True Primary Sjögren's Syndrome in a Subset of Patients with Hepatitis C Infection: A Model Linking Chronic Infection to Chronic Sialadenitis

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Abstract

Background: Hepatis C virus infection is presently an exclusion criterion to classify Sjögren's syndrome; however, there are distinct clinicopathologic and biologic similarities between HCV-related and SS-related chronic inflammation of mucosa-associated lymphoid tissue and lymphoproliferation that suggest common pathogenetic pathways.

Objectives: To determine whether a subset of patients with sicca syndrome and HCV infection may present a true primary SS rather than a distinct clinicobiologic entity.

Methods: We extensively characterized 20 consecutive patients with positive anti-HCV antibodies and heavy subjective dry eye and/or dry mouth symptoms, plus positive unstimulated sialometry and/or Shirmer's test. We then compared these features with those in HCV-negative primary SS controls (classified according to the latest American-European Consensus Group Classification Criteria for SS).

Results: Of the 20 HCV-positive patients with sicca manifestations, 12 (60%) had positive anti-SSA/SSB antibodies (3/12 by enzyme-linked immunosorbent assay and 6/12 by immunoblot) and/or positive salivary gland biopsy (at least 1 focus/4 mm^2), which met the strict classification criteria for SS, as in the case of HCV-negative SS controls. Comparing the HCV-positive SS subset with HCV-negative SS controls showed similar female to male ratio (11/1 vs. 46/4), major salivary gland swelling (17% vs. 26%), positive antinuclear antibodies (75 vs. 94%) and positive rheumatoid factor (58 vs. 52%). Significant differences ($P < 0.05$) were seen in mean age (69 vs. 56 years), liver disease (50 vs. 2%), lung disease (25 vs. 0%), anti-SSA/SSB positivity (25 vs. 90%), and low C3 or C4 (83 vs. 36%). HCV-positive SS patients exhibited a trend for more frequent chronic gastritis (50 vs. 22%), fibromyalgia (33 vs. 14%), peripheral neuropathy (33 vs. 18%), purpura (33 vs. 19%) and cryoglobulinemia (33 vs. 6%).

Conclusions: A major subset of HCV-positive patients with definite subjective sicca symptoms and positive objective tests may indeed present a true, though peculiar, subset of SS. There are strict similarities with key clinical, pathologic and immunologic findings of definite HCV-negative SS. Other features appear more characteristic of HCV infection. When also considering that HCV is sialotropic and may be treated, HCV-related chronic sialadenitis represents a unique opportunity to clarify key pathogenetic events occurring in the large majority of HCV-negative SS; and similarities to typical primary SS, rather than differences, should be taken into account.

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In the past decade, a number of reports indicated that hepatitis C virus infection may be associated with salivary gland chronic inflammation and with dry eye and dry mouth symptoms, which are usually mild [1–4, reviewed in 2]. HCV is sialotropic [5,6], and mice transgenic for HCV envelope genes develop sialadenitis resembling Sjögren's syndrome [7]. In addition, salivary gland lymphoma is associated with HCV infection [5,8,9]. All these data led to the hypothesis that HCV is a relevant local trigger for chronic inflammation and B cell proliferation in the salivary gland mucosa-associated lymphoid tissue microenvironment, in accordance with the currently accepted models of B cell lymphomagenesis [5,10].

Whether the picture of chronic sialadenitis and sicca syndrome associated with HCV infection represents either a subset of SS or a distinct clinicobiologic entity is still a matter of debate. HCV infection is presently an exclusion criterion to classify Sjögren's syndrome [11–13]. The lack of anti-SSA/SSB antibodies in most patients with HCV infection and sicca syndrome, in conjunction with the mild or poorly characterized sicca manifestations in many of the published series [2], and the lack of HCV positivities in American series of SS patients [11], contributed to the latest American-European Consensus Classification approach [13]. On the other hand, in clinical practice there are also HCV-infected patients who present with severe subjective and objective sicca features and with positive autoantibodies (including antinuclear antibodies, anti-SSA/SSB antibodies, or rheumatoid factor) [3], leading to the perception and clinical diagnosis of true SS.

To better address this issue, we extensively characterized consecutive patients with HCV infection and definite subjective dry eye/mouth symptoms [12] plus positive sialometry and/or Shirmer's test, as compared to controls with primary SS who were negative for HCV, classified according to recent American-European Consensus Criteria [13]. According to minor salivary gland tissue biopsy and anti-SSA/SSB testing many of the patients with HCV infection satisfied the latest classification criteria for SS [13], and distinct similarities in clinical, pathologic and immunologic findings were noticed in HCV-unrelated SS controls. Some features, on the other hand, appeared more characteristic of HCV infection.

Overall, when carefully selected *a priori* (presence of subjective and objective sicca manifestations by means of approved questionnaires and tests) [12], many HCV-positive patients with sicca syndrome are characterized by clinicopathologic and biologic features of SS, and likely represent a true (though peculiar) subset of SS. When also considering that HCV may be effectively treated

HCV = hepatitis C virus
SS = Sjögren's syndrome

[14], this subset provides a unique opportunity to clarify key pathogenetic events occurring in the large majority of HCV-negative SS patients in whom the local trigger of the MALT disorder remains unknown [10]. Similarities with typical primary SS cases, rather than differences, should be taken into account.

Patients and Methods

Our study group comprised 20 consecutive patients with severe dry mouth and/or dry eye symptoms (positive subjective symptoms according to the questionnaire in the 1993 European Classification Criteria for SS) [12], plus positive unstimulated sialometry (=1.5 ml/15 min) and/or positive Shirmer's test ($\leqslant$ 5 mm 5 min) [12], and serologic evidence of HCV infection (positive anti-HCV antibodies by third-generation enzyme-linked immunosorbent assay, confirmed by second- or third-generation recombinant immunoblot assay) [8]. HCV RNA was sought in the serum by nested polymerase chain reaction according to published procedures [8]. The patients – 19 females and 1 male, with a mean age of 68.2 years (range 51–80) – all HIV-negative heterosexuals, were lacking a mixed cryoglobulinemia syndrome. Other exclusion criteria for SS were missing (absence of lymphoma, sarcoidosis, graft versus host disease, use of anti-cholinergic drugs).

The HCV-positive patients underwent minor salivary gland biopsy and anti-ENA testing (by ELISA), since positivity of at least one of the two is required for classification of primary SS, according to the latest American-European Classification Criteria [13]. Controls were represented by 50 consecutive, unselected primary SS patients, classified according to the same criteria [13] and lacking serologic evidence of HCV infection.

All the patients and controls were extensively characterized by clinical and instrumental evaluation of clinical features (major salivary gland swelling, liver and kidney disease, interstitial lung disease, arthralgias/arthritis, chronic gastritis, purpura, peripheral neuropathy, and fibromyalgia), and laboratory features (antinuclear antibodies by indirect immunofluorescence on Hep-2 cells, positive if $\geqslant$ 1:160; anti-ENA antibodies by ELISA; RF by nephelometry, positive if >20 IU/ml; serum cryoglobulins; and low C3 and C4), according to published methods [8,15].

Statistical analysis was performed by either the Mann-Whitney test or the chi-squared test (with Yates correction), with 95% confidence interval, and statistical significance of $P < 0.05$.

Results

Of the 20 patients with HCV infection and sicca features, 12 (60%) had positive anti-SSA/SSB and/or positive salivary gland biopsy (at least 1 focus/4 mm^2), and thus satisfied the strict criteria for SS, as for HCV-negative primary SS controls [13]. Data regarding these 12 SS-positive, HCV-positive cases are presented in Tables 1 and 2. HCV RNA was amplified in the serum of 10/11 cases (data not available in the remaining case).

A comparison of these patients with the HCV-negative primary SS controls revealed several similarities: female to male ratio (11/1 vs. 46/4), presence of major salivary gland swelling, positive ANA, and positive RF [Table 3]. Significant differences ($P < 0.05$) were seen in mean age (69 vs. 56 years), presence of liver disease (50% vs. 2%), interstitial lung disease (25% vs. 0), anti-SSA/SSB positivity (25 vs. 90%, by ELISA), and low C3 or C4 (83 vs. 36%) [Table 3]. Also noticed was a trend among HCV-positive patients for more frequent chronic gastritis, fibromyalgia, peripheral neuropathy, purpura and cryoglobulinemia [Table 3].

Putative false positivities by the anti-SSA/SSB ELISA assay were excluded, as shown by the lack of autoantibody detection in different groups of controls (healthy subjects, anti-HCV-positive cases without sicca features, and patients with rheumatoid arthritis without sicca features; data not shown).

Anti-ENA antibodies were also sought by immunoblot (INNO-LIA ANA Update, Innogenetics) in the 12 HCV-positive patients with SS, and in 9 of the HCV-negative SS controls (8/9 anti-SSA/SSB-positive, and 1/9 SSA/SSB negative by ELISA). Immunoblot detected anti-SSA antibodies in 6/12 HCV-positive cases (including the 3

MALT = mucosa-associated lymphoid tissue
ELISA = enzyme-linked immunosorbent assay
RF = rheumatoid factor
ANA = antinuclear antibody

Table 1. Clinical features of patients with HCV infection and primary Sjögren's syndrome

Pt	Age	Gender	Parotid swelling	Fibromyalgia syndrome	Liver disease	Nephritis	Lung disease	Chronic gastritis	Peripheral neuropathy	Purpura	Arthralgia/ arthritis
1	80	F	–	+	–	–	Hemorrhagic alveolitis	–	+	+	+
2	67	F	–	–	Primary biliary cirrhosis		Fibrosis	–	–	–	–
3	52	F	+	–	Chronic persistent hepatitis			+	+	–	+
4	67	F	–	+	Chronic persistent hepatitis			+	–	–	+
5	79	F	–	–	Chronic persistent hepatitis		Interstitial lung disease	–	–	–	+
6	71	M	–	–	–	+	Interstitial lung disease	–	–	+	+
7	60	F	–	–	–	–	–	+	–	–	+
8	71	F	–	–	Chronic persistent hepatitis	–	–	–	+	+	–
9	79	F	+	–	–	–	–	+	–	–	+
10	55	F	–	+	Chronic persistent hepatitis	–	–	+	+	–	+
11	72	F	–	+	–	–	–	–	–	–	+
12	72	F	–	–	–	–	–	+	–	+	+
			2/12 (17%)	4/12 (33%)	6/12 (50%)	1/12 (8%)	3/12 (25%)	6/50 (50%)	4/12 (33%)	4/12 (33%)	10/12 (83%)

Table 2. Autoantibodies, objective tests for ocular and oral dryness and salivary gland histopathologic features in patients with HCV infection and primary Sjögren's syndrome

Pt	ANA	Anti-ENA abs by ELISA	Anti-ENA abs by immunoblot	Cryoglobulinemia	RF	Schirmer's test	Unstimulated sialometry	Minor salivary glands grading by Chisholm and Mason
1	1:1280	–	SSA	+	+	+	ND	3°
2	1:160	SSA	SSA	–	–	+	ND	1°
3	1:160	SSA	SSA	–	+	ND	+	2°
4	–	–	–	–	–	–	+	3°
5	1:160	–	–	–	+	–	+	3°
6	–	–	–	–	+	–	+	3°
7	1:2560	–	SSB	+	+	+	+	4°
8	–	–	–	–	+	+	+	4°
9	1:5120	SSA	SSA / SSB	–	–	+	+	4°
10	–	–	–	+	+	+	–	4°
11	1:1280	–	–	–	+	+	+	4°
12	1:320	–	SSB	+	+	–	+	3°
	8/12 (66%)	3/12 (25%)	6/12 (50%)	4/12 (33%)	9/12 (75%)	7/11 (63%)	9/10 (90%)	10/12 (83%)

ANA = antinuclear antibodies (detected by indirect immunofluorescence on Hep-2 cells), anti-ENA abs = anti-extractable nuclear antigen antibodies, RF = rheumatoid factor (positive if = 20 IU/ml).

Table 3. Clinical and biologic features of patients with primary Sjögren syndrome, with or without HCV infection

	Primary SS HCV-positive (n = 12)	Primary SS HCV-negative (n = 50)	*P**
Age (mean ± SD)	69 ± 9.1	56 ± 13.4	0.0075°
Gender F/M	11/1	46/4	NS
Parotid swelling	2 (17%)	13 (26%)	NS
Fibromyalgia syndrome	4 (33%)	7 (14%)	NS
Liver disease	6 (50%)	1 (2%)	0.0001
Nephritis	1 (8%)	2 (4%)	NS
Lung disease	3 (25%)	0	0.004
Chronic gastritis	6 (50%)	11 (22%)	NS
Purpura	4 (33%)	5 (19%)	NS
Peripheral neuropathy	4 (33%)	9 (18%)	NS
Arthralgia/arthritis	10 (83%)	36 (72%)	NS
ANA	9 (75%)	47 (94%)	NS
Anti-SSA/SSB abs			
ELISA	3 (25%)	45 (90%)	0.0001
Immunoblot	6 (50%)	n. d.	
Rheumatoid factor	7 (58%)	26 (52%)	NS
Cryoglobulinemia	4 (33%)	2/30 (6%)	NS
Low C3 and/or C4	10 (83%)	18 (36%)	0.008

* χ^2 test corrected by Yates (CI: 95%)

° Non-parametric Mann-Whitney U test (CI: 95%).

cases positive by ELISA; Table 3) and anti-SSA/SSB antibodies in 8/9 HCV-negative SS controls (i.e., the same patients with anti-SSA/SSB-positive by ELISA; data not shown).

Discussion

Whether chronic sialadenitis and heavy sicca syndrome associated with HCV infection represent either a subset of SS or a distinct clinical entity is still uncertain. In the present study we show that when investigating carefully selected HCV-positive individuals with definite, subjective and objective ocular and/or oral sicca manifestations, primary SS (according to the more recent and strict classification criteria) [13] may often be detected. Both clinical and biologic evidence in this series support the notion that such patients represent a true subset of SS: in particular, the high female to male ratio, the frequent positivity of ANA, including anti-SSA/SSB antibodies, and the usual, focal lymphocytic pathologic picture in the salivary glands, which strictly represents that of primary SS [16,17]. Although other features appear more characteristic of HCV infection and definitely identify HCV-associated SS as a subset that does not reflect the typical primary SS case, similarities with typical primary SS cases rather than differences should be seriously considered.

The mean age of our HCV-positive SS patients was higher than that of SS controls, concurring with previous observations [2–4]. It is possible that HCV infection takes a longer time to induce chronic inflammation or sicca features when compared to other triggering events in HCV-unrelated SS. Of note, the mean age of patients with HCV-related mixed cryoglobulinemia appears to be similar [18]. It is difficult to explain the heavy sicca manifestations based only on the older age, though age may be a co-factor. Most elderly patients do not complain of heavy sicca symptoms, as also noticed in consecutive patients with polymyalgia rheumatica followed in our center (data not shown).

With regard to the rate of anti-SSA/SSB detection in HCV-positive SS cases in this study (25% and 50% by ELISA and immunoblot, respectively), this was found to be lower in the 12 patients with the final diagnosis of SS than in SS HCV-negative controls. However, this rate was higher than in several previously published series of HCV-infected patients with sicca syndrome. Similarly, the fraction of ANA-positive cases previously reported [reviewed in 2] was lower than in the present series. On the other hand, in a recent paper by Ramos Casals et al. [3], anti-SSA and anti-SSB positivity was detected in 17% and 14% of well-characterized cases with HCV infection and sicca syndrome,

respectively (surprisingly, despite this and other findings, the authors did not support the concept of a true HCV-associated SS). Thus, higher rates of detection of anti-SSA/SSB antibodies in HCV-positive patients may be due to careful patient selection when suspecting SS (definite sicca syndrome according to a standardized questionnaire, with subjective and objective manifestations in this study) [12]. Furthermore, the laboratory assay used appears to be relevant as well. It should now be verified whether conventional assays miss some low titer anti-SSA/SSB positivities in HCV-positive patients with sicca features, and whether additional tests may better detect such SS-related autoantibodies in ANA-positive cases. This point is of major relevance, since the lack of anti-SSA/SSB antibodies in most patients with HCV infection and sicca syndrome contributed to the latest American-European Consensus Classification approach for SS, where HCV infection represents an exclusion criterion [13].

In addition, even if anti-SSA/SSB autoantibody positivity is missing, both the high frequency of ANA positivity and the pathologic picture of focal sialadenitis (grade 3 or 4 according to Chisholm and Mason) [12] in HCV-positive subjects with definite sicca syndrome strongly support a diagnosis of SS. In fact, when subjective and objective dryness is present, patients with grade 3 or 4 sialadenitis may be classified as primary SS by the recent American-European Criteria, despite the lack of anti-SSA/SSB antibody detection [13].

Three other striking points support the position that, in the presence of the aforementioned clinicopathologic and biologic features, the patient should be considered as having true SS, HCV-related, and that SS should not be excluded simply because HCV infection is detected. First, common biologic features are shared between SS-related/HCV-unrelated B cell lymphoproliferative disorders and HCV-related B cell disorders (in particular, the preferential expansion B cell clones with definite immunoglobulin gene rearrangements), thus indicating common pathogenetic pathways [10,19]. Secondly, a viral triggering event is highly suspected in the etiopathogenesis of SS [16], and thus the identification of HCV as a salivary gland candidate in a fraction of cases is consistent with the hypothesis [10]. Of note, the biologic and clinical implications of antigen eradication are crucial in MALT inflammation and lymphoproliferation [10], and should be investigated in the salivary gland microenvironment of HCV infection. Finally, B cell lymphoma of the salivary gland, i.e., the most common localization of B cell lymphoma complicating the course of SS, is also associated with HCV infection [9], which may then elicit both the autoimmune sialadenitis and the lymphoproliferative complication. Thus, the developments in the "HCV model" of chronic sialadenitis may prove of major value for understanding the biologic events sustaining chronic inflammation and B cell lymphoproliferation in the large majority of SS patients in whom the key antigenic trigger(s) remains unknown.

This is certainly the case of RF-positive B cell proliferation, since many molecular features are shared between HCV-positive mixed cryoglobulinemia/B cell lymphoma and B cell clonal expansion in myoepithelial sialadenitis in HCV-negative SS [10,19]. Thus, pathogenetic hypotheses to explain the preferential expansion of RF-positive B cell clones in the course of HCV infection may also be valid for SS-related lymphoproliferation [10,20,21]. In early 2000, it was reported that B cell clones expanded in HCV-related mixed cryoglobulinemia, and B cell lymphoma may show immunoglobulin heavy chain complementarity-determining region 3 sequences (i.e., a crucial antibody region for antigen binding), which are significantly similar to the CDR3 region of human RF as well as of anti-HCV antibodies directed to the E2 antigen of HCV [20,21]. This may implicate either a molecular mimicry mechanism, or the simultaneous binding of two different antigens (i.e., IgG and HCV) by different binding sites of the RF Fab [22]. Subsequent results in larger series of patients and including the analysis of immunoglobulin kappa chain genes were consistent with this hypothesis, as was the evidence that the *VH1-69* gene is over-represented in anti-HCV antibodies directed to the E2 antigen [23,24]. In fact, the *VH1-69* gene is well recognized to be preferentially expressed by B cell clones in HCV-related mixed cryoglobulinemia and B cell lymphoma, e.g., in clones presenting the WA idiotype [18]. Strikingly, Quinn and co-workers [25] recently showed that the surface immunoglobulin expressed by HCV-associated B cell lymphoma may indeed bind the viral E2 envelope protein. Additional pathogenetic mechanisms may include HCV internalization in the B cell, processing and presentation to T cells, as well B cell infection and deregulation by HCV, and B cell stimulation by means of HCV-CD81 binding or by HCV-very low density lipoprotein complexes. Overall, advances in the study of HCV-related lymphoproliferation proved relevant for research hypotheses in SS lymphoproliferation and pathogenesis [10,19].

Finally, HCV infection may also favor the development of peculiar clinico-laboratory features in SS, e.g., the presence of liver disease, purpura, decreased serum complement levels, and mixed cryoglobulinemia. The present results concur fully with previous reports on this topic [2] and underscore that HCV-associated SS represents a minor subset of SS with some distinct clinical and biologic features. In the present series, we also highlight the presence of chronic gastritis and fibromyalgia, which were not thoroughly investigated in previous studies. Chronic gastritis is frequently noticed in SS, with gastric MALT lymphocytic infiltrates resembling the salivary infiltrates [15]. In addition, HCV may localize in the gastric epithelium and may therefore favor chronic gastritis and gastric lymphoproliferation, as recently shown [20]. Secondly, fibromyalgia features are often encountered both in SS and in chronic infection [16]. Thus, the frequent detection of chronic gastritis and fibromyalgia in HCV-positive SS patients is not unexpected. The identification of HCV infection again represents a starting point to better investigate the pathobiology of these SS features.

In conclusion, both the present results and previously published clinicopathologic and biologic data indicate that sicca syndrome in the course of HCV infection shows similarities with clinical, pathologic and immunologic findings of definite HCV-negative SS. Thus, when strict classification criteria for SS are satisfied, including

CDR3 = complementarity-determining region 3
Ig = immunoglobulin

positive salivary gland biopsy and/or anti-SSA/SSB antibodies [13], there is a strong rationale to consider this entity as a true subset of SS, even if HCV-infected subjects present some additional, distinctive features when compared with HCV-negative SS cases. Studying this subset of patients may prove of major value when investigating the issue of chronic MALT inflammation, autoimmunity and lymphoproliferation in the large majority of SS patients, where the key antigenic trigger(s) remains unknown. Also for this reason, the similarities with typical primary SS cases, rather than the differences, should be taken into account.

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The great question .... Which I have not been able to answer, despite my thirty years of research into the feminine soul, is "What does a woman want?"

Sigmund Freud (1865-1939) Austrian physician who pioneered the study of the unconscious mind