Cryoglobulinemia in Systemic Lupus Erythematosus: A Retrospective Study of 213 Patients

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Abstract

Objectives

The clinical value of cryoglobulinemia (CG) in systemic lupus erythematosus (SLE) is largely unknown. The aim of this retrospective study was to describe the characteristics of CG in SLE, its impact on SLE phenotype, and the features associated with cryoglobulinemic vasculitis (CryoVas) in SLE patients.

Methods

This retrospective study conducted in a French university hospital, reviewed the data from 213 SLE patients having been screened for CG between January, 2013 and December, 2017. SLE patients positive for CG were compared to SLE patients without CG. Patients were classified as CryoVas using the criteria of De Vita *et al*.

Results

Of the 213 SLE patients included (mean age 28 years, female sex 85%), 142 (66%) had at least one positive CG in their history, 67% of them having a persistent CG at follow-up. CG was type III in 114 (80%) cases and type II in 27 (19%) cases. The mean concentration of the cryoprecipitate was 40mg/L (range 0-228). Patients with CG had significantly more complement consumption for C3, C4, and CH50 and received more corticosteroids ($p = 0.002$) and rituximab ($p = 0.02$) during follow-up. Among patients with CG, 21 (15%) developed a CryoVas. These patients had a higher SLEDAI at SLE diagnosis than CG + patients without CryoVas (17 vs. 13.6, respectively, $p = 0.03$). The clinical involvement of patients with CryoVas was mainly cutaneous (purpura, ulcers, digital ischemia) and articular, without any death at follow-up. Severe manifestations of CG included glomerulonephritis in 1/21 (5%) patient and central nervous system involvement in 4/21 (19%) patients. A response to first line treatments was observed in 12/13 (92%) patients; but relapses were observed for 3 of them.

Conclusion

CG is frequent in SLE, but mostly asymptomatic. CryoVas features involve mostly joints, skin, and general symptoms. CryoVas in SLE appears to be a specific condition, with a low prevalence of neuropathy, membranoproliferative glomerulonephritis, and severe involvement.

Highlights

- Cryoglobulinemia is frequent in SLE; but mostly asymptomatic.

- 66% of SLE patients were tested positive for cryoglobulins, and 15% of the SLE patients with cryoglobulinemia developed a cryoglobulinemic vasculitis.
- Features of the cryoglobulinemic vasculitis mainly involved skin, joints, and general signs. Severe manifestations of vasculitis were rare.

Introduction

Systemic lupus erythematosus (SLE) is one of the most frequent connective tissue diseases and affects predominantly young women [1, 2]. Its clinical presentation is heterogeneous and can involve many organs such as skin, joints, kidney, central nervous system, or blood cells. The SLE biological autoimmune disorders include the presence of antinuclear antibodies (ANA) in almost all patients and double-stranded DNA (dsDNA) antibodies in two thirds of patients, usually associated with low complement levels [3]. Other immunological features include cryoglobulin in 19–83% of patients [4–12].

Cryoglobulins are immunoglobulins characterized by reversible precipitation at a low temperature. The main clinical consequences of cryoglobulinemia (CG) are due to small vessel vasculitis inducing cutaneous, renal, articular, and neurological involvements [13]. CG is associated with B cell lymphoproliferative diseases, chronic infections (mainly hepatitis C virus (HCV)), and autoimmune diseases. In 1974, Brouet *et al*. proposed a classification of CG according to its monoclonal (type I) and/or polyclonal (type II and III) composition [14]. SLE is one of the most frequent autoimmune disease associated with CG along with Sjogren's syndrome [15].

The data in the literature concerning the phenotype of SLE patients with CG is scarce and not up-to-date, and the clinical impact of CG on SLE remains poorly described [10, 16]. The few studies published are limited in terms of external validity (out-of-date), inclusion of HCV patients [16], and sample size [10].

This retrospective cohort study aimed to describe the clinical and biological phenotype of SLE patients positive for CG, including patients with a cryoglobulinemic vasculitis (CryoVas).

Material And Methods

Patient selection and study design

A retrospective study was conducted in a tertiary care center. The data of all patients who had ANA ≥ 1/160 and at least one positive antibody among dsDNA antibodies, anti-Sm antibodies, or anti-SSA antibodies, between January 1, 2013 and December 31, 2017, were retrospectively analyzed. Patients without CG research or patients positive for HCV were excluded. Medical charts were reviewed to assess the diagnosis of SLE according to the Systemic Lupus International Collaborating Clinics (SLICC) criteria [17] and/or the European League Against Rheumatism (EULAR) 2019 criteria [18].

The SLE patients were classified according to their CG status. The first group included SLE patients with the presence of CG reported at least once in their history (SLE CG+). The second group included SLE

patients with negative CG reported in their history (SLE CG-). The clinical and biological data of the included patients were considered until June 30, 2020.

The database was reported to the national data protection agency (*Commission Nationale de l'Informatique et des Libertés*, CNIL) and the study was approved by the ethics committee of the *Hospices Civils de Lyon* (validation number 21_5338). Patients were informed and could express their refusal to participate in the study.

Clinical variables

Data for demographic, clinical, and biological items were collected by use of standardized forms. The date of SLE diagnosis was registered for each patient. Clinical impairments of SLE during the medical history were collected. Specific treatments including steroids, immunosuppressive, immunomodulatory, and biologic therapies were investigated as well as their medical indication (SLE or CryoVas). SLE disease activity was assessed retrospectively using the SLE Disease Activity Index (SLEDAI) [19] at SLE diagnosis, at the date of first positive CG, and at the date of an eventual CryoVas. A flare was defined by an SLEDAI > 4. Clinical data potentially linked to CG at the time of the first positive CG were collected. The dates of first positive CG and CryoVas were registered. Patients were classified as having CryoVas using the 2011 criteria of De Vita *et al* [20], involving clinical and laboratory items. Clinical impairments of the CryoVas were collected. Patients were classified as having central nervous system involvements in case of cerebral vasculitis, myelitis, or meningoencephalitis attributed to the CryoVas. If a biopsy of skin, kidney or peripheral nervous system was performed, a histology compatible with CryoVas was searched for. Deaths of any cause and loss to follow-up were registered. Cardiovascular events (acute coronary syndrome, stroke) and severe infections (intravenous antimicrobial treatment or hospitalization for infection) were collected as well as their subtypes.

Laboratory parameters

Cryoglobulin tests, purification, and characterization were performed in the immunology laboratory of the *Hospices Civils de Lyon* according to the local protocol previously published [21]. Blood samples were collected by venipuncture for cryoglobulin testing and complement exploration. Samples were then rapidly sent to the laboratory at 37°C and maintained in an incubator at 37°C to clot for a minimum of 2 hours. Samples were centrifuged and serum was preserved at 4°C for 7 days. Visual observation at day 7 allowed the detection of any cryoprecipitate. In that case, cryoprecipitates were isolated by + 4°C centrifugation (2200g, 15 min) and purified by 3 washes with cold phosphate buffered saline (PBS, pH 7.4, + 4°C) to remove serum and proteins which had not precipitated. Pellets were then dissolved at 37°C in 500 µL PBS and conserved at 37°C for further analysis. Characterization of the cryoprecipitate was performed by electrophoresis-immunofixation to type cryoglobulins with anti-γ, anti-α, anti-µ, anti-λ and anti-κ antisera (SAS-3®, Helena Bioscience, Gateshead, UK). In the dissolved cryoprecipitate conserved at 37°C, IgG, IgM, and/or IgA concentrations as well as Rheumatoid Factor (RF) activity (anti-IgG IgM) were assayed by immunonephelemetry (BNprospec®, Siemens, Marburg, Germany). Serum RF (normal < 20UI/mL), complement C3 (normal range 0.8–1.58 g/L) and C4 (normal range 0.16–0.39 g/L) were

quantified by immunonephelometry (BNprospec®), and complement functional activity (CH50) was quantified on Optilite® (The Binding Site, Birmingham, UK; normal range 41–95 U/mL).

Anti-dsDNA antibodies were determined using a radioimmunological test (Farr test anti-dsDNA, Trinity Biotech, Wicklow, Ireland) with a cut-off at 10 IU/mL. ANA (indirect immunofluorescence on HEp2 cells, Biorad®, Hercules, CA, USA), complement, anti-dsDNA test, gammaglobulins, and RF levels were assessed at the time of the first positive CG or 3 months before or after the first research for CG if no CG was documented. The other autoimmune parameters (anti-RNP, SSA, Sm, CCP, antiphospholipid antibodies) were registered as positive if persistent across the medical history of the patient and if the levels were superior to the laboratory's reference ranges. Antiphospholipid antibodies were registered as positive if persistent at least 12 weeks later, following Sapporo criteria for antiphospholipid syndrome [22].

The viral status against hepatitis B virus (HBV), HCV, and Human Immunodeficiency Virus (HIV) was also registered. HBV status was considered positive if HbS antigen was present, and HCV status was considered positive if anti-HCV antibodies were present, regardless of the viral load status. The other laboratory parameters (urine sediment, creatinine levels, blood cell counts, etc…) were determined using the routine procedures of the *Hospices Civils de Lyon* laboratory. Lymphopenia was considered if total lymphocytes were inferior to 1 G/L, leukopenia if white cell counts were inferior to 4 G/L, and thrombocytopenia if platelets were inferior to 100.10^9/L. These parameters had to be present at least twice and persistent at a minimum of 12 weeks during the medical history to be registered.

Statistical analysis

Baseline characteristics were assessed using descriptive statistics. Continuous variables were described by their means and standard deviations and compared using t-tests. Categorical variables were described as numbers and percentages and compared with χ^2 or Fisher's exact test. For all statistical analyses, $p < 0.05$ was considered significant. Analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria)

Results

Studied population

Amongst ANA positive patients, a sample of 224 patients with SLE was identified. Three patients were excluded because of HCV infection and 8 were excluded due to the absence of CG testing in the medical file. After reviewing medical charts, 213 patients with SLE were included in the study, 85% of them being women. The mean age at SLE diagnosis was 29.2 (± 12.8) years. The mean duration of follow-up was 13.2 (± 8.9) years. CG was positive at least once in 142/213 patients (66%; Table 1). Twenty-one (10%) patients were lost to follow-up before June 30, 2020 (12 from the SLE CG + group and 8 from the SLE CG- group). Two patients died (one in each group).

Table 1
– SLE clinical involvements according to the presence of cryoglobulinemia

	SLE CG+ (n = 142)	SLE CG- (n = 71)	p-value
Female sex, n (%)	124 (87%)	56 (79%)	0.11
SLE diagnosis			
Age at SLE diagnosis, (years), mean (± SD)	29.1 ± 12.3	28.1 ± 13.7	0.15
SLEDAI score at SLE diagnosis, mean (± SD)	14.2 ± 7.5	12.5 ± 7.5	0.051
SLE clinical manifestations			
Acute cutaneous lupus, n (%)	69 (49%)	34 (48%)	0.92
Subacute cutaneous lupus, n (%)	41 (29%)	13 (18%)	0.10
Chronic cutaneous lupus, n (%)	26 (18%)	11 (15%)	0.61
Oral ulcers, n (%)	29 (20%)	13 (18%)	0.72
Alopecia, n (%)	36 (25%)	15 (21%)	0.50
Cutaneous vasculitis unrelated to CG, n (%)	1 (1%)	8 (11%)	0.0008
Raynaud's phenomenon, n (%)	70 (49%)	27 (38%)	0.12
Joints, n (%)	132 (93%)	65 (92%)	0.71
Pericarditis, n (%)	34 (24%)	16 (23%)	0.82
Myocarditis, n (%)	7 (5%)	3 (4%)	1.00
Pleuritis, n (%)	19 (13%)	11 (15%)	0.68
Cardiac valvulopathy, n (%)	9 (6%)	2 (3%)	0.85
Intra-alveolar hemorrhage, n (%)	2 (1%)	3 (4%)	0.62
Pulmonary hypertension, n (%)	2 (1%)	3 (4%)	0.84
Nephritis, n (%)	64 (45%)	27 (38%)	0.33
Peripheral nervous system, n (%)	4 (3%)	3 (4%)	1.00
Central nervous system, n (%)	17 (12%)	11 (15%)	0.47
Psychosis, n (%)	27 (19%)	13 (18%)	0.91
Associated autoimmune disease			
Antiphospholipid syndrome, n (%)	37 (26%)	11 (15%)	0.08

SLE: systemic lupus erythematosus, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia

	SLE CG+ (n = 142)	SLE CG- (n = 71)	p-value
Sjogren's syndrome, n (%)	28 (20%)	11 (15%)	0.45
Other connective tissue disease, n (%)	14 (10%)	2 (3%)	0.07

SLE: systemic lupus erythematosus, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia

Immunological characteristics of the cryoglobulinemia

The CG was mostly type III (114/142, 80%), and 73/142 (51%) patients with type III CG had polyclonal IgG and polyclonal IgM. Type II cryoglobulinemia (27/142, 19%) consisted of monoclonal IgMκ and polyclonal IgG or IgM in 15/142 (11%) patients. The mean total Ig concentration of the CG was 40mg/L (range 0-228; Table 2), the mean Ig concentration being 44mg/L (range 8-228) for type II and 39mg/L (range 0-111) for type III. RF activity in the cryoprecipitate was negative for 125/142 (88%) patients. Six (4%) of the 142 patients had a positive RF in the serum and 3 (2%) had a positive RF in the cryoprecipitate (Table 2). For 14 patients, the CG isotype was not available because of old data. For the specific CryoVas group, the mean total concentration of the cryoprecipitate was 31.1mg/L (range 8.6–81.8) compared to 41.2mg/L (range 6.1–228) in the SLE CG + without CryoVas group (p = 0.6). The proportion of CG type II and CG type III was not significantly different between the CryoVas group (19% and 81%, respectively) and the SLE CG + without CryoVas group (19% and 80%, respectively; Table 5).

Table 2
– Immunological characteristics of the cryoglobulin in SLE patients

	SLE CG+ (n = 142)
Type I cryoglobulinemia, n (%)	1 (1%)
Type II cryoglobulinemia, n (%)	27 (19%)
Type III cryoglobulinemia, n (%)	114 (80%)
Ig concentration in cryoprecipitate (mg/L)	
Total, mean (range)	40 (0-228)
IgG, mean (range)	21 (0-107)
IgA, mean (range)	1 (0–27)
IgM, mean (range)	19 (0-228)
Type I cryoglobulinemia	
Monoclonal IgMκ, n (%)	1 (1%)
Type II cryoglobulinemia isotypes	
Monoclonal IgMκ + polyclonal IgG/IgM, n (%)	15 (11%)
Monoclonal IgGκ + polyclonal IgG/IgM, n (%)	4 (3%)
Monoclonal IgMλ + polyclonal IgG/IgM, n (%)	4 (3%)
Monoclonal IgGλ + polyclonal IgG/IgM, n (%)	2 (1%)
Type III cryoglobulinemia isotypes	
Polyclonal IgG + polyclonal IgM, n (%)	73 (51%)
Polyclonal IgG, n (%)	22 (15%)
Polyclonal IgG + polyclonal IgM + polyclonal IgA, n (%)	3 (2%)
Polyclonal IgM, n (%)	1 (1%)
RF activity	
Negative RF in cryoprecipitate, n (%)	125 (88%)
Positive RF in cryoprecipitate, n (%)	3 (2%)
Ig: immunoglobulins, RF: rheumatoid factor	

Within the SLE CG + group, 96/142 (67%) patients had a persistent CG, 32/142 (23%) patients had a transient CG, and 14/142 (10%) had a positive CG without additional confirmatory testing.

SLE manifestations according to the presence of cryoglobulinemia

There was no significant difference between the SLE CG + and SLE CG- groups concerning nephritis, arthritis, dermatitis, and Raynaud's phenomenon. There was a trend towards an increase in SLEDAI score at SLE diagnosis in the SLE CG + group compared to SLE CG-(14.2 versus 12.5, respectively, p = 0.051, Table 1).

The frequency of associated autoimmune diseases was not significantly different between SLE CG + and SLE CG- groups: the most frequent were antiphospholipid syndrome (26% and 15%, respectively, p = 0.08) and Sjogren's syndrome (20% and 15%, respectively, p = 0.45, Table 1). Two SLE CG + patients had a chronic HBV infection (Table 3). Regarding the other CG underlying conditions, five patients had a lymphoid hemopathy, one had a myelodysplastic syndrome, and eleven presented other autoimmune diseases.

Table 3
– SLE biological features in patients according to the presence of a cryoglobulin

	SLE CG+ (n = 142)	SLE CG- (n = 71)	p-value
Immunology			
RF in serum positive, n (%)	6 (4%)	5 (7%)	0.51
RF in serum, UI/L, mean (± SD)	119 ± 100	107 ± 110	0.69
Hypogammaglobulinemia, n (%)	6 (4%)	3 (4%)	1.00
Hypergammaglobulinemia, n (%)	87 (61%)	34 (48%)	0.06
Gammaglobulins, g/L, mean (± SD)	16.2 ± 6.0	16.8 ± 7.0	0.89
Decreased complement C3, n (%)	96 (68%)	32 (45%)	0.002
Decreased complement C4, n (%)	107 (75%)	33 (46%)	0.00003
Decreased CH50, n (%)	91 (64%)	28 (39%)	0.0006
Farr test, UI/L, mean (± SD)	72.2 ± 88.0	42.7 ± 51.2	0.07
Anti-SSA antibodies, n (%)	72 (51%)	32 (45%)	0.44
Anti-Sm antibodies, n (%)	55 (39%)	22 (31%)	0.27
Anti-RNP antibodies, n (%)	65 (46%)	27 (38%)	0.28
Infections			
HBV infection, n (%)	2 (1%)	0 (0%)	0.90
HIV infection, n (%)	0 (0%)	0 (0%)	1.00
Cytopenia			
Leukopenia, n (%)	36 (25%)	22 (31%)	0.38
Lymphopenia, n (%)	89 (63%)	40 (56%)	0.37
Thrombopenia, n (%)	36 (25%)	18 (25%)	1.00
Autoimmune hemolytic anemia, n (%)	23 (16%)	9 (13%)	0.50
RF: rheumatoid factor, HBV: hepatitis B virus, HIV: human immunodeficiency virus			

In the SLE-CG + compared to the SLE CG-,group, there was a significantly higher frequency of decreased C3 (68% versus 45%, respectively, p = 0.002), decreased C4 (75% versus 46%, respectively, p = 0.00003), and low CH50 (64% versus 39%, respectively, p = 0.0006). In the SLE CG + group, there was also a trend towards more hypergammaglobulinemia (61% versus 48%, p = 0.063) and anti-dsDNA antibodies (72.2 UI/L versus 42.7 UI/L, p = 0.07; Table 3).

Clinical signs related to cryoglobulinemia

In the SLE CG + group, the first positive CG was found at a mean of 6.4 years after the diagnosis of SLE. In the SLE CG + group, the most observed clinical signs potentially related to CG at CG diagnosis were general symptoms, Raynaud’s phenomenon, and articular disorders. The specific signs of vasculitis in the SLE CG + group were mainly cutaneous manifestations, including purpura, ulcers, digital ischemia, and livedo. Severe manifestations included ischemic stroke in 3/142 (2%) patients, kidney failure in 12/142 (8%) patients, and central nervous system involvement in 9/142 (9%) patients (Table 4).

Table 4
– Clinical signs related to cryoglobulinemia

	SLE CG+ (n = 142)
Cryoglobulinemia diagnosis	
Years between SLE diagnosis and first CG, mean (± SD)	6.4 ± 7.4
Years between SLE diagnosis and first clinical signs linked to CG, mean (± SD)	6.6 ± 9.7
SLEDAI score at SLE diagnosis, mean (± SD)	14.2 ± 7.5
SLEDAI score at CG diagnosis, mean (± SD)	14.9 ± 8.9
Clinical signs at the time of the first positive CG	
General symptoms, n (%)	54 (38%)
Myalgia, n (%)	31 (22%)
Purpura, n (%)	9 (6%)
Raynaud's phenomenon, n (%)	48 (34%)
Acrocyanosis, n (%)	15 (11%)
Digital ischemia, n (%)	9 (6%)
Necrosis, n (%)	9 (6%)
Ulcers, n (%)	16 (11%)
Livedo, n (%)	23 (16%)
Arthralgia, n (%)	87 (21%)
Peripheral nervous system, n (%)	3 (2%)
Central nervous system, n (%)	9 (6%)
Stroke, n (%)	3 (2%)
Proteinuria > 1g/24h, n (%)	22 (15%)
Proteinuria > 3 g/24h, n (%)	16 (11%)
Hematuria, n (%)	33 (23%)
Kidney failure, n (%)	12 (8%)
Gastro-intestinal involvement, n (%)	3 (2%)
CG: cryoglobulinemia, SLEDAI: systemic lupus erythematosus activity index	

Table 5
– Characteristics of SLE patients with cryoglobulinemia according to CryoVas status

	SLE CG + with CryoVas (n = 21)	SLE CG + without CryoVas (n = 121)	p-value
Female sex, n (%)	16 (76%)	108 (89%)	0.15
SLE and CG diagnosis			
Age at lupus diagnosis, (years), mean (± SD)	28.0 ± 14.1	30.0 ± 12.0	0.54
Years between SLE diagnosis and first positive CG, mean (± SD)	7.6 ± 9.1	5.9 ± 7.1	< 0.0001
Years between SLE and CryoVas diagnosis, mean (± SD)	8.5 ± 9.7	NA	
SLEDAI score at SLE diagnosis, mean (± SD)	17.0 ± 7.5	13.6 ± 7.4	0.03
SLEDAI score at CG diagnosis, mean (± SD)	17.9 ± 9.0	14.3 ± 8.9	0.08
SLEDAI score at CryoVas diagnosis, mean (± SD)	23.7 ± 9.9	NA	
Associated autoimmune disorder			
Antiphospholipid syndrome, n (%)	7 (33%)	30 (25%)	0.42
Sjogren's syndrome, n (%)	4 (19%)	24 (20%)	1.00
SLE clinical involvements			
Acute cutaneous lupus, n (%)	13 (62%)	56 (46%)	0.19
Subacute cutaneous lupus, n (%)	10 (48%)	31 (26%)	0.04
Chronic cutaneous lupus, n (%)	5 (24%)	21 (17%)	0.54
Oral ulcerations, n (%)	5 (24%)	24 (20%)	0.54
Alopecia, n (%)	9 (43%)	27 (22%)	0.046
Raynaud's phenomenon, n (%)	15 (71%)	55 (45%)	0.03
Joints, n (%)	21 (100%)	111 (92%)	0.36
Pericarditis, n (%)	11 (52%)	23 (19%)	< 0.0001
Myocarditis, n (%)	2 (10%)	5 (4%)	0.28

RF: rheumatoid factor, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia, CryoVas: cryoglobulinemic vasculitis, IS: immunosuppressive, NA: not applicable, TNF: tumor necrosis factor

	SLE CG + with CryoVas (n = 21)	SLE CG + without CryoVas (n = 121)	p-value
Pleuritis, n (%)	2 (10%)	17 (14%)	0.74
Valvulopathy, n (%)	3 (14%)	6 (5%)	0.13
Intra-alveolar hemorrhage, n (%)	1 (5%)	1 (1%)	0.27
Pulmonary arterial hypertension, n (%)	0 (0%)	1 (1%)	1.00
Nephritis, n (%)	9 (43%)	55 (45%)	0.97
Peripheral nervous system, n (%)	2 (10%)	2 (2%)	0.08
Central nervous system, n (%)	3 (14%)	14 (12%)	0.72
Psychosis, n (%)	5 (24%)	22 (18%)	0.55
Leukopenia, n (%)	6 (29%)	30 (25%)	0.71
Lymphopenia, n (%)	15 (71%)	74 (61%)	0.37
Thrombopenia, n (%)	6 (29%)	30 (25%)	0.71
Autoimmune hemolytic anemia, n (%)	3 (14%)	20 (17%)	1.00
Immunology			
RF in serum positive, n (%)	1 (5%)	5 (4%)	1.00
RF in serum, UI/L, mean (± SD)	25 ± 4.2	137 ± 100	0,02
RF in cryoglobulin positive, n (%)	0 (0%)	3 (2%)	1.00
Hypogammaglobulinemia, n (%)	0 (0%)	6 (5%)	0.59
Hypergammaglobulinemia, n (%)	16 (76%)	71 (59%)	0.13
Gammaglobulins, g/L, mean (± SD)	17.4 ± 4.0	15.9 ± 5.9	0.17
Decreased complement C3, n (%)	17 (81%)	79 (65%)	0.16
Decreased complement C4, n (%)	19 (90%)	88 (73%)	0.08
Decreased CH50, n (%)	17 (81%)	74 (61%)	0.08
Farr test, UI/L, mean (± SD)	86.4 ± 82.1	69.9 ± 86.3	0.48
Total concentration of cryoprecipitate, mg/L, mean (range)	31.1 (8.6–81.8)	41.2 (6.1–228)	0.06

RF: rheumatoid factor, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia, CryoVas: cryoglobulinemic vasculitis, IS: immunosuppressive, NA: not applicable, TNF: tumor necrosis factor

	SLE CG + with CryoVas (n = 21)	SLE CG + without CryoVas (n = 121)	p-value
Type I cryoglobulinemia, n (%)	0 (0%)	1 (1%)	
Type II cryoglobulinemia, n (%)	4 (19%)	23 (19%)	1.00
Monoclonal IgMκ + polyclonal IgG, n (%)	0 (0%)	14 (12%)	0.13
Type III cryoglobulinemia, n (%)	17 (81%)	97 (80%)	1.00
Polyclonal IgG + polyclonal IgM, n (%)	12 (57%)	61 (50%)	0.43
Polyclonal IgG, n (%)	4 (19%)	18 (15%)	0.74
Cryoglobulinemia clinical signs			
General symptoms, n (%)	13 (62%)	41 (34%)	0.02
Myalgia, n (%)	10 (48%)	21 (17%)	0.004
Purpura, n (%)	9 (43%)	0 (0%)	< 0.0001
Raynaud phenomenon, n (%)	11 (52%)	37 (31%)	0.055
Acrocyanosis, n (%)	4 (19%)	11 (9%)	0.24
Digital ischemia, n (%)	6 (29%)	3 (2%)	0.02
Necrosis, n (%)	8 (38%)	1 (1%)	< 0.0001
Ulcers, n (%)	8 (38%)	8 (7%)	0.0004
Livedo, n (%)	7 (33%)	16 (13%)	0.047
Joints, n (%)	18(86%)	69 (57%)	0.01
Peripheral nervous system, n (%)	2 (10%)	1 (1%)	0.057
Central nervous system, n (%)	4 (19%)	5 (4%)	0.03
Proteinuria > 1g/24h, n (%)	4 (19%)	18 (15%)	0.74
Proteinuria > 3 g/24h, n (%)	2 (10%)	14 (12%)	1.00
Hematuria, n (%)	3 (14%)	30 (25%)	0.41
Kidney failure, n (%)	1 (5%)	11 (9%)	1.00

RF: rheumatoid factor, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia, CryoVas: cryoglobulinemic vasculitis, IS: immunosuppressive, NA: not applicable, TNF: tumor necrosis factor

	SLE CG + with CryoVas (n = 21)	SLE CG + without CryoVas (n = 121)	p-value
Gastro-intestinal involvement, n (%)	1 (5%)	0 (0%)	
IS and immunomodulatory treatments			
Number of IS treatments, median (range)	2 (0–7)	2 (0–6)	0.50
Corticosteroids, n (%)	20 (95%)	117 (97%)	0.56
Methotrexate, n (%)	7 (33%)	49 (40%)	0.52
Azathioprine, n (%)	9 (43%)	35 (29%)	0.20
Mycophenolate mofetil, n (%)	11 (52%)	50 (41%)	0.35
Belimumab, n (%)	2 (10%)	26 (21%)	0.25
Anti-TNF agents, n (%)	1 (5%)	2 (2%)	0.38
Cyclophosphamide, n (%)	7 (33%)	39 (32%)	0.92
Rituximab, n (%)	8 (38%)	16 (13%)	0.01
Calcineurin inhibitors, n (%)	2 (10%)	6 (5%)	0.34
Thalidomide, n (%)	1 (5%)	9 (7%)	1.00
Lenalidomide, n (%)	0 (0%)	1 (1%)	
Plasmapheresis, n (%)	0 (0%)	5 (4%)	1.00

RF: rheumatoid factor, SLEDAI: systemic lupus erythematosus activity index, CG: cryoglobulinemia, CryoVas: cryoglobulinemic vasculitis, IS: immunosuppressive, NA: not applicable, TNF: tumor necrosis factor

Cardiovascular events and severe infections in SLE patients according to the presence of cryoglobulinemia

In the SLE CG + group, 14/142 (10%) patients presented a stroke versus 4/71 (6%) in the SLE CG- group (p = 0.3) during follow-up. In each group, 4% of the patients had an acute coronary syndrome in their medical history. Severe infections were reported in 37/142 (26%) SLE CG + patients versus 16/71 (23%) SLE CG- patients. The most frequent types of infection were digestive infections in the SLE CG + group and pneumonia in the SLE CG- group (See supplemental Table 1). Among SLE CG + patients, there was no significant difference in terms of severe infections or cardiovascular events between patients with CryoVas and those without.

Immunomodulatory and immunosuppressive treatments received by patients with and without cryoglobulinemia

There was a trend towards an increased number of immunosuppressive and immunomodulatory treatments in SLE CG + patients compared to SLE CG- ones (median 2 versus 1, p = 0.053) at follow-up. The SLE CG + group received significantly more corticosteroid (96% versus 85%, p = 0.002) and rituximab (17% versus 6%, p = 0.02) administrations than SLE CG- patients (see supplemental Table 2).

Characteristics of patients with CryoVas

In the SLE CG + group, 21/142 (15%) patients presented a CryoVas according to de Vita's criteria [20], and 7/21 were documented with biopsy findings of histological vasculitis (skin biopsy = 5, nerve biopsy = 1, muscle biopsy = 1). No kidney biopsy retrieved lesions of membranoproliferative glomerulonephritis, and no death occurred. The delay between SLE diagnosis and first positive test for CG was significantly longer in the CryoVas group than in the group without CryoVas (7.6 years versus 5.9 years, respectively, p < 0.0001). The SLEDAI score was significantly higher at SLE diagnosis in the CryoVas group (17.0 versus 13.6, p = 0.03), but was not significantly different at the time of CG diagnosis (17.9 versus 14.3, p = 0.08). The mean SLEDAI score at CryoVas diagnosis was 23.7 (± 9,9, Table 5).

In terms of SLE clinical involvements, there were significantly more cutaneous signs in the CryoVas group, such as subacute cutaneous lupus (48% versus 26%, p = 0.04), alopecia (43% versus 22%, p = 0.046), and Raynaud's phenomenon (71% versus 45%, p = 0.03). Concerning clinical signs potentially related to CG, patients with CryoVas had significantly more general symptoms (62% versus 34%, p = 0.02), myalgia (48% versus 17%, p = 0.004), articular involvement (86% versus 57%, p = 0.01), purpura (43% versus 0%, p < 0.00001), digital ischemia (29% versus 2%, p = 0.02), cutaneous necrosis (38% versus 1%, p < 0.0001), cutaneous ulcers (38% versus 7%, p = 0.0004), central nervous system involvement (19% versus 4%, p = 0.003), and livedo (33% versus 13%, p = 0.047). In the CryoVas group, severe manifestations included kidney failure and central nervous system involvement in 1/21 (5%) and 4/21 (19%) patients, respectively. Moreover, patients with CryoVas presented significantly more pericarditis across their SLE medical history (52% versus 19%, p < 0.001). The proportion of lupus nephritis was not significantly different between patients with CryoVas and those without (43% vs 45%, respectively, p = 0.97; Table 5).

The biological parameters, including complement and CG characteristics, were not significantly different between SLE CG + patients with CryoVas and those without (Table 5).

CryoVas specific treatments

Among patients with CryoVas, 13/21 (62%) patients received a specific treatment for CryoVas. Among them, 3 received corticosteroids alone, and 10 received corticosteroids with immunosuppressive drugs at first line treatment (rituximab = 4, azathioprine = 3, methotrexate = 2, cyclophosphamide = 1). Cutaneous involvement motivated the initiation of these specific treatments for 11/13 (85%) patients (see supplemental Table 3). Patients with CryoVas received significantly more rituximab than SLE CG + patients without CryoVas (38% versus 13%, p = 0.01; Table 5).

A response to first line treatment was observed for 12/13 (92%) patients; but relapses were observed for 3 (75%) of them. Side effects related to the specific treatment of CryoVas occurred in 3/13 patients, and included cytopenia and drug-induced hepatitis (see supplemental Table 3).

Discussion

Among the 213 SLE patients included in the present study, 66% had at least one positive test for CG, 67% of them having a persistent CG. Most of the CG were type III and were associated with low complement levels. Among the 142 SLE CG + patients, 15% developed a CryoVas. No death was observed in the CryoVas group and 92% of the CryoVas patients responded to the first line treatment for CryoVas, mainly initiated based on cutaneous indication.

The prevalence of CG in SLE patients observed in the present series is higher than that previously reported by Garcia-Carrasco *et al*. [16] (25%) and Karimifar *et al*. [10] (48%). This could be explained by the improvement in laboratory techniques for the detection of CG [23]. It is important to note that the present series is the first to study CG in SLE patients after exclusion of HCV patients, HCV being a major cause of CG [21]. The prevalence of CG in SLE reported herein is also higher than in other connective tissue diseases. Previous series described 16% of CG in Sjogren's syndrome [24] and 3% of CG in systemic sclerosis [25]. Moreover, 15% of the SLE CG + and 10% of all the SLE patients from the present series developed a CryoVas, which constitutes a high rate of vasculitis for SLE patients. Previous studies reported vasculitis in 11–39% of SLE patients [16, 26, 27]. Interestingly, among 242 CryoVas patients, Terrier *et al.* [28] found only 5 (2%) patients with SLE. Overall, these results indicate that CG is frequent in SLE, with a non-negligible proportion of CryoVas. Nevertheless, CryoVas in SLE remains a scarce condition for which clinical data is poorly described in the literature.

The majority of CG were type III, even in the CryoVas group. This predominance of type III CG is consistent with previous data about CG in SLE [14, 21]. Unlike Garcia-Carrasco *et al*. [16], there was no significant increase in RF herein, which was positive in the cryoprecipitate or in the serum in less than 5% of SLE CG + patients, even in patients with CryoVas. This is consistent with the results of the study by Kolopp-Sarda *et al*. [21], showing the absence of RF activity in 81% of mixed CG, and only 4.6% of RF activity in patients with anti-DNA antibodies. The decrease in complement fractions described herein in SLE CG + patients is also consistent with the two main previous studies [10, 16].

Concerning SLE clinical involvements, there were more cutaneous and articular clinical signs in the CryoVas group. Unexpectedly, more pericarditis were found in the CryoVas group, a finding not reported previously, which could be due to multiple statistical testing (i.e. type 1 error). Concerning the relation between SLE activity and the presence of CG, SLE was more active at diagnosis in the CryoVas group. Similarly to Karimifar *et al*. [10], no association between SLEDAI score and the presence of CG was found herein. However, the present study identified a higher use of corticosteroids in SLE CG + patients, which could be an indirect argument for increased SLE activity in SLE CG + patients. Several studies had reported a potential association between CG and SLE nephritis [5, 29, 30], which was not observed herein,

even in patients with CryoVas. Concerning the CryoVas features, these were mainly articular, general, and cutaneous clinical signs. Severe manifestations such as neurological and renal involvements were rare. Surprisingly, there was more central than peripheral nervous system involvement in patients with CryoVas, which is uncommon for this vasculitis. Terrier *et al.* [28] found that, in patients with CryoVas, there was 52% of peripheral nervous system involvement and only 2% of central nervous system involvement. In a recent series of 71 Sjogren's syndrome with CryoVas, patients had 2.8% and 25.4% of central and peripheral nervous system involvement, respectively [31]. Thus, it seems that the clinical phenotype of CryoVas in SLE is specific and rarely severe. Indeed, no CryoVas patient died during follow-up and only 13/21 patients received immunosuppressive therapy for CryoVas, mainly for cutaneous indication.

The management of CryoVas related to SLE remains largely unknown, as the prevalence of this condition is low. In the present series, patients received both small molecule drugs and rituximab, in addition to corticosteroids for treatment induction, with a high response rate. The 2009 EULAR guidelines recommend the use of an immunosuppressive drug for the management of non-infectious mixed CryoVas, without mentioning the particular case of CryoVas in SLE [32].

This study is, to our knowledge, the largest study addressing CG in SLE. A consecutive series of patients was included, which increased the external validity of the study. Importantly, patients with HCV, an obvious cause of CG, were excluded. There are, however, several limitations to this single center and retrospective study. The inclusion of the patients was based on the laboratory results of a large university hospital (*Hospices Civils de Lyon)*, possibly inducing a selection bias by capturing patients at higher risk of severe involvements and unfavourable outcome. Moreover, patients with secondary Sjogren's syndromes were included, which can be a major cause of CG, even if the number of Sjogren's syndromes was balanced between the two groups. Finally, the distinction between features related to CG or SLE may be difficult because of the overlap in the clinical involvements between these two conditions.

In conclusion, CG is frequent in SLE but mostly asymptomatic. CG in SLE is mainly type III and associated with more complement consumption. CryoVas occurred in 15% of SLE CG + patients. CryoVas in SLE appeared to be a specific condition, with a low prevalence of neuropathy, membranoproliferative glomerulonephritis, and severe involvement.

Abbreviations

CG
cryoglobulinemia
CryoVas
cryoglobulinemic vasculitis
SLE
systemic lupus erythematosus
Ig

immunoglobulin
RF
rheumatoid factor
ANA
antinuclear antibodies
HIV
human immunodeficiency Virus
HBV
hepatitis B Virus
HCV
hepatitis C Virus
SLEDAI
systemic lupus erythematosus activity index
SLICC
systemic lupus international collaborating clinics
EULAR
European League Against Rheumatism

Declarations

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Availability of data and materials : The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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