

Cryoglobulinemia in Systemic Lupus Erythematosus: Prevalence and Clinical Characteristics in a Series of 122 Patients

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Objectives: To determine the prevalence and nature of cryoglobulins in 122 patients with systemic lupus erythematosus (SLE) and identify the clinical and immunologic features related to their presence.

Methods: In a cross-sectional study, we investigated 122 consecutive patients (106 women and 16 men) with SLE who fulfilled the 1982 revised criteria of the American College of Rheumatology for the classification of SLE. All patients had documented medical histories and underwent a medical interview as well as a routine general physical examination by a qualified internist, and their clinical and serologic characteristics were collected on a protocol form. Serum samples were obtained at 37°C, and cryoglobulinemia was estimated by centrifugation at 4°C after incubation for 7 days in all patients. The type of cryoglobulinemia was identified by agarose gel electrophoresis and immunofixation.

Results: Cryoglobulins were detected in the sera of 31 SLE patients (25%); 20 patients (65%) had a cryocrit lower than 1%, 8 (26%) had percentages ranging between 1% and 5%, and only 3 patients (9%) had a cryocrit over 5%. Only cutaneous vasculitis (39% v 16%; $P = .01$) was more prevalent in patients with than in those without cryoglobulins. Rheumatoid factor (RF) (42% v 15%; $P =$

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.002) and low CH50 levels (84% v 49%; $P < .001$) were more prevalent in SLE patients with cryoglobulins. Hepatitis C virus (HCV) infection was investigated in 24 of the 31 cryoglobulinemic SLE patients and was detected in 5 (21%). In comparison, 4 (5%) of the 75 noncryoglobulinemic SLE patients studied were positive ($P = 0.035$; odds ratio, 4.67). Patients with a cryocrit greater than 1% showed a higher frequency of HCV infection than those with a cryocrit less than or equal to 1% (46% v 0%, $P = .01$).

Conclusions: Cutaneous vasculitis, RF, hypocomplementemia, and HCV infection were associated with cryoglobulins in SLE patients. Testing for HCV infection is therefore recommended for patients with SLE and cryoglobulinemia to identify this subset of patients for prognostic and therapeutic reasons.

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INDEX WORDS: Cryoglobulinemia; systemic lupus erythematosus; hepatitis C virus.

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is the most clinically and serologically diverse of the systemic autoimmune diseases because it may affect any organ of the body and display a broad spectrum of clinical and immunologic manifestations. The diversity of clinical features includes articular and mucocutaneous involvement, renal disease, hematologic abnormalities, and central nervous system disease (1). However, it is now thought that this condition can be divided into more homogeneous subsets of pathogenic, therapeutic, and prognostic significance (2-5).

Mixed cryoglobulins (MC) are cold-precipitable proteins containing immunoglobulin (Ig) G and anti-IgG rheumatoid factor (RF), usually an IgM of monoclonal (type II) or polyclonal (type III) origin (6). Cryoglobulins have been observed in a wide variety of diseases, including malignancies, infections, and systemic autoimmune diseases (7). These proteins are currently considered to be circulating immune complexes, because they contain antigens, antibodies, or other immunoreactants related to the pathogenesis of the disease they accompany (8). Although cryoglobulins have been described in some SLE patients with immune complex-mediated pathology, their prevalence, nature, and potential correlation with the clinical and serologic manifestations of SLE have been little studied (9).

The objectives of the current study were to determine the prevalence and nature of cryoglobulins in a large series of SLE patients and to

identify the clinical and immunologic features related to their presence.

PATIENTS AND METHODS

Patients

We studied 122 consecutive SLE patients (106 women and 16 men; mean age at onset, 31 years; range, 14 to 87 years) in our unit. All patients fulfilled the 1982 revised criteria of the American College of Rheumatology for the classification of SLE (10). All had documented medical histories and underwent a medical interview and a routine general physical examination by a qualified internist. Clinical and serologic characteristics of all these patients were collected on a protocol form.

Laboratory Studies

Blood samples were obtained and kept at 37°C for 30 minutes before separation. Serum was prepared by centrifuging at 37°C for 10 minutes at 2,500 rpm. Fresh centrifuged serum was incubated at 4°C for 7 days after collection and examined for cryoprecipitation. The cryocrit was obtained by centrifuging at 2,000 rpm (750g) for 30 minutes at 4°C. The cryoprecipitate was diluted in warm saline for 1 hour. Finally, dissolved cryoprecipitate was identified by agarose gel electrophoresis and immunofixation.

Other immunologic tests included antinuclear antibodies (ANA) (indirect immunofluorescence using mouse liver, kidney, and stomach as substrates), antibodies to double-stranded DNA (dsDNA) (Farr's technique), precipitating antibod-

ies to the extractable nuclear antigens Ro/SS-A and La/SS-B (ELISA) and RF (latex fixation and Waaler-Rose tests). Complement factors (C3 and C4) were estimated by nephelometry (Behring BNA nephelometer) and total hemolytic complement assay (CH50) by Lachmann's hemolytic technique (11). IgG and IgM anticardiolipin antibodies were measured by an ELISA technique as described by Gharavi et al (12) with minor modifications (13). The lupus anticoagulant (LA) was measured by coagulation assays following the recommendations of the LA subcommittee of the Scientific and Standardization Committee of the International Society of Thrombosis and Hemostasis (14).

Hepatitis C virus (HCV) antibodies were determined by a third generation ELISA (AxSYM System 3.0, Abbot Laboratories, IL). Positivity was confirmed by a third generation recombinant immunoblot assay (Chiron RIBA HCV 3.0 SIA; Ortho-Clinical Diagnostics, Chiron Corp, Emeryville, CA) and with detection of HCV-RNA by polymerase chain reaction (Amplicor HCV; Roche Diagnostic System, Nutley, NJ).

Statistical Analysis

We used conventional chi-square and Fisher's exact tests to analyze qualitative differences. For comparison of quantitative parameters, Student *t* test was used in large samples of similar variance, and the nonparametric Mann-Whitney *U* test for small samples. A value of $P < .05$ indicated statistical significance. The odds ratio (OR) was calculated to assess the risk of appearance of each variable, with a confidence interval (CI) of 95%. This statistical analysis was performed by the SPSS program (SPSS Inc, Chicago, IL) with the information stored in the database program.

RESULTS

Cryoglobulins were detected in the sera of 31 SLE patients (25%), of whom 25 were women and 6 were men. SLE onset occurred between the ages of 11 and 70 years (mean, 32 years) and at study initiation, the mean age of patients was 37 years (range, 11 to 74 years) and the mean duration of SLE was 63 months (range, 6 to 240 months). The most common clinical manifestations were arthritis in 27 patients (87%), fever in 15 (48%), cutaneous vasculitis in 12 (39%), malar rash in 12 (39%), photosensitivity in 11 (36%), serositis in 11 (36%),

and Raynaud's phenomenon in 10 (32%). Renal involvement was present in 11 patients (36%) and renal biopsies performed in 10 of these 11 cases showed diffuse proliferative nephritis (class IV) in 5 patients (50%), mesangial nephritis (class II) in 2 (20%), focal proliferative nephritis (class III) in 1 (10%), membranous nephritis (class V) in 1 (10%) and minimal disease (class I) in 1 (10%). Finally, the most frequent immunologic features were ANA in 31 patients (100%), anti-dsDNA in 28 (90%), hypocomplementemia in 26 (84%), RF in 13 (42%), and anti-Ro/SS-A in 7 patients (39%).

Most of the 31 SLE patients showed very low percentages of cryocrit: 20 patients (65%) had a cryocrit lower than 1%, 8 (26%) had percentages ranging from 1% to 5%, and only 3 patients (9%) had a cryocrit over 5%. Cryoglobulins were characterized when sufficient cryoprecipitate ($> 5\%$) was available. Thus, for the 3 patients with a cryocrit greater than 5%, their cryoprecipitates were separated by high-resolution agarose electrophoresis. Two cryoprecipitates were type II mixed cryoglobulins, containing monoclonal IgM κ with RF activity and polyclonal IgG. The electrophoresis of the remaining cryoprecipitate identified a monoclonal IgG λ and, at the time of the study, this patient did not have clinical or laboratory evidence of hematologic malignancy.

The clinical and immunologic characteristics of patients, divided according to the presence or absence of cryoglobulins, are shown in Tables 1 and 2. When the clinical features of SLE were analyzed according to the presence or absence of cryoglobulins, only cutaneous vasculitis (39% *v* 16%; $P = .01$; OR, 3.20; CI, 1.17 to 8.77) was more prevalent in patients with cryoglobulins. For immunologic features, RF (42% *v* 15%; $P = .002$; OR, 3.97; CI, 1.43 to 10.85) and low CH50 levels (84% *v* 49%; $P < .001$; OR, 5.32; CI, 1.79 to 19.06) also were more prevalent in SLE patients with cryoglobulins. HCV infection was detected in 5 (21%) of the 24 cryoglobulinemic SLE patients investigated (in the remaining 7 patients with SLE and cryoglobulinemia, HCV testing was not possible because these were lost to follow-up), compared with 4 (5%) of the 75 noncryoglobulinemic SLE patients ($P = .035$; OR, 4.67; CI, 0.9 to 25.49). In Table 3, we describe the clinical and immunologic SLE features of the 5 patients with SLE, HCV infection, and cryoglobulinemia. Four patients had arthritis, 3 serositis, 2 nephropathy, and 2 cutaneous SLE

Table 1: Clinical Manifestations of Systemic Lupus Erythematosus in Patients With and Without Cryoglobulinemia

Manifestations	SLE Patients With Cryoglobulinemia n = 31 (%)	SLE Patients Without Cryoglobulinemia n = 91 (%)	P Value
Malar rash	12 (39)	47 (52)	—
Discoid lesions	0 (0)	5 (6)	—
Subacute cutaneous lesions	4 (13)	7 (8)	—
Photosensitivity	11 (36)	41 (45)	—
Oral ulcers	8 (26)	28 (31)	—
Arthritis	27 (87)	74 (81)	—
Serositis	11 (36)	25 (28)	—
Nephropathy	11 (36)	30 (33)	—
WHO type I	1 (10)	4 (14)	—
WHO type II	2 (20)	3 (11)	—
WHO type III	1 (10)	5 (19)	—
WHO type IV	5 (50)	8 (30)	—
WHO type V	1 (10)	7 (26)	—
Neurologic involvement	6 (19)	11 (12)	—
Thrombocytopenia	5 (16)	21 (23)	—
Hemolytic anemia	5 (16)	7 (8)	—
Fever	15 (48)	37 (41)	—
Raynaud's phenomenon	10 (32)	20 (22)	—
Livedo reticularis	4 (13)	3 (3)	—
Thrombosis	2 (7)	13 (14)	—
Myositis	2 (7)	4 (4)	—
Lung involvement	0 (0)	5 (6)	—
Cutaneous vasculitis	12 (39)	15 (16)	.01
Sicca syndrome	7 (23)	22 (24)	—

Abbreviations: SLE, systemic lupus erythematosus; WHO, World Health Organization.

features. Hypocomplementemia and ANA were detected in all 5 patients, positive anti-dsDNA antibodies in 3, and RF in 2. No differences in the presentation of cryoglobulinemia were found in SLE patients with and without HCV infection.

Finally, we analyzed the main clinical and serologic features related to cryoglobulinemia in cryoglobulinemic SLE patients, according to the amount of cryocrit (less than or greater than 1%) (Table 4). SLE patients with a cryocrit greater than 1% showed a higher frequency of HCV infection than those with a cryocrit less than or equal to 1% (46% v 0%, $P = .01$).

DISCUSSION

A cryoglobulin is a serum protein or proteins that precipitates when serum is incubated at a temperature of less than 37°C. Cryoglobulins un-

dergo reversible precipitation at cold temperature. Although fibrinogen or C-reactive protein–albumin complexes may cryoprecipitate, this report describes only immunoglobulins (Ig). The cold-induced precipitation of serum proteins was first described in 1933 (15), and Lerner and Watson introduced the term *cryoglobulinemia* in 1947 (16). In 1966, Meltzer and Franklin (17) described the typical clinical symptoms associated with cryoglobulinemia, particularly the triad of purpura, arthralgia, and weakness. The existence of circulating cryoglobulins (cryoglobulinemia) is not always related to the presence of symptomatology, and we use the term *cryoglobulinemic syndrome* when patients with cryoglobulinemia have clinical manifestations. Since the initial report in 1990 of the association between MC and HCV infection, it has become clear that most of the so-called “essential”

Table 2: Immunologic Features of Systemic Lupus Erythematosus in Patients With and Without Cryoglobulinemia

Parameter	SLE Patients With Cryoglobulinemia n = 31 (%)	SLE Patients Without Cryoglobulinemia n = 91 (%)	P Value
ANA	31 (100)	90 (99)	—
High anti-dsDNA antibodies	28 (90)	80 (88)	—
Anti-Ro/SS-A antibodies	7 (23)	20 (22)	—
Anti-La/SS-B antibodies	3 (10)	5 (5)	—
Anti-U1-snRNP antibodies	3 (10)	14 (15)	—
Anti-Sm antibodies	2 (7)	12 (13)	—
Rheumatoid factor	13 (42)	14 (15)	.002
Low C3	14 (45)	31 (34)	—
Low C4	19 (61)	38 (42)	—
Low CH50	26 (84)	45 (49)	<.001
IgG aCL	2 (6)	15/85 (18)	—
IgM aCL	3 (10)	8/85 (9)	—
Lupus anticoagulant	4 (13)	16/85 (19)	—
HCV infection	5/24 (21)	4/75 (5)	.035

Abbreviations: aCL, anticardiolipin antibodies; ANA, antinuclear antibodies; dsDNA, double-stranded DNA; HCV, hepatitis C virus; Ig, immunoglobulin.

cryoglobulinemias are in fact associated with HCV infection. The discovery of the relationship between HCV infection and MC shows the striking association between a viral infection and an autoimmune disease and, moreover, a potential link between autoimmune and lymphoproliferative disorders.

Cryoglobulinemia has been studied in some systemic autoimmune diseases, such as rheumatoid arthritis (18,19), systemic sclerosis (20), polyarthritis nodosa (21) and, especially, in Sjögren's

syndrome (SS) (22). Recently, we reported that the cryoglobulinemia observed in some cases of "primary" SS was due to HCV infection (23). The clinical significance of cryoglobulinemia in SLE has been little studied, and its prevalence has ranged from 16% to 83% in small series of patients (24-30) (Table 5). The cryoproteins found in SLE contain immunoglobulins, mostly IgG and IgM, and complement components (24,26,31,32). Many studies have been performed with the aim of de-

Table 3: Clinical and Immunologic Features of the 5 Patients With SLE, HCV Infection, and Cryoglobulinemia

Patient	Sex	Age (yr)	Clinical Features	Hematologic Features	Immunologic Features
1	F	45	Arthritis, serositis	Leukop, lymph	ANA, RF, hypocompl
2	F	32	Malar rash, arthritis, nephropathy	Lymph	ANA, dsDNA, hypocompl
3	F	69	Malar rash, photosensitivity, arthritis	—	ANA, dsDNA, hypocompl
4	M	57	Arthritis, serositis, nephropathy, purpura	Leukop, lymph	ANA, RF, hypocompl
5	M	31	Serositis	Leukop, lymph	ANA, dsDNA, hypocompl

Abbreviations: ANA, antinuclear antibodies; dsDNA, anti-double-stranded DNA antibodies; F, female; hypocompl, hypocomplementemia; leukop, leukopenia; lymph, lymphopenia; M, male; RF, rheumatoid factor.

Table 4: Prevalence of the Main Clinical and Serologic Features Related to Cryoglobulinemia in Systemic Lupus Erythematosus Patients According to Percentage of Cryocrit

	Cryocrit < 1% n = 20 (%)	Cryocrit > 1% n = 11 (%)	P Value
Nephropathy	6 (30)	5 (46)	—
Cutaneous vasculitis	10 (50)	2 (18)	—
Hypocomplementemia	16 (80)	10 (91)	—
RF	6 (30)	7 (64)	—
HCV infection	0/13 (0)	5 (46)	.01

Abbreviation: HCV, hepatitis C virus; RF, rheumatoid factor.

fining the antibody specificity and the presumptive antigens in the cryoglobulins. The most frequently found autoantibodies in cryoprecipitates of patients with SLE were anti-dsDNA, anti-single-stranded anti-DNA and, rarely, antiribonucleoprotein. These autoantibodies are more concentrated in cryoprecipitates than in serum and are correlated with the autoantibodies found in the elution of glomeruli of patients with lupus nephritis (33). In addition, we found a type I cryoglobulinemia in one of our SLE patients. Usually, patients with SLE show type II or type III cryoglobulinemia (26,28), although type I cryoglobulinemia has been described infrequently (26). One patient with SLE and type I cryoglobulinemia was diagnosed with multiple myeloma (34). In light of this report, lymphoproliferative malignancies should be considered in patients with SLE and cryoglobulinemia.

In this study, we found a prevalence of cryoglobulins of 25% in a large series of SLE patients, most of whom showed very low amounts of circulating cryoglobulins (cryocrit < 1%). Similarly,

Gripenberg et al (35) also found low levels of cryoglobulins (< 0.05 g/L) in 81% of the SLE patients analyzed. Additionally, some studies have found an association between the amount of cryoprecipitates and the disease activity in SLE (24,36). We found no correlation between clinical SLE features and cryoglobulinemia in our SLE patients, but, interestingly, we found that a cryocrit greater than 1% was more frequent in those SLE patients with HCV infection.

We also analyzed the clinical SLE features according to the presence or absence of cryoglobulinemia and found a higher prevalence of cutaneous vasculitis in cryoglobulinemic SLE patients. In previous studies, cryoglobulinemia has been related to disease activity and severity (particularly, nephritis) (28,33,37), although other researchers found no significant differences (35). We found no significant increase of nephropathy in our SLE cryoglobulinemic patients, and no differences were found in the comparison of nephritis types according to the World Health Organization classifica-

Table 5: Prevalence of Cryoglobulinemia in Patients With SLE: Reported Series

Author	Year	SLE Patients	Cryoglobulins (%)
Christian et al (24)	1963	12	10 (83)
Lee et al (25)	1964	57	9 (16)
Statsny et al (26)	1969	31	11 (35)
Barnett et al (27)	1970	18	16 (89)
García-Bragado et al (28)	1980	16	3 (19)
Ortega-González et al (29)	1984	36	12 (33)
Sikander et al (30)	1989	22	15 (68)
Present series	2000	122	31 (25)
Total		314	107 (34)

Abbreviation: SLE, systemic lupus erythematosus.

tion. The presence of cryoglobulinemia appeared to have no influence in the renal involvement of our SLE patients.

Furthermore, we found a higher frequency of some immunologic markers in cryoglobulinemic SLE patients (RF and hypocomplementemia). The association between hypocomplementemia and cryoglobulins is well known. Adu and Williams (38) showed the ability of SLE cryoglobulins to activate complement in vitro, suggesting that these immune complexes can activate complement in vivo and thus may contribute to tissue damage in this disease. Roberts et al (39) suggested that, in SLE patients with diffuse proliferative glomerulonephritis, immune-bound antibodies in cryoglobulins and glomerular immune deposits appear to activate complement via the classic and alternative pathways. Interestingly, we found a higher frequency of RF in cryoglobulinemic SLE patients, probably related to the RF activity of some cryoglobulinemic component. RF might be an immunologic marker that suggests the existence of cryoglobulinemic in SLE patients.

Finally, we analyzed the possible role of HCV in the cryoglobulinemia of our SLE patients. The last study of this subject was performed before the development of a test for the detection of anti-HCV antibodies in 1989. Given the strong associ-

ation between mixed cryoglobulinemia and HCV infection (40-43), the cryoglobulinemia observed in some SLE patients might be associated with HCV infection. In this study, 21% percent of our SLE patients with cryoglobulinemia had HCV infection compared with 5% without cryoglobulinemia, suggesting that cryoglobulinemia in SLE is associated with HCV infection in some cases. Patients with cryoglobulinemic syndrome may show several features commonly observed in SLE, such as arthritis, nephropathy, or hypocomplementemia. In these patients, cryoglobulinemia related to chronic HCV infection (as may have happened in patients 1 and 4 of Table 3) may produce a "lupus-like syndrome", mimicking "true" SLE according to the 1982 revised criteria for SLE classification.

In summary, we found that cutaneous vasculitis, RF, hypocomplementemia, and HCV infection were associated with the presence of cryoglobulins in SLE patients. These features may identify a subset of SLE patients with cryoglobulinemia. Additionally, the cryoglobulinemia observed in some cases of SLE may be due to HCV infection. Testing for HCV infection is therefore recommended for patients with SLE and cryoglobulinemia, to identify those patients who may need to be managed differently.

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