cells [29] and has been demonstrated in normal tissue [30], but the higher prevalence of HCMV-IE proteins and the greater intensity of HCMV-IE, HCMV-LA and pp65 in the analyzed samples in this study indicate that an active HCMV infection, most likely often due to a reactivation, is abundant in the salivary glands of patients with SD. Notably, eight out of 14 of the SD biopsies with HCMV-IE protein with a score of 4 were also HCMV IgM positive –seven had probable HCMV reactivation and one a primary infection, further indicating that they had an ongoing active infection. Furthermore, the staining pattern of SD patient biopsies showed mainly nuclear staining of HCMV-IE in glands,

vessels, and inflammatory cells, while both nuclear and cytoplasmic HCMV-IE staining was observed in tissue specimens from sicca symptom control patients. An isotype control antibody was used to control for background staining. We did not include a negative tissue control in the IHC analysis, as we had no access to salivary gland tissue biopsies from healthy subjects. Samples from healthy donors should be included in future prospective studies.

Serologically, we observed intriguing patterns. While HCMV IgG seroprevalence remained similar between SD patients and healthy controls, HCMV IgM seroprevalence was significantly higher in SD patients (32.1 % vs 13.4 %, $P = 0.04$). Serological evidence (IgM and IgG positivity) of HCMV reactivation was observed in 28 % of SD patients, suggesting a potential link between viral activity and disease progression. In serum samples collected at the 10-year follow-up from patients with SD, both HCMV-IgM and -IgG could be detected at the time of diagnosis and at the 10-year follow-up in paired samples from two patients. This may indicate some immune abnormality in these individuals, or repeated HCMV reactivations. Notably, persistent autoantigen-specific IgM is also observed in patients with SD [31] as part of the chronic inflammatory disease. It should be noted that IgM and IgG positivity also may indicate a new infection with a different HCMV strain. A primary or reactivated infection may show different avidity of IgM and IgG antibodies [32], however avidity testing was not performed in this study.

The presence of HCMV proteins in patients with seemingly incompatible serological profiles was observed in two patients with SD and one patient with sicca. These individuals showed HCMV protein positivity in their salivary glands despite being HCMV-IgM and -IgG negative. This incompatibility of HCMV serology and viral nucleic acid or protein detection has been reported in both healthy individuals [33,34] and cancer patients [35], but it is not clear why some individuals lack detectable antibodies to this virus. However, no saliva was collected for detection of HCMV-DNA in this cohort of the patients. We earlier reported that about 30 % of patients with glioblastoma who were HCMV protein positive in their tumors and also had good memory T cell responses against HCMV, were serologically negative for HCMV [35].

Reactivated HCMV may drive inflammation through multiple pathways. The virus induces expression of inflammatory mediators [36,37], various inflammatory cytokines and chemokines [38]. In chronic inflammatory diseases and autoimmune diseases, a self-perpetuating inflammatory cycle may be created, where latently infected monocytes migrate to inflamed tissues and differentiate into macrophages or dendritic cells, reactivating HCMV in the process. Of particular interest is the correlation between elevated IL-17 expression and SD severity [39], as this cytokine has emerged as an important cytokine in patients with autoimmune diseases (ref). Afshari et al. reported elevated IL-17 mRNA levels in HCMV-infected liver transplant patients [40]. This suggests a need for further research exploring the potential link between HCMV and IL-17 in the pathogenesis of SD.

HCMV-IgG titers were significantly higher in SD patients compared to healthy controls ($p < 0.0001$). The significance of this observation is uncertain, as hypergammaglobulinemia is common in patients with SD [41]. However, Brauner et al. [42] found that treatment naïve SD patients demonstrated higher influenza-specific antibody titers after H1N1 vaccination compared to healthy controls. Naïve B cells from untreated patients showed hyperreactivity, with overexpression of immune-related genes and enhanced capacity for Ig class switching when triggered by TLR7 and TLR9 agonists in vitro. Vaccination led to increased Sjögren-associated autoantibody titers and elevated EBV antibody levels in SD patients. Since latent HCMV reactivation may be triggered by inflammation [4], it is possible that HCMV reactivation triggers the hyper-responsive B cells in untreated SD patients, resulting in higher HCMV IgG titers. HCMV interacts with TLR7/9 in plasmacytoid dendritic cells and stimulate IFN-α and B cell proliferation [43], potentially promoting autoantibody production and inflammation in SD patients. Correlating HCMV IgG titers to total Ig levels would provide valuable information, but is not possible in this study due to lack of sufficient patient samples.

The healthy control group consisted of 50 blood donors, with equal sex distribution, and 17 healthy females that were age-matched to the SD patients. A surprisingly high proportion of the healthy age-matched female controls were HCMV-IgM positive –seven of 17 (41.2 %), of which five had a primary HCMV infection. This was an unexpected result, as primary HCMV infection is generally rare in this age group. In comparison, only two of the 50 healthy blood donors were HCMV-IgM positive, both considered to be caused by HCMV reactivation.

This study has several limitations. The retrospective design, small sample size, and lack of clinical scoring such as ESSDAI and ESSPRI at the time of sample collection constrain the broader interpretability of our findings. Sample material was limited to salivary gland tissue biopsies and serum samples. Nevertheless, our study provides valuable new data. Studies of the possible association between HCMV infection and SD are few and contradictory and mainly based on serology [2,44]. In contrast to our findings, Sorgato et al. [45] failed to detect HCMV by IHC in salivary gland tissues from patients with RA/SD. We used an optimized IHC technique for detection of HCMV protein expression in tissue specimens that was originally developed for detection of HCMV proteins in tumor tissue specimens [28,46], while Sorgato used a conventional immunostaining protocol. Future descriptive analyses of HCMV in SD should include more comprehensive analyses, including molecular techniques to validate protein and antibody findings, keeping in mind that discrepancies in CMV nucleic acid and protein findings have been reported across different tissue types [47,48] and samples should be assessed from both patients with SD and healthy controls.

## 5. Conclusion

To our knowledge, our study is the first study showing significantly higher prevalence of HCMV proteins in the salivary glands of SD patients compared to patients with sicca symptoms without SD, indicating an active HCMV infection at the site of inflammation in these patients. While investigating HCMV reactivation in salivary glands in vivo presents methodological challenges, these results underscore the critical need for functional studies exploring HCMV's potential role in triggering autoimmune responses and exacerbating inflammatory processes. Continued research in this direction could provide valuable insights that will contribute to the overall understanding and management of Sjögren's disease.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2025.110545.

## CRediT authorship contribution statement

**Mattia Russel Pantalone:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Xinling Xu:** Writing – review & editing, Writing – original draft, Data curation. **Nerea Martín Almazán:** Writing – review & editing. **Christina Gerstner:** Writing – review & editing. **Marie Fischer:** Writing – review & editing, Resources. **Marika Kvarnström:** Writing – review & editing, Resources. **Cecilia Söderberg-Nauclér:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Marie Wahren-Herlenius:** Writing – review & editing, Conceptualization. **Afsar Rahbar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Data curation, Conceptualization.

## Ethics approval

Ethical approval for the study was obtained from the Ethics Committee at Karolinska Institutet, Stockholm, Sweden (approval no. Dnr 2008/518–31, Dnr 2014/1916–32, Dnr 01–420, Dnr 98–367, Dnr 2017/373–31/2).

## Funding

This study was supported by The Swedish Medical Research Council Grant # 2022–02724 and 2021–02503 and was financially supported by InFLAMES Flagship Programme of the Academy of Finland (decision number: 337530).

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Cecilia Soderberg-Naucler reports financial support was provided by The Swedish Medical Research Council. Cecilia Soderberg-Naucler reports financial support was provided by InFLAMES Flagship Program of the Academy of Finland. Marie Wahren-Herlenius reports financial support was provided by The Swedish Medical Research Council. Marie Wahren-Herlenius reports financial support was provided by Swedish Heart and Lung Association. Cecilia Soderberg-Naucler reports a relationship with Immunor AS that includes: equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We acknowledge Vijole Ottosson for excellent technical support.

## Data availability

Data will be made available on request.

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