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Infection and autoimmunity in Sjogren's syndrome: A clinical study and comprehensive review

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ABSTRACT

Sjögren's syndrome (SS) is an autoimmune disease characterized primarily by lymphocytic infiltration of the exocrine glands, and autoantibody production. Multiple environmental factors affecting an individual with a genetic susceptibility may trigger the development of SS. Herein, we aimed to evaluate links between the different pebbles in the mosaic of SS. Demographic, clinical data and blood samples were gathered from 82 consecutive patients with SS, and 139 healthy controls. Samples were analyzed for infectious serology and auto-antibodies as well as for relevant genetic mutations (TAP genes) and cytokines levels. An immune response (IgG) against Epstein–Barr virus (EBV) early antigen (EA) was positively associated with SS (OR 4; 95% CI: 1.82–8.83, $p = 0.001$) while a protective effect of IgG anti-cytomegalovirus (CMV) was observed (OR 0.3; 95%CI: 0.16–0.74, $p = 0.009$). Anti-Ro/SSA, anti-LA/SSB, anti-nuclear, anti-gliadin, anti-TTG-IgG and anti-RNP antibodies were statistically more prevalent among SS patients than controls. Notably, the presence of anti-Ro/SSA and anti La/SSB correlated with anti-EBVEA IgG (OR 3.1; 95%CI: 1.08–8.74) and (OR 3.9; 95%CI: 1.37–10.96) respectively. Autoantibodies, cytokines and several genetic markers correlated with clinical manifestation of SS. Our data suggest that infectious agents may play both a causative and protective role in the pathogenesis of SS. Moreover certain autoantibodies, cytokines and specific TAP alleles correlate with clinical manifestations of SS, and may enable better prediction and/or directed therapy once confirmed in future studies.

1. Introduction

Sjögren's syndrome (SS) is a progressive autoimmune disease (AD) affecting mainly females in their 4th to 5th decades of life [1]. The dominant picture is epithelitis caused by lymphocyte infiltration of exocrine glands, however, extra-glandular manifestations may affect above 50% of patients [2]. SS may be observed alone or associated with other autoimmune diseases (i.e., polyautoimmunity) [3,4]. Reports of prevalence range from 0.1 to 4.8%, depending on different geographical and ethnical areas [5]. While the pathogenesis of SS remains unclear, it has been postulated that a genetic predisposition with epigenetic modification lies in the base of the disease, which is further triggered by environmental factors [6–10]. This has been termed the 'mosaic of autoimmunity' [11,12]. In other words a prone individual with a genetic tendency, and in some cases a subclinical immune dysregulation, will encounter at several points of his life immune stimuli such as hormone disbalance, infections, chemicals and physical agents and emotional stress that will eventually trigger autoimmunity [13,14]. A genetic influence on SS has been reported mainly based in aggregation of ADs in family members of patients with SS [14,15]. Genes involved in both innate and adaptive immunity in SS play a crucial role into

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the susceptibility towards the disease [16,17] with HLA locus disclosing the strongest association [18]. There are likely also subgroups of patients with Sjogren's syndrome with different outcomes [19–22]. Among environmental factors, a wealth of data has linked infections, mainly viruses to this matter. Some infections such as hepatitis B virus (HBV) or hepatitis C virus (HCV), parvovirus B19, tuberculosis, malaria, and human immunodeficiency virus may mimic SS [23], while others, most notably of the *herpesviridae* family, have a tropism for salivary and lacrimary glands [24,25]. Immune mediated changes in the salivary epithelium of SS, characterized by a type-I interferon (IFN) signature, including the over expression of type I IFN-inducible genes, further support the role of viral infection in SS [26,27]. Moreover, numerous epidemiological and clinical studies have linked infections, especially Epstein–Barr virus (EBV), but also retroviruses, cytomegalovirus (CMV), HBV, HCV, Coxsackie virus, and Human T-lymphotropic virus Type 1, to the pathogenesis of SS [23,28,29]. Autoantibodies are a major component of SS [6,30,31], and the prevalence of anti-nuclear antibodies (ANA) in SS is ~80% [32]. Wide arrays of platforms are available nowadays for the assessment of autoantibodies of the ANA family [33]. Of which the multiplex assay has been assessed and validated in previous studies [34]. Notably, different prevalence and levels of a specific antigen were documented among healthy and diseased populations and may depend on various genetics and environmental exposures, such as the levels of anti-dsDNA antibodies evaluated in different healthy populations by the Bioplex system [34]. The presence of anti-Ro/SSA or anti-La/SSB is one of the criteria for the diagnosis and classification of SS, and can be detected 60–80% of patients as well [35], and they may appear 5 years before clinical disease [36]. Autoantibodies may have a direct pathological role in SS, perhaps by damaging tissues directly [37], inhibiting the activity of Ro52 antigens which are involved in ubiquitination and regulation of apoptosis and cell cycle [38], or by opsonizing cells before phagocytosis [39]. More interestingly, the La antigen, a target of autoantibody production in SS, is involved in processing viral RNA [40]. In order to further evaluate the interplay between genetic and environmental factors, we analyzed blood specimens of SS patients and healthy controls. Autoantibodies, infectious serology, cytokines, and genetic mutations were analyzed and correlated with demographic and clinical data.

2. Methods

2.1. Patients

In this cross sectional study, we collected sera from 82 consequent SS patients, and 139 healthy controls. All patients fulfilled the revised classification criteria for SS [41]. Controls were healthy individuals not related to patients with SS, and without inflammatory or autoimmune diseases, or a history of a chronic infectious disease, including tuberculosis and human immunodeficiency virus. Patients and controls were assessed at the Center for Autoimmune Diseases (CREA), in Colombia, and their clinical characteristics have been published previously [42]. We gathered demographic (age, gender) and clinical data (e.g., age of disease onset, disease duration, extent of glandular and extra-glandular dermal, musculoskeletal, central nervous system, and thyroid involvement). Information regarding recent lab results was obtained (complete blood count [leucopenia defined as white blood cells <4000 K/microL] and chemistry), and blood was drawn for further testing (see methods below: autoantibodies, anti-infectious serology and genetic polymorphisms). The study received approval from the hospital's ethics committee.

2.2. Anti-infectious antibody testing

Anti-infectious agents were tested by two methods: **1. Multiplexed assay**: Screening for IgG and IgM antibodies against Epstein–Barr virus (EBV), *Toxoplasma gondii*, and cytomegalovirus (CMV), Rubella, and *Treponema* was performed using the Bio-Rad BioPlex 2200 (Bio-Rad Laboratories, Hercules CA, USA). The technology and protocol have been evaluated and described by our group and by others [43,44].

Briefly, the BioPlex 2200 is a fully automated, random-access analyzer built on synthesis of multiplex, magnetic beads and flow cytometry technologies. The EBV kit uses three different populations of beads screening for Abs directed against EBV nuclear antigen (EBVNA-1), EBV viral capsid antigen (EBVCA), and EBV early antigen diffuse (EBVEA). For syphilis the beads screen for Abs against different epitopes of the TPr protein from *Treponema*. Finally, ToRC packs screen for antibodies against *T. gondii,* Rubella and CMV. **2. Enzyme immunoassay**: Screening for IgG antibodies against hepatitis B virus (HBV), *Helicobacter pylori*, and hepatitis C virus (HCV) was performed using ELISA kits. Tests for anti-HBV core protein (recombinant HBc antigen) and anti-*H. pylori* were performed using MONOLISA anti-HBc Plus and PYLORI DETECT IgG commercial kits respectively (Bio-Rad, Hercules CA, USA) according to the manufacturer's protocol as described in our previous work [45]. Sera were tested for anti-HCV (recombinant HCV antigen: c22-3, c200 and NS5) using the HCV encoded antigen ORTHO HCV Version 3.0 ELISA test system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol, and as previously described by us [46].

2.3. Auto-antibody testing

A large profile belonging to 4 groups of autoantibodies was tested in all sera, utilizing the BioPlex 2200 system, explained above. The autoantibodies included: anti-nuclear antibodies (ANA IgG: Anti-dsDNA, Sm, chromatin, ribosomal-P, RNP, SmRNP, Ro/SSA, La/SSB, centromere, Scl-70, Jo-1), vasculitis associated (IgG: Anti- glomerular basement membrane [GBM], proteinase 3 [PR3], myeloperoxidase [MPO]), gastrointestinal associated (IgG, IgA: Anti-*Saccharomyces cerevisiae* [ASCA], gliadin [AGA], tissue transglutaminase [tTg]), and thrombophilia associated (IgG, IgM: Anti-cardiolipin [CL], B2 glycoprotein 1 [B2GP1], complex of both [CL-B2], phosphatydilserine-B2GP1 complex [PS-B2], phosphatidylethanolamine [PE], prothrombin [PT], phosphatydilserine–prothrombin complex [PS–PT]). The results are expressed in Antibody Index (AI) units, and cutoff levels according to the company instructions.

2.4. TAP typing and cytokine evaluation

Genotype data of TAP1 and TAP2 gene polymorphisms, cytokine levels and rheumatoid factor levels were available for 35 patients. These analyses were performed at time of the biopsy sample for diagnosis as it was previously described. Briefly, first, TAP polymorphisms were identified by amplification refractory mutation system-PCR (ARMS-PCR) [42]. Second, levels of Interleukin-10 (IL-10), IL-12 (p70), IL-4, Interferon γ (IFN-γ), and Tumor necrosis factor-α (TNF-α) were determined by ELISA according to the manufacturer instructions (OptEIATM, Pharmingen, San Diego) as it was described [42]. Finally, total and IgA rheumatoid factor (RF) were measured by turbidimetry and ELISA, respectively (Immco Diagnostics, Buffalo, NY, USA) as it was previously described [42].

2.5. Statistical analysis

Data analysis was performed using SPSS 17.0. Results are shown in averages ± standard deviation, and in percentages. Comparison

between SS patients and healthy controls and among subphenotypes in the group of SS patients was done. Differences among groups were established by Chi-square or Fisher's tests as appropriate. The differences between media values were established by *T* test or Mann–Whitney Test as appropriate in continues variables according with the result of Shapiro–Wilk Test. Crude odds ratios (OR) were estimated and reported with its correspondent 95% confidence interval (CI). A level of 5% was used to define statistical significance.

3. Results

3.1. Demographic

In this work we compared 82 SS patients with 139 healthy controls. In both there was a significant female predominance (97% and 92%), and the average age was 40 ± 8 and 45 ± 11 years old, receptively. The mean age of disease onset in the SS groups was 4, while the average time from onset to the current study was 6 years (range 5.4–1.3).

3.2. Anti-infectious antibodies prevalence

When the presence of infectious serology in patients and controls was compared, a difference was demonstrated regarding anti-EBVEA IgG and anti-CMV IgG antibodies (Table 1). More SS patients disclosed a higher percentage of anti-EBVEA IgG antibody, (OR 4; 95%CI: 1.82–8.83, $p = 0.001$), but a less anti-CMV IgG (OR 0.3; 95% CI: 0.16–0.74, $p = 0.009$) than healthy controls (Table 1).

3.3. Autoantibodies prevalence

GI-related autoantibodies – SS patients had more anti-gliadin and anti-transglutaminase (IgG) antibodies 14.6% vs. 1.4%, (OR 11.8; 95% CI: 2.57–54.31, $p < 0.001$) and 6.7% vs. 1.6% (OR 8.9; 95% CI: 1.02–78.09, $p = 0.027$ respectively). Other GI autoantibodies including anti-gliadin, and anti-transglutaminase of the IgA subtype did not significantly differ between the groups (Table 2).

Vasculitis associated autoantibodies and Antiphospholipid Autoantibodies – did not differ significantly between the groups.

Anti nuclear antibodies – In this study, ANA was defined as positive if any of the anti-cellular antibodies were detected by Bioplex testing (Anti-dsDNA, Sm, chromatin, ribosomal-P, RNP, SmRNP, Ro/SSA, La/SSB, centromere, Scl-70, Jo-1). Notably, 83% of SS patients had ANA positivity by Bioplex testing (Table 3), and not surprisingly, significant differences in antibody levels were observed between SS patients and the controls. SS patients had 40–60% of anti-Ro/SSA (including SSA52 and SSA60 antigens) and anti-SSB, which were much more prevalent than control, 1–2%. In addition, the SS group had slightly, but significant higher rates of anti-RNP antibodies than the controls (11% vs 3%), (Table 3). A relatively high ANA sero-positivity was documented in the control group, and was accounted for high anti-DNA (38%) and anti-Chromatin (37%) levels, which were comparable to those of the SS group. These unexpected high levels of antibodies among healthy subjects was addressed in our former study [34].

3.4. Interactions between autoantibodies and anti-infectious agents

We analyzed the direct association between anti-infections antibodies and autoantibodies among patients, Table 4. A significant positive association was demonstrated between the presence of anti-Ro/SSA/SSA52 and EBV associated antibodies namely: anti-EBVNA IgG (OR 9.5; 95%CI: 1.14–79.03), anti-EBVEA IgG (OR 3.1; 95%CI: 1.08–8.74) and anti-EBVCA IgG (OR 12.3; 95%CI: 1.51–100.73), as well as between anti-La/SSB and anti-EBVEA (OR 3.9; 95%CI: 1.37–10.96). In addition, anti-SmRNP was positively associated with anti-CMV IgM (OR 14; 95%CI: 1.47–134.1). In addition, the presence of anti-RNP-68 was negatively associated with anti-EBVEA (OR 0.8; 95%CI: 0.65–0.99).

3.5. Interactions between SS manifestations, serological and genetic markers

The main clinical manifestations of the SS patients are displayed in Table 5.

Table 1
Anti-infectious serology – control vs. Sjögren's syndrome.

Infectious agents	Control (%) ($n = 139$)	SS (%) ($n = 82$)	*P*	OR	CI
anti-HBV	11	6	*NS*		
anti-HP	81	80.5	*NS*		
anti-TOXO IgM	3	1	*NS*		
anti-CMV IgM	16	26	*NS*		
anti-TOXO IgG	39	46	*NS*		
anti-CMV IgG	93	80.5	*0.009*	0.317	0.16–0.74
anti-TPr15 IgG	6	4	*NS*		
anti-TPr17 IgG	1	0	*NS*		
anti-TPr47 IgG	6	4	*NS*		
anti-Syph IgG	6	4	*NS*		
anti-EBVCA IgM	7	6	*NS*		
anti-EBVCA IgG	93	85	*NS*		
anti-EBVEA IgG	8	26	*0.001*	4.006	1.82–8.83
anti-EBVNA IgG	91	88	*NS*		

SS – Sjögren's syndrome, *P* – *P* value, OR – odds ratio, CI – confidence interval, NS – not significant, HBV – hepatitis B virus, HP – *Helicobacter pylori*, TOXO – toxoplasma, CMV – Cytomegalovirus, TPr(15,17,47) – *Treponema pallidum* r(15,17,47), Syph – syphilis antibody, EBVCA – Epstein Barr virus core antigen, EBVEA – Epstein Barr virus early antigen, EBVNA – Epstein Barr virus nuclear antigen.

Table 2
Auto-antibodies – control vs. Sjögren's syndrome.

Auto antibodies	Control (%) ($n = 139$)	SS (%) ($n = 82$)	*P*	OR	CI
Vasculitis					
anti-GBM	0	0	*NS*		
anti-MPO	1	2	*NS*		
anti-PR3	1	0	*NS*		
GI auto-Ab					
anti-GA IgG	1	15	*0.001*	11.829	2.58–54.32
anti-SCA IgG	4	7	*NS*		
anti-TTG IgG	0	6	*0.006*	0.939	0.89–0.99
anti-GA IgA	4	5	*NS*		
anti-SCA IgA	2	4	*NS*		
anti-TTG IgA	0	0	*NS*		
aPL					
anti-CL IgM	1	0	*NS*		
anti-B2GPI IgM	0	1	*NS*		
anti-CL_B2 IgM	10	16	*NS*		
anti-PS_B2 IgM	0	1	*NS*		
anti-PT_PT IgM	8	5	*NS*		
anti-PE IgM	0	0	*NS*		
anti-CL IgG	1	2.5	*NS*		
anti-B2GPI IgG	9	1	*0.004*	1.094	1.04–1.15
anti-CL_B2 IgG	4	1	*NS*		
anti-PS_B2 IgG	5	11	*NS*		
anti-PT_PT IgG	1	1	*NS*		
anti-PE IgG	1	1	*NS*		

HC – healthy controls, SS – Sjögren's syndrome, *P* – *P* value, OR – odds ratio, CI – confidence interval, NS – not significant, GBM – glomerular basement membrane, MPO – myeloperoxidase, PR3 – proteinase 3, GI_auto Ab – gastrointestinal auto antibodies, GA – gliadin antibodies, SCA – *Saccharomyces cerevisiae* antibody, TTG – tissue transglutaminase antibody, aPL – antiphospholipid, CL – cardiolipin, B2GPI – β2 glycoprotein I.

Table 3
Anti-nuclear antibodies – control vs. Sjögren's syndrome.

Anti nuclear antibody	Control (%) $n = 139$)	SS (%) ($n = 82$)	*P*	Or	CI
anti-dsDNA	34	27	*NS*		
anti-Chrom	38	37	*NS*		
anti-SSA	2	62	0.001	75.129	22.01–256.50
anti-SSA52	1	46	*0.001*	120.045	16.01–899.82
anti-SSA60	2	60	*0.004*	71.354	20.92–243.37
anti-SSB	2	38	0.001	27.758	8.13–94.77
anti-RIBOP	0	4	*NS*		
anti-RNP	3	11	*0.018*	4.192	1.25–14.08
anti-Sm	1	4	*NS*		
anti-CentB	0	7	*NA*	NA	NA
anti-SmRNP	1	6	*NS*		
anti-SCL70	0	1	*NA*	NA	NA
anti-JO1	0	0	*NS*		
Any_ANA	46	83	*0.001*	5.768	2.97–11.21

HC – healthy controls, SS – Sjogren's syndrome, *P* – *P* value, OR – odds ratio, CI – confidence interval, NS – not significant, ANA – anti nuclear antibodies, dsDNA – double stranded DNA, Chrom – Chromatin, RNP68 – ribonucleoprotein 68, SM – smith, CENTB – centromere b, NS – no significant, NA – not assessed. Any-ANA – positive for at least one ANA.

Several key clinical manifestations were associated with different serological markers assessed in this study (i.e. antibodies, anti-infectious agents and cytokines), as well as with certain genetic polymorphisms (see below).

Disease onset – Our results suggest that if SS patients were negative for anti-Ro/SSA ($p = 0.012$) or urticaria or vasculitis were the presenting symptoms ($p < 0.001$ and $p < 0.013$, respectively), the age of disease onset was older than average (44 ± 15). For instance, the highest difference was found within the group of patients with no presence of Ro/SSA the mean age of onset was 49 ± 15 years, and for urticaria and vasculitis was 45 ± 13 years.

Keratoconjunctivitis sicca – While all SS patients had Keratoconjunctivitis sicca (KCS) at some point during their disease, only 62% had this symptom as an initial presentation. Interestingly, patients who presented with KCS had higher blood levels of IFN-γ than those who did not have it at the onset (31 pg/ml ± 9 vs. 22.5 pg/ml ± 7, $p = 0.04$).

Raynaud's syndrome – SS patients who suffered from Raynaud's syndrome had higher anti-Ro/SAA antibody titers: 4.9 ± 3.2 vs. 3.1 ± 3.5AI (OR 4.7; 95%CI: 1.20–18.88, $p = 0.03$), and rheumatoid factor positivity (RF > 10units) (OR 6; 95%CI: 1.21–29.52, $p = 0.026$), when compared to SS patients without Raynaud's syndrome. In addition, the presence of Raynaud's syndrome in SS patients inversely correlated to sera interleukin-4 levels: levels were 5.7 pg/ml in SS with Raynaud's syndrome vs. 11.6 pg/ml in those without ($p = 0.04$).

Vasculitis among SS patients was significantly related with the presence of anti-phospholipid-antibodies (anti-b2GPI or anti-cardiolipin) (OR 5.4; 95%CI: 1.55–19.11, $p = 0.01$), ASCA IgG (OR 6.5; 95%CI: 1.14–36.78, $p = 0.05$) and the TAP2*02:01 allele (OR 11.4, 95%CI: 1.20–108.28, $p = 0.03$).

Arthritis was linked to the presence of TAP1*04:01 allele (OR 8; 95%CI: 1.27–50.04, $p = 0.03$) among SS patients, while patients without arthritis tend to carry the TAP1*01:01 allele (OR 0.1; 95%CI: 0.01–0.61, $p = 0.02$).

Extra-glandular Manifestations in general positively correlated with the presence of anti-RNP antibodies (OR 5.2; 95%CI: 1.18–22.81, $p = 0.027$).

Neurological manifestations – among SS patients both Peripheral and Central nervous system involvement (PNS, CNS) correlated with higher titers of ASCA IgG 2.1 ± 3.2 vs. 0.4 ± 1 (OR 11.5; 95%CI: 1.89–69.89, $p = 0.01$) compared to those without neurologic involvement. In addition the TAP1*04:01 allele was significantly more prevalent in patients with neurological involvement (OR 15.6; 95%CI: 1.33–182.09, $p = 0.03$).

Leucopenia – the presence of leucopenia, in SS patients, correlated with levels of interleukin-12. Average interleukin-12 levels were 16.5 ± 10,4 pg/ml in patients with leucopenia vs. 8.8 ± 7.94 pg/ml in those without, $p = 0.009$.

Table 4
Correlations between anti-infectious serology and antibodies in patients with Sjögren's syndrome.

Infections	Antibody	*P*	Or	CI
anti EBVCA IgG	SSA52	0.004	12.333	1510–100,738
anti EBVEA IgG	SSB	0.017	3.882	1374–10,966
anti EBVEA IgG	SSA52	0.042	3.083	1087–8746
anti EBVNA IgG	SSA52	0.017	9.514	1145–79,031
anti CMV IgM	SMRNP	0.014	14.118	1478–134,819

P – *P* value, OR – odds ratio, CI – confidence interval, EBVCA – Epstein Barr virus core antigen, EBVEA – Epstein Barr virus early antigen, EBVNA – Epstein Barr virus nuclear antigen, CMV – Cytomegalovirus antigen.

Table 5
Clinical and laboratory manifestations of patients with Sjögren's syndrome.

Clinical manifestations	Number of patients (%) ($n = 82$)
SICCA at onset	61.7%
Musculoskeletal manifestations	67.9%
Arthritis	18.5%
Arthralgia	48.8%
Dermatologic manifestations	17%
Purpura	3.7%
Urticaria	6.2%
Reynaud	16%
Vasculitis	16%
Neurological manifestations	11.1%
Leucopenia[a]	16%
Autoimmune thyroid disease[b]	30.9%

[a] WBC < 4000.
[b] According to laboratory findings.

4. Discussion

In the present study we compared frequencies and levels of anti-infections and auto-antibodies of SS patients and healthy controls. In addition, in a sub-analysis of our cohort of SS patients, we analyzed the interactions of clinical manifestations with auto-antibodies, anti-infectious antibodies, cytokines, and some genetic markers, in the aim of elucidating the autoimmune 'mosaic' of this disease. If we consider the triangle of genetics, environmental (infectious, vitamins), and immunological factors (autoantibodies, cytokines), it seems that their integration will not only determine which disease will develop, but also the clinical manifestations that differ between individuals with the same autoimmune disease.

Our results disclosed an association between SS and previous infection with EBV. These results echo studies presented by others (see below). The presence of anti-EBVNA IgG and anti-EBVCA IgG antibodies in both patients and controls was high, as expected in most world populations. However the presence of anti-EBVEA antibodies, which is indicative of viral replication in its lytic phase, was more prevalent in the SS group. Newkirk MM et al. [47] demonstrated a relationship between anti-EBVEA and SS patients in the past, and Pasoto SG et al. [48] had found a relationship to SS, especially with the joint manifestations. The EBVEA antigen, also called BHRF, is structurally and functionally similar to the proto-oncogene bcl-2, and has been found to bind to DNA, and act as a cofactor of the viral DNA polymerase [49]. Other autoimmune

diseases such as SLE, systemic sclerosis and the anti-phospholipid syndrome were also linked to high levels of anti-EBVEA antibodies [50,51]. Furthermore, we found a positive relation between the hallmark SS antibodies namely anti-Ro/SSA and anti-La/SSB and past infection with EBV. A direct correlation between anti-Ro/SSA and anti-La/SSB and EBV infectivity was this autoimmune disease. Another expected finding is the higher prevalence of anti-celiac antibodies and anti-nuclear antibodies among SS patients in this study [52].

However, in contrast to previous studies, we found that in our specific cohort the prevalence and titers of IgG to CMV were higher in controls than among patients. This may suggest a protective role of past infection with CMV, in SS. Notably, several studies have shown an increase in IgG and IgM of CMV in SS patients compared to controls [43], whereas others have been able to induce a SS-like disease in autoimmune prone mice (the NZM2328 type), following the injection of murine CMV, intra-peritoneal [53]. In a Danish study, only complement fixation antibodies and not Ig class antibodies to CMV were more prevalent in SS patients than controls [54], and in other reports, anti-CMV antibodies negatively correlated with SS [28]. Taking it all together it seems that the specific genetic characteristics of the study population as well as the exposure to various viral strains may have an important role in the association between CMV infection and SS.

In the current study we found that the presence of anti-RNP antibodies may define a subgroup of SS. Anti-RNP positivity among SS was associated with anti-CMV IgM antibodies and negatively with anti-EBVEA. Clinically a higher rate of extra glandular manifestations was observed in this subgroup which may resemble somewhat the mixed connective tissue disease (MCTD) spectrum. Another group of SS patients, which had Raynaud's phenomenon, had lower levels of IL-4, which plays an integral role in the development and onset of SS-like disease in the NOD mouse model [55] and perhaps in humans. The Raynaud's phenomenon has been reported in 13–30 percent of patients with SS [56]. Another finding is that our SS patients presenting with KCS had higher levels of blood INF-γ. Interestingly, studies demonstrated higher levels of cytokines, including INF-γ, in the lacrimal and salivary fluid of SS patients and non-SS patients with KCS, compared to control ones [24,42,57]. Perhaps this rational may facilitate more experiments with anti-INF-treatments for SS patients, or KCS, in the future. In this study as well as in a former one using the same cohort we have found a high level of anti-dsDNA in the control group. The presence of anti-dsDNA was evaluated in different populations by the Bioplex platform and a high prevalence was documented among healthy subjects from Latin America and specifically from Colombia [34]. This unexpected very high prevalence of these antibodies was suggested to be the cause of environmental exposure (e.g. infections) and differ significantly from other healthy populations in which accepted low levels were documented using the same method at the same time. Importantly, in the current study although the prevalence of anti-dsDNA was high (both in controls and in SS patients) it did not differ between groups as expected.

TAP1 and TAP2 genes codified for TAP proteins which are needed for transporting peptides to the MHC molecules before being presented, and as so are candidates for studying in autoimmune diseases. One study reported that TAP2*Bky2 allele was associated with the production of anti-Ro/SAA antibodies in SLE Japanese patients [58]. Strong linkage disequilibrium among the loci TAP2 and DQB1 was found in Colombian SS patients, and it was suggested that a specific region between these loci is strongly associated with a predisposition to develop SS [59]. Moreover, a previous case–control study did not find any relationship between TAP polymorphisms and SS disease [42,59,60]. However, herein we analyzed TAP polymorphisms in 42% of our SS patient cohort and found significant correlation between some alleles and SS clinical manifestations. These results support the concept of genetic-environmental interplay in SS. Alas, the small number of subjects analyzed is a limitation and these genetic links should be verified in further studies.

5. Conclusions

This study, to our knowledge, is the first to attempt to clarify and unravel the perplex mosaic of SS, by evaluating demographic, clinical, infectious, genetic and immunological (autoantibodies and cytokines) aspects, in one disease cohort. This was performed in a small, albeit homogenic and well characterized group of SS patients of a common ethnic background. The limitation of our study is the small groups of patients which underwent genetic evaluation. Future studies should continue to examine autoimmune diseases, such as SS, in the context of these complex ties and possibly a specific combination of markers will enable to foresee a clinical course of a given patient, and even help guide treatment strategies.

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