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Cryoglobulinemic vasculitis in primary Sjögren's Syndrome: Clinical presentation, association with lymphoma and comparison with Hepatitis C-related disease

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ABSTRACT

Objective: To describe the clinical spectrum of cryoglobulinemic vasculitis (CV) in primary Sjögren's syndrome (pSS), investigate its relation to lymphoma and identify the differences with hepatitis C virus (HCV) related CV.
Methods: From a multicentre study population of consecutive pSS patients, those who had been evaluated for cryoglobulins and fulfilled the 2011 classification criteria for CV were identified retrospectively. pSS-CV patients were matched with pSS patients without cryoglobulins (1:2) and HCV-CV patients (1:1). Clinical, laboratory and outcome features were analyzed. A data driven logistic regression model was applied for pSS-CV patients and their pSS cryoglobulin negative controls to identify independent features associated with lymphoma.
Results: 1083 pSS patients were tested for cryoglobulins. 115 (10.6%) had cryoglobulinemia and 71 (6.5%) fulfilled the classification criteria for CV. pSS-CV patients had higher frequency of extraglandular manifestations and lymphoma (OR=9.87, 95% CI: 4.7–20.9) compared to pSS patients without cryoglobulins. Purpura was the commonest vasculitic manifestation (90%), presenting at disease onset in 39% of patients. One third of pSS-CV patients developed B-cell lymphoma within the first 5 years of CV course, with cryoglobulinemia being the strongest independent lymphoma associated feature. Compared to HCV-CV patients, pSS-CV individuals displayed more frequently lymphadenopathy, type II IgMk cryoglobulins and lymphoma (OR = 6.12, 95% CI: 2.7–14.4) and less frequently C4 hypocomplementemia and peripheral neuropathy.
Conclusion: pSS-CV has a severe clinical course, overshadowing the typical clinical manifestations of pSS and higher risk for early lymphoma development compared to HCV related CV. Though infrequent, pSS-CV constitutes a distinct severe clinical phenotype of pSS.

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Introduction

Cryoglobulinemic vasculitis (CV) is a life threatening immune complex mediated small vessel vasculitis, involving primarily the skin, kidneys, and peripheral nerves, leading to end stage organ/tissue damage, if untreated [1,2]. It can be of infectious origin, with hepatitis C virus (HCV) infection being the most common cause, or associated with autoimmune diseases where the most frequent underlying condition is primary Sjögren's syndrome (pSS). As new, effective therapeutic modalities for the management of HCV infection keep emerging, pSS will soon become the leading cause of CV [2,3]. CV is associated with the presence of serum cryoglobulins of which, type II containing an IgMκ monoclonal rheumatoid factor (mRF) predominate in pSS, while both type II and III are detected in HCV and other autoimmune diseases. Previous studies have clearly shown that type II cryoglobulinemia in pSS is associated with more systemic manifestations, mainly vasculitis [4], and higher risk for future lymphoma development [5]. Thus, cryoglobulinemia is at the crossroad of the two most serious complications of pSS, that is, systemic vasculitis and B-cell non- Hodgkin's lymphoma (NHL). With the advent of new treatments, targeting successfully B-cells and NHL [6], the investigation and mapping of the clinical spectrum of pSS-CV may provide a concise strategic plan for early diagnosis and treatment for this subset of pSS. To this end, older studies have described the clinical picture of pSS patients in association with cryoglobulinemia [7–9], while after the introduction of CV criteria [10,11] some studies have focused on CV [12–16]. However, even after the application of CV classification criteria, the clinical picture of pSS-CV is still obscured due to the fact that many of the previous studies have a small number of pSS patients.

Herein, we present the clinical phenotype of CV in an integrated study population of Greek-Italian pSS patients, investigate the possible role of CV and cryoglobulinemia in NHL development in pSS and compare the clinical manifestations of pSS-CV with HCV-CV.

Patients and methods

Study design

This is a retrospective, matched case-control study in a multicenter population of consecutive pSS patients who fulfilled the 2016 ACR/EULAR classification criteria [17] and were followed up from May 1984 until March 2019, in 5 centers from Greece and Italy (University of **U**dine, Pisa, **A**thens, **H**arokopio, **I**oannina) (**UPAHI** group). The study was approved by the local ethical committees of all the Institutions involved, after obtaining patients' informed consent and in compliance to general data protection regulations (GDPR). One thousand eighty-three patients had been evaluated for serum cryoglobulins and 71 of them fulfilled the 2011 classification criteria for CV [10]. Cryoglobulins were evaluated after blood collection, quantitation, immunodiffusion and immunofixation, as described previously [4]. All pSS patients were HCV-RNA negative. The cumulative clinical, laboratory and histologic data of pSS-CV patients were compared with two control groups: a) pSS patients, repeatedly negative for serum cryoglobulins, matched (1:2 ratio) according to gender, age at pSS onset and disease duration from pSS onset and b) patients with HCV related CV, being RNA positive at CV diagnosis and without any associated autoimmune rheumatic disease, matched (1:1) according to age and gender. All patients with HCV related CV were diagnosed, treated and followed-up at the Infectious Disease Unit, Department of Clinical and Biomedical Sciences Hospita L. Sacco, Milan, Italy. In addition, pSS cryoglobulin-positive patients who did not fulfill the 2011 CV classification criteria, were compared with a pSS cryoglobulin negative control group, matched (1:2 ratio) according to gender, age and disease duration from pSS onset. All the laboratory, objective tests or minor salivary gland biopsy of pSS patients, were performed in the context of standard of care, according to physicians' judgment. pSS onset was defined as the year when the patient recalled the first disease related manifestation, such as Raynaud's phenomenon, arthritis, sicca symptoms, salivary gland enlargement or purpura. CV onset was defined as the time point of the appearance of the first CV related manifestation, according to the 2011 classification criteria. Groups were compared on the basis of cumulative clinical (dry mouth, dry eyes, salivary gland enlargement, Raynaud's phenomenon, lymphadenopathy, arthralgia/myalgia, arthritis, palpable purpura, liver involvement, kidney involvement, central and peripheral nervous system involvement, lymphoma), laboratory (anti Ro/SSA antibodies, anti La/SSB antibodies, rheumatoid factor, cryoglobulinemia, low serum C4 complement levels, monoclonal gammopathy) and histologic (focus score, germinal centers) features. Systemic organ involvement was based on the ESSDAI definitions and/or biopsy specimens [18]. Fatigue, dryness and pain were assessed as defined by the ESSPRI [19]. Since different pathogenetic mechanisms are operating in glandular and extraglandular manifestations of pSS, they have been classified as glandular (dry mouth, dry eyes, salivary gland swelling), non-specific manifestations (fatigue, arthralgia/myalgia, arthritis, Raynaud´s phenomenon), peri-epithelial (interstitial nephritis, primary biliary cholangitis, small airways disease), immune complex mediated (extra-epithelial) (purpura, skin ulcers, glomerulonephritis, vasculitic involvement of peripheral and/or central nervous system) and NHL [20].

Statistical and data driven analysis

Statistical analysis for categorical data was performed by $\chi 2$ test with Yates correction or Fisher exact when cell counts <5 patients and for numerical data *t*-test or Mann-Whitney, after Shapiro-Wilk normality test. In order to handle the multiple comparison testing, p-values have been also adjusted with Bonferroni correction. The Fast-Correlation based feature selection (FCBF) algorithm was applied on the dataset of pSS-CV patients and their pSS cryoglobulin negative controls, to identify potentially independent variables associated with lymphoma [21]. The FCBF preselection algorithm is a correlation based tool identifying, among several potentially independent variables, those with the weakest association amongst them and the strongest correlation with the outcome of interest that is NHL. Subsequently, the strongest preselected group of the FCBC derived potentially independent variables, has been used for constructing a binary multivariable logistic regression model to identify independent variables/features associated with lymphoma. The implementation of the FCBF-based multivariable logistic regression approach along with the statistical analysis was performed using Python 3.6 and GraphPad 7.0a.

Based on the post hoc sample size and study power calculation conducted according to the Fleiss method, assuming 90% study power and 95% two-sided levels of confidence, the present study sample size could detect an effect size (Odds Ratio) of 5.00 between patient groups (EpiInfo, CDC, Atlanta, Georgia, USA).

Results

Patient characteristics

Serum cryoglobulins were detected in 115/1083 patients (10.6%) of whom 71 (61.7%) fulfilled the 2011 CV classification criteria, while 44 (38.3%) had pSS with cryoglobulinemia but did not meet the CV criteria. Early ($\leq$ 35 years) pSS onset had 19.7% (n = 14/71) of pSS-CV patients, while 12.7% (n = 9/71) had late ($\geq$ 65 years) pSS onset. Among pSS-CV patients, 97% were females (n = 69/71) and 3% males (n = 2/71). The median age of pSS-CV patients, calculated at pSS onset, was 50 years (range: 21–75). The median duration from pSS onset in pSS-CV patients was 16 years (range: 0–37). None of the pSS or RNA-HCV positive control patients fulfilled criteria for another systemic autoimmune disease. pSS cryoglobulin positive patients without

vasculitis were also predominantly females [95.5%, (n = 42/44) vs 4.5%, (n = 2/44)] with a median age at pSS onset of 50 years (range: 11–79) and median disease duration from pSS onset of 13 years (range: 0–42).

The clinical phenotype of pSS-cryoglobulin positive patients with and without CV

The clinical picture of 71 pSS-CV patients was compared with that of 141 pSS cryoglobulin negative matched control patients. pSS-CV patients exhibited higher frequency of fatigue (59.2% vs 43%, p = 0.041, OR = 1.92, 95% CI: 1.08–3.52), Raynaud's phenomenon (47.9% vs 32.6%, p = 0.044, OR = 1.89, 95% CI: 1.06–3.39), salivary gland enlargement (53.6% vs 33.3%, p = 0.007, OR = 2.31, 95% CI: 1.29–4.21) and interstitial renal disease (10% vs 1.5%, p = 0.007, OR = 7.55, 95% CI: 1.59–36.4), compared to pSS-cryoglobulin negative patients respectively. No difference was found in sicca manifestations between the 2 groups. As anticipated, pSS-CV patients had increased prevalence of extra-epithelial manifestations of a vasculitic origin, including purpura (90.1% vs 14.9%, $p < 0.0001$, OR = 52.24, 95% CI: 21.43–125.4), vasculitic ulcers (12.7% vs 0.71%, $p < 0.001$, OR = 20.32, 95% CI: 3.17–224.4), peripheral nervous system vasculitic involvement (25.4% vs 1.5%, $p < 0.0001$, OR = 21.74, 95% CI: 5.12–96.01), glomerulonephritis, mainly of membranoproliferative type, (11.4% vs 0.71%, $p < 0.001$, OR = 18.06, 95% CI: 2.64–201.8) and lymphadenopathy (31% vs 7.1%, $p < 0.001$, OR = 5.88, 95% CI: 2.63–12.99). NHL, mainly of MALT type, was more frequent in the pSS-CV group (47.9% vs 8.5%, $p < 0.0001$, OR = 9.87, 95% CI: 4.7–20.9). Minor salivary gland biopsies of pSS-CV patients displayed a higher proportion of germinal centers (35% vs 11.3%, p = 0.043, OR = 4.21, 95% CI: 1.20–13.45) and a higher focus score [median: 2.05 (range: 0–9) vs 1.45 (range: 0–7)] compared to pSS cryoglobulin negative controls. The laboratory analysis disclosed that almost all pSS-CV patients had positive rheumatoid factor (95.7% vs 61.3, $p < 0.0001$, OR = 14.09, 95% CI: 4.58–44.33) as well as low C4 complement levels (88.6% vs 31.5, $p < 0.0001$, OR = 16.82, 95% CI: 7.47–35.57). Monoclonal gammopathy was also more prevalent in the pSS-CV group (45.5% vs 7.6%, $p < 0.0001$, OR = 10.17, 95% CI: 4.41–22.17). The presence of anti-Ro/SSA and anti-La/SSB autoantibodies was comparable between the two groups. A more detailed comparison of the clinical, laboratory, serological and histologic features between the 2 groups are presented in Table 1. After applying Bonferroni correction, fatigue,

Table 1
Comparison of clinical, laboratory and histologic features between pSS-CV and pSS patients with negative serum cryoglobulins.

	pSS-CV	pSS-Cryo negative	*P*-value	*P*-value*
Number of patients	71	141		
Gender (female%)(n)	97.2 (69/71)	96.5 (136/141)	1	1
Disease duration (from SS onset _years)	16 (range: 0–36)	15 (range: 0–34)	0.720	1
Media age (years)	50 (range: 21–75)	50 (range: 20–77)	0.887	1
Clinical Features% (n)				
Dry mouth	97.2 (69/71)	93.6 (132/141)	0.342	1
Dry eyes	98.6 (70/71)	92.9 (131/141)	0.104	1
SGE	53.6 (37/69)	33.3 (47/141)	0.007	0.252
Lymphadenopathy	31 (22/71)	7.1 (10/141)	<0.0001	<0.001
Fatigue	59.2 (42/71)	43(55/128)	0.041	1
Arthralgia/myalgia	71.8 (51/71)	63.8 (90/141)	0.312	1
Arthritis	25.7 (18/70)	18.3 (23/126)	0.295	1
Myositis	0(0/71)	1.4 (2/141)	0.552	1
Raynaud's phenomenon	47.9 (34/71)	32.6 (46/141)	0.044	1
Purpura	90.1 (64/71)	14.9 (21/141)	<0.0001	<0.0001
Vasculitic ulcer	12.7 (9/71)	0.71 (1/141)	<0.001	0.009
PNS	25.4 (18/71)	1.5 (2/130)	<0.0001	<0.0001
CNS	2.8 (2/71)	2.3 (3/129)	1	1
Liver				
Sclerosing Cholangitis	0(0/71)	0(0/141)	1	1
AIH	1.4 (1/71)	1.4 (2/141)	1	1
PBC	1.4 (1/71)	2.8 (4/141)	0.666	1
Lung				
ILD	8.6 (6/70)	6.8 (9/133)	0.853	1
Small airways disease	13(7/54)	6.6 (8/121)	0.273	1
Kidney				
Interstitial renal disease	10 (7/70)	1.5 (2/138)	0.007	1
Glomerulonephritis	11.4 (8/70)	0.71 (1/141)	<0.001	0.026
Splenomegaly	2.8 (2/71)	10.8 (8/74)	0.260	1
Lymphoma	47.9 (34/71)	8.5 (12/141)	<0.0001	<0.0001
Biological features% (n)				
Focus score (median)	2.05 (34/71)	1.42 (78/141)	0.025	0.9
Germinal centers	35 (7/20)	11.3 (6/53)	0.043	1
Anti-Ro	90 (63/70)	85(119/140)	0.429	1
Anti-La	60 (42/70)	46(64/139)	0.078	1
Anti-Ro/La	88.4 (61/69)	83.6 (117/140)	0.472	1
RF	95.7 (67/70)	61.3 (84/137)	<0.0001	<0.0001
Low C4	88.6 (62/70)	31.5 (41/130)	<0.0001	<0.0001
Hypergammaglobulinemia	69.7 (46/66)	56.9 (74/130)	0.114	1
Monoclonal gammopathy	45.5 (30/66)	7.6 (10/132)	<0.0001	<0.0001
Leukopenia	17.4 (12/69)	10 (14/140)	0.193	1
Thrombocytopenia	3(2/67)	2.2 (3/134)	1	1

Cryo: cryoglobulinemia, SGE: salivary gland enlargement, PNS: peripheral nervous system, CNS: central nervous system, AIH: autoimmune hepatitis, PBC: primary biliary cirrhosis, ILD: interstitial lung disease, RF: rheumatoid factor.
*: Bonferroni adjusted p-value.

Raynaud's phenomenon, salivary gland enlargement, interstitial renal disease, focus score and germinal centers were found with no statistically significant difference between the 2 groups (Table 1).

The FCBF algorithm, that constitutes the data driven analytic approach, performed analysis of 36 features/variables and disclosed 8 strong potentially independent variables [lymphadenopathy, cryoglobulinemia, positive rheumatoid factor (RF), salivary gland enlargement (SGE), dry mouth, arthritis, CNS involvement and lung-bronchocentric involvement accounting for small airways disease]. Narrowing of the analysis, using a combined FCBF/multivariable logistic regression model, showed that lymphadenopathy, cryoglobulinemia and positive RF are independent lymphoma associated features (supplementary Table 1). The performance of the model disclosed 70.6% sensitivity, 94.7% specificity and 84.5% accuracy area under the curve (AUC = 85.5%), after a 10-fold cross validation approach (supplementary Figure 1).

The most common presenting manifestations, according to the 2011 CV classification criteria, of pSS-CV patients were arthralgia/myalgia (49%), followed by arthritis (43.7%) and purpura (39.1%) (Fig. 1A). More than half of the patients (58.2%) with CV developed the first CV specific manifestation within the first year of pSS onset (Fig. 1B). In one third of pSS-CV patients (33.3%), lymphoma was observed within 5 years of CV onset, while in 13.3% of them, the appearance of lymphoma was a late sequel (>15 years of CV duration) (Fig. 1C).

1A) Most common presenting clinical manifestations of CV after applying the 2011 CV classification criteria, 1B) Chronological presentation of first CV related manifestation after pSS onset, 1C) Time distribution of lymphoma development during the course of CV.

The comparison between pSS cryoglobulin positive patients without CV and pSS cryoglobulin negative controls is presented in Table 2. The pSS cryoglobulin positive group without CV, had a higher frequency of monoclonal gammopathy (22% vs 7.3%, p = 0.040, OR = 3.56, 95% CI: 1.16–11.16) and NHL (29.5% VS 5.9%, $P<0.001$, OR = 6.26, 95% CI: 2.13–17.67). Data regarding lymphoma and type of cryoglobulins were available in 27 of 44 pSS cryoglobulin positive patients without CV. Type II cryoglobulinemia, containing an IgMκ monoclonal RF, was present in 87.5% (n = 7/8) of pSS patients with lymphoma and 47.3% (n = 9/19) in those without, while type III cryoglobulinemia was more prevalent in non-lymphoma (52.7%, n = 10/19) compared to lymphoma (12.5%, n = 1/8) pSS patients. After Bonferroni adjustment, monoclonal gammopathy was found with no statistically significant difference between the 2 groups (Table 2).

Overall, the 71 pSS-CV patients with either mild/non-specific or serious manifestations were treated as follows: corticosteroids (CS) 86%, hydroxychloroquine (HCQ) 80.3%, azathioprine (AZA) 21.1%, methotrexate (MTX) 22.5%, cyclophosphamide (CyC) 8.5%, rituximab (RTX) 18.3% and plasmapheresis 7%. Notably, pSS-CV patients without lymphoma received more frequently AZA, MTX and CyC compared to those with lymphoma (32.4% vs 8.8%, 27% vs 17.6% and

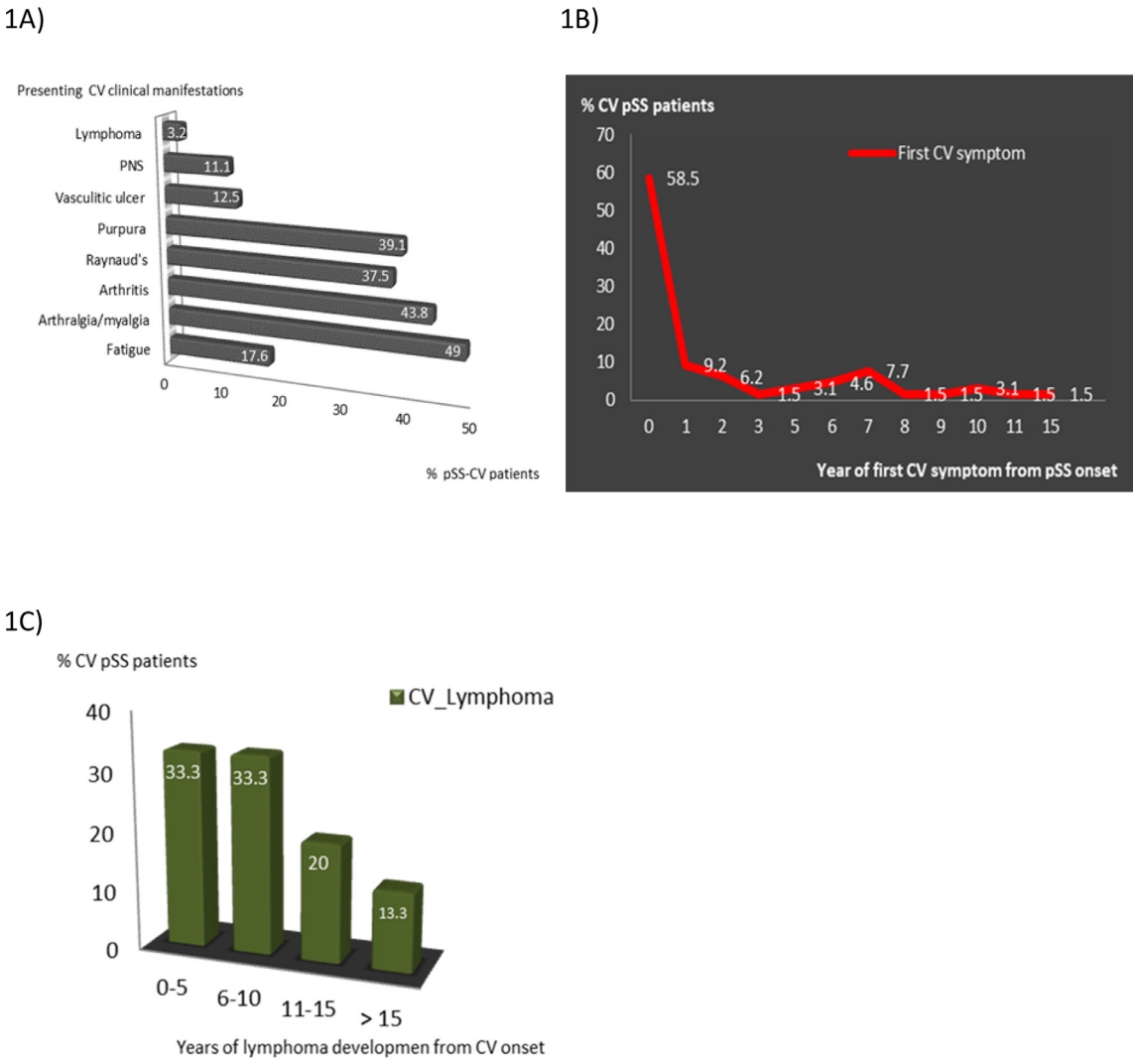

Fig. 1. The clinical course of pSS-CV patients.

Table 2
Clinical, laboratory and histologic features of cryoglobulin positive pSS patients without CV compared to pSS cryoglobulin negative patients.

	pSS-Cryo without CV	pSS-Cryo negative	*P*-value	*P*-value*
Number of patients	44	84		
Gender (female)% (n)	95.5 (42/44)	95.2 (80/84)	0.700	1
Median age (years)	50 (range:11–79)	51	0.695	1
Median disease duration (from SS onset _years)	13 (range:0–42)	11.5	0.628	1
Clinical features% (n)				
Dry mouth	95.5 (42/44)	95.2 (80/84)	1	1
Dry eyes	97.7 (43/44)	95.2 (80/84)	0.659	1
SGE	48.8 (21/43)	29.8 (25/84)	0.054	1
Lymphadenopathy	13.6 (6/44)	6 (5/84)	0.253	1
Fatigue	36.8 (14/38)	43.6 (37/80)	0.444	1
Arthralgia/myalgia	56.8 (25/44)	53.6 (45/84)	0.870	1
Arthritis	10.8 (4/38)	8.9 (7/79)	0.746	1
Myositis	0 (0/44)	0 (0/84)	1	1
Raynaud's phenomenon	38.6 (17/44)	28.6 (24/84)	0.337	1
Purpura	6.8 (3/44)	15.5 (13/84)	0.253	1
Vasculitic ulcer	2.3 (1/44)	1.2 (1/84)	1	1
PNS	4.5 (2/44)	3.6 (3/82)	1	1
CNS	2.3 (1/44)	2.4 (2/82)	1	1
Liver				
Sclerosing Cholangitis	0 (0/44)	2.4 (2/784)	0.545	1
AIH	0 (0/44)	0 (0/84)	1	1
PBC	0(0/42)	3.6 (3/84)	0.550	1
Lung				
ILD	9.1 (4/44)	4.9 (4/81)	0.450	1
Small airways disease	0 (0/40)	6.5 (5/77)	0.163	1
Kidney				
Interstitial renal disease	4.5 (2/44)	1.2 (1/83)	0.275	1
Glomerulonephritis	2.3 (1/44)	1.2 (1/83)	1	1
Splenomegaly	0 (0/44)	0 (0/84)	1	1
Lymphoma	29.5 (13/44)	6(5/84)	<0.001	0.023
Biological features% (n)				
Anti-Ro/SSA	70.5 (31/44)	71.4 (60/84)	0.928	1
Anti-La/SSB	25 (11/44)	41.7 (35/84)	0.094	1
RF	70.7 (29/41)	53(44/83)	0.090	1
Low C4	53.6 (22/41)	36.3 (29/80)	0.100	1
Hypergammaglobulinemia	59(23/39)	55(44/80)	0.831	1
Monoclonal gammopathy	22 (9/41)	7.3 (6/82)	0.040	1
Leukopenia	4.5 (2/44)	8.3 (7/84)	0.717	1
			0.122	1

Cryo: cryoglobulinemia, SGE: salivary gland enlargement, PNS: peripheral nervous system, CNS: central nervous system, AIH: autoimmune hepatitis, PBC: primary biliary cirrhosis, ILD: interstitial lung disease, RF: rheumatoid factor.
*: Bonferroni adjusted p-value.

10.8% vs 5.9 respectively). The 141 pSS cryoglobulin negative controls have received: CS 31.4%, HCQ 46.4%, AZA 4.3%, MTX 7.1%, CyC 0.7% and RTX 4.3%. Interestingly, pSS-CV patients with serious manifestations such as glomerulonephritis, peripheral neuropathy and vasculitic ulcers were treated with steroids, hydroxychloroquine, B cell depletion therapy and very few cases with cyclophosphamide or plasmapheresis. A more detailed description of treatment is presented in supplementary Table 2. The therapeutic regimen of the 44 cryoglobulin positive without CV pSS patients versus (vs) their controls included: CS 38.6% vs 39%, HCQ 45.5% vs 65.9%, AZA 9.1% vs 6.1%, MTX 2.3% vs 7.3%, CyC 4.5% vs 2.4% and RTX 4.5% vs 8.5% (supplementary Table 3).

Comparison of pSS-CV vs HCV-CV patients

The differences between the 71 pSS-CV and the 76 HCV-CV matched patients are presented in Table 3. As expected, dry eyes (98.6% vs 21.1%, $p < 0.0001$, OR = 262.5, 95% CI: 42.14–2680), dry mouth (97.2% vs 18.4%, $p < 0.001$, OR = 152.8, 95% CI: 34.41–657.8) and salivary gland enlargement (53.6% vs 0%, $p < 0.0001$) were more frequent among pSS-CV patients compared to HCV-CV. In addition, lymphadenopathy (31% vs 4%, $p < 0.001$, OR = 10.78, 95% CI: 3.29–35.17), arthritis (25.7% vs 10.8%, $p = 0.035$, OR = 2.85, 95% CI: 1.17–6.86), Raynaud's phenomenon (47.9% vs 22.4%, $p = 0.002$, OR = 3.18, 95% CI: 1.59–6.45), interstitial renal disease (10% vs 1.4%, $p = 0.030$, OR = 8.11, 95% CI: 1.36–92.47), hypergammaglobulinemia (69.7% vs 26.2%, $p < 0.001$, OR = 6.49, 95% CI: 2.98–13.41) and type II IgMκ cryoglobulinemia (97% vs 80.3%, $p = 0.035$, OR = 7.86, 95% CI: 1.15–85.55) were more often observed in pSS-CV compared to HCV-CV patients. On the contrary, HCV infection was associated with increased frequency of fatigue (98.6% vs 59.2%, $p < 0.0001$, OR = 47.64, 95% CI: 8.18–495.9), peripheral nervous system vasculitic involvement (71.6% vs 25.4%, $p < 0.0001$, OR = 7.43, 95% CI: 3.59–15.57) and low C4 (98.5% vs 88.6%, $p = 0.033$, OR = 8.51, 95% CI: 1.22–95.82), compared to pSS. The prevalence of lymphoma development was significantly higher in pSS-CV compared to HCV-CV patients (47.9% vs 13%, $p < 0.0001$, OR = 6.12, 95% CI: 2.7–14.4). After Bonferroni correction, arthritis, Raynaud's phenomenon, interstitial renal disease, low C4 and type II cryoglobulinemia were found with no statistically significant difference between the 2 groups (Table 3).

Discussion

Cryoglobulinemic vasculitis is a rare disease presenting in approximately 4–11% of pSS patients [16,22,23]. Despite the rarity, the disease exhibits two major clinical elements leading to poor outcome in pSS, which are systemic vasculitis and lymphoma development. This information has been concluded by several case and cohort studies in the past [4,16,23–25]. However, the major determining factor of those studies was the presence of cryoglobulinemia while more

Table 3
Comparison of the clinical, laboratory and histologic features between pSS-CV and HCV-SS patients.

	pSS-CV	HCV-CV	*P*-value	*P*-value*
Number of patients	71	76		
Gender (female)% (n)	97.2 (69/71)	97.4 (74/76)	1	1
Median age (years)	50 (range: 21–75)	51 (range: 29–62)	0.718	1
Clinical Features% (n)				
Dry mouth	97.2 (69/71)	18.4 (14/76)	<0.0001	<0.0001
Dry eyes	98.6 (70/71)	21.1 (16/76)	<0.0001	<0.0001
SGE	53.6 (37/69)	0(0/75)	<0.0001	<0.001
Lymphadenopathy	31 (22/71)	4(3/75)	<0.0001	<0.001
Fatigue	59.2 (42/71)	98.6 (69/70)	<0.0001	<0.0001
Arthralgia/myalgia	71.8 (51/71)	73.7 (56/76)	0.797	1
Arthritis	25.7 (18/70)	10.8 (8/74)	0.035	1
Myositis	0(0/71)	0(0/75)	1	1
Raynaud's phenomenon	47.9 (34/71)	22.4 (17/76)	0.002	0.062
Purpura	90.1 (64/71)	88.2 (67/76)	0.902	1
Vasculitic ulcer	12.7 (9/71)	25(19/76)	0.090	1
PNS	25.4 (18/71)	71.6 (53/74)	<0.0001	<0.0001
CNS	2.8 (2/71)	1.4 (1/71)	1	1
Liver				
Sclerosing Cholangitis	0(0/71)	0(0/76)	1	1
AIH	1.4 (1/71)	0(0/70)	1	1
PBC	1.4 (1/71)	0(0/72)	0.496	1
Lung				
ILD	8.6 (6/70)	1.3 (1/75)	0.056	1
Small airways disease	13 (7/54)	4(3/75)	0.093	1
Kidney				
Interstitial renal disease	10 (7/70)	1.4 (1/74)	0.030	0.93
Glomerulonephritis	11.4 (8/70)	23.7 (18/76)	0.085	1
Splenomegaly	2.8 (2/71)	10.8 (8/74)	0.097	1
Lymphoma	47.9 (34/71)	13(9/69)	<0.0001	<0.001
Biological features% (n)				
RF	95.7 (67/70)	87.1 (61/70)	0.128	1
Low C4	88.6 (62/70)	98.5 (66/67)	0.033	1
Cryoglobulinemia type II	97 (32/33)	80.3 (61/76)	0.035	1
Hypergammaglobulinemia	69.7 (46/66)	26.2 (17/65)	<0.0001	<0.0001
Monoclonal gammopathy	45.5 (30/66)	55.5 (20/36)	0.442	1
Leukopenia	17.4 (12/69)	14.7 (10/68)	0.845	1
Thrombocytopenia	3(2/67)	11.4 (8/70)	0.097	1

SGE: salivary gland enlargement, PNS: peripheral nervous system, CNS: central nervous system, AIH: autoimmune hepatitis, PBC: primary biliary cirrhosis, ILD: interstitial lung disease, RF: rheumatoid factor.
*: Bonferroni adjusted p-value.

precise data according to the international and validated classification criteria for CV are limited [9,10,14,16,26,27]. Thus, the clinical presentation of pSS-CV, as well as the characteristics that rule the outcome and the definition of the clinical phenotype of pSS-CV are still unmet needs.

The present study was conducted to address the clinical presentation of CV in unselected patients with pSS. The tools that were used include: a) an integrated Greek-Italian population, from 5 clinical centers evaluated by physicians, highly experienced with pSS-CV, b) application of unified and validated criteria for both pSS and CV, c) carefully selected triple matched 1:2 controls from the same clinical center, and d) analysis of the results by applying not only the classic statistics, but beyond that, a data driven approach with an unbiased selection of variables, to point out features associated with CV which are involved in lymphoma development. HCV infection often mimics pSS, sharing common clinical manifestations, including sicca symptoms and CV. To address this challenging diagnostic question, we compared the clinical picture of pSS-CV with that of HCV-CV.

To our knowledge this is the largest study of pSS-CV patients fulfilling the 2011 CV classification criteria. The prevalence of cryoglobulins is within the range reported by previous studies, [7–9,14,16] corresponding to 10% of the evaluated pSS patients. The clinical picture described in this report is in line with previous studies, but some points are presented for the first time and deserve special attention: a) approximately 60% of pSS-CV patients had their first CV manifestation within the first year from pSS onset, b) pSS-CV is heralded by non-specific clinical manifestations, such as arthralgia/myalgia, arthritis or Raynaud's phenomenon, c) high prevalence of skin vasculitis, extending from the one third of patients at disease onset to almost all patients after many years of follow up and d) a time-related pattern for the appearance of glomerulonephritis and peripheral neuropathy was not observed. One-third of the associated NHL cases occurred during the first 5 years after CV onset, but the diagnosis of NHL for the majority of pSS-CV patients, spread out in 20 years, since CV diagnosis. Based on these observations, since pSS-CV is usually heralded by non-specific manifestations of pSS, it is strongly recommended that, pSS patients must be evaluated properly at pSS onset for the presence of cryoglobulins. Following early diagnosis, pSS-CV patients should undergo a close follow up for many years, since internal organ vasculitis or lymphoma can very well be late sequels. These findings can also start a discussion, whether early intervention with targeted B-cell treatments, might be instituted after the detection of cryoglobulins, even if this is associated with non-specific symptoms, in an attempt to prevent overt vasculitis and/or lymphoma. Finally, the present study has clearly shown that pSS-CV and HCV-CV, are distinct entities, since pSS-CV patients present much more frequently with sicca manifestations, lymphadenopathy, arthritis and lymphoma, mainly in the context of type II cryoglobulinemia with an IgMκ mRF [28].

In accordance with previous reports [16,26,29], purpura is the most prevalent CV-specific manifestation of pSS-CV. This study, adds that purpura is also the most common CV-specific presenting

manifestation occurring in one-third of patients within the first year after pSS onset, thus explaining why purpura at pSS diagnosis is an excellent predictor for future lymphoma development [30]. Although the clinical expression of pSS-CV is spreading across time, this study showed a temporal clustering of CV and pSS onset in 60% of pSS-CV patients. In line with this, one third of pSS-CV will eventually develop a non-Hodgkin lymphoma of B cell origin within the first 5 years of pSS onset. Taken together, it appears that this subset of pSS patients possess a discrete B cell monoclonal population, producing an IgMκ monoclonal RF, very early during pSS disease course. Importantly, previous data point out that the monoclonal component is composed within the salivary glands [31,32], explaining why salivary gland enlargement in several reports is an independent predictor of future lymphoma development. The production and perpetuation of mRFs within the inflamed salivary glands are probably attributed to the formation of ectopic germinal center like structures (EGCS), which are more frequently found in pSS-CV patients compared to cryoglobulin negative controls. On the contrary, in HCV-related cryoglobulinemia [12], B-cell clonal expansion is mainly localized within the bone marrow and the liver [33]. Compared to HCV-CV, pSS-CV patients carry an increased risk of lymphoma that could be explained by the occurrence of MALT lymphomas unrelated to cryoglobulinemia [12] and by the more intense and persistent autoreactive B cell activation as attested by the higher frequency of lymphadenopathy, hypergammaglobulinemia and specific autoantibodies, observed in pSS-CV patients. On the other hand, HCV-CV patients had a remarkably increased peripheral nervous system involvement which could be attributed to 2 distinct underlying mechanisms: a) inflammation of the vasa nervorum not only due to cryoglobulins but also to anti-HCV/HCV immune complex deposition, and b) direct HCV mediated inflammation of the nerves [34–36].

Previous studies have clearly shown that cryoglobulinemia is associated with increased morbidity and mortality, serving also as one of the strongest laboratory predictors for future lymphoma development, either with or without CV [13,30,37]. The answer to the question whether cryoglobulinemia or CV serves better as lymphoma risk factor is still unaddressed. Indeed, previous studies, using small number of patients, showed that neither CV nor cryoglobulinemia were proven risk factors for lymphoma in the multivariate model, although CV was correlated with increased mortality [16]. In our dataset, we performed a data driven analysis to identify lymphoma associated features including both cryoglobulinemia and the presence of CV. Although, cryoglobulinemia was emerged as an independent feature, this finding is random, since in our specific dataset both cryoglobulinemia and CV possess the same power of significance, according to the FCBC algorithm and, eventually one of them, but not both could be selected as a potentially independent variable. The FCBF algorithm categorizes on a mathematical based manner, features and variables with minimal inter-correlation and therefore it was unexpected to pre-select both cryoglobulinemia and CV as potentially independent variables for the logistic regression (LR) model. Thus, it was highly unlikely to compare cryoglobulinemia and CV as independent variables for any type of LR model, since the first variable is a prerequisite for the second. Finally, to address this question, we analyzed concomitantly the group of the 44 pSS cryoglobulin positive patients without vasculitic tissue damage and compared it with the 71 pSS CV patients. Around half of the pSS-CV patients had NHL, while that was true only for one fourth of cryoglobulin positive CV negative pSS patients. In the latter group the determining factor for lymphoma development was the presence of mRF in the cryoprecipitate (87.5% of patients with lymphoma) i.e. type II cryoglobulinemia and not type III. In the pSS-CV patients where the prevalence of lymphoma was double, the presence of IgMκ mRF type II cryoglobulinemia was 97%. At this point, we feel it is important to emphasize that the capacity of serum cryoglobulins to precipitate in tissues may be affected by many factors: a) the quantity of circulating cryoglobulins as a net balance between production and clearance [38,39], b) the degree of the affinity-avidity of the monoclonal component with rheumatoid factor activity against the polyclonal IgG component [40], c) the physicochemical properties of cryoglobulins such as sialylation of the Fc portion [41] and d) several environmental factors including temperature, pH and the presence of plasma hyperfiltration conditions in specific tissues [40,42]. Thus, the cryoprecipitable IgMκ mRF, the common denominator between the two groups, operates as either a double or single sword edge in patients with pSS-CV and pSS with cryoglobulinemia only, regarding lymphoma development. In the second case, it represents only the B-cell clonal expansion, whilst in the first case it is the major element responsible for the generation of the complex disease of CV that can be seen as a composite index, born by nature. This can explain why in several previous studies, many items of CV served as predictive factors for future lymphoma development. Another issue that remains inadequately addressed in the literature, is the net effect of specific treatment modalities on lymphoma development. In this series of pSS-CV patients, the majority of those with severe vasculitic involvement were treated with corticosteroids, hydroxychloroquine, B cell depletion therapy (rituximab) and plasmapheresis. In very few cases cyclophosphamide was administered, while methotrexate (MTX) and azathioprine (AZA) were more commonly used in pSS-CV patients compared to cryoglobulin negative controls. pSS-CV patients without lymphoma have been also treated more frequently with MTX and AZA compared to those with lymphoma. Therefore, it seems that the additional risk from the excess use of MTX and AZA among pSS-CV patients compared to controls is low, suggesting that systemic immunosuppression confers low risk to lymphoma development.

In this work, we present our results with and without Bonferroni correction that represents one of the most widely used methods for p-values adjustment to avoid type I error in the context of multiple comparisons. However, it has been supported that Bonferroni correction is very stringent and augments the occurrence of type II error [43]. Therefore, many researchers including the authors of this manuscript, choose not to make any adjustments for multiple comparisons and present data on the original form [43], while part of the scientific community believes that Bonferroni correction is of limited use in biomedical science [44]. For all reasons mentioned above, we show the original and Bonferroni adjusted p-values, adopting in this way all scientific opinions. At the end, CV was proven a major risk factor for lymphoma development. The statistical significance of this element was not lost after Bonferroni correction, a finding that further strengthens our conclusions. On the other hand, the non-specific features of CV such as Raynaud's phenomenon and fatigue did not retain statistical significance after adjustment, implying that a type II error may have occurred.

The current study has some limitations. Even if the group of pSS-CV patients is the largest ever described, its number is still relatively small and therefore, a larger group would allow a better power analysis. While our study was amply powered, the resulting confidence intervals of effect estimates, support the necessity for the refinement of exact effect size estimates, in further prospective investigations. The fact that not all pSS patients included in the total study population have been evaluated for cryoglobulins, probably underestimates the real prevalence of both cryoglobulinemia and CV, pointing out a selection bias. However, the evaluated pSS patients represent more serious cases and therefore, our results can be generalized, as they reflect the real clinical practice. In addition, the inclusion of non-specific manifestations in the 2011 classification criteria of CV is an inner deficit, leading to overestimation of such manifestations in the context of pSS CV, since they can be attributed to either the CV or pSS itself. Such limitations, regarding not only the clinical phenotyping of CV in pSS but also the need for validation, are expected to be overcome through a large multicenter study population in the context of the HarmonicSS project. Another limitation is the heterogeneity in

terms of ethnicity, genetic background and environmental influence between Greek and Italian pSS patients who participated in the study. Finally, the FCBF/LR data driven analysis was applied on the dataset of pSS-CV and cryoglobulin negative patients, of whom 46 had lymphoma, a relatively small number that is anticipated to affect the sensitivity level of the model.

In summary, CV associated with pSS constitutes a specific clinical phenotype of pSS, associated with both an inflammatory component, clinically expressed as vasculitis and NHL development. The presence and type of cryoglobulins should be evaluated early and during follow up in every patient with pSS. In the majority of patients, CV is appeared early after pSS onset, with non-specific clinical manifestations or purpura. The major determining factor of CV is the IgMκ mRF of type II mixed cryoglobulinemia, offering the opportunity to use B-cell targeted treatments that have the potential to halt the progress of the disease. Finally, the clinical expression of pSS-CV, has certain differences compared to HCV related CV, reflecting biologic differences which may guide the physicians in the differential diagnosis and the proper therapeutic interventions.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.semarthrit.2020.07.013.

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