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Rituximab-Associated Flare of Cryoglobulinemic Vasculitis

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Background: Patients with cryoglobulinemic vasculitis (CV) can develop disease flare after rituximab administration. The objective of our study was to describe the prevalence, clinical characteristics, predisposing factors, and outcomes of patients with rituximab-associated flare of CV.

Methods: We conducted a retrospective study in a tertiary referral center until March 25, 2020.

Results: Among 64 patients with CV who received rituximab therapy in our center, 14 (22%) developed disease flare. Median age was 67.5 years. Seven patients (50%) had type II CV and the other half had either type I ($n = 6$) or type III ($n = 1$). Twelve patients (86%) had an underlying B-cell lymphoproliferative disorder as the cause of their CV. CV flare occurred after a median time of 5.5 days (range: 2–8 days). The organ systems most involved were the skin ($n = 10$), kidneys ($n = 5$), and peripheral nerves ($n = 3$). Five patients (36%) developed acute kidney injury (AKI), 3 of whom presented with nephritic syndrome secondary to biopsy-proven membranoproliferative glomerulonephritis. Treatment was directed against the underlying disease in addition to supportive care. Patients who developed flare were more likely to have B-cell lymphoproliferative disorder as the underlying etiology of their CV ($P = 0.03$). Eight patients (57%) died after a median time of 27 months.

Conclusions: Rituximab-associated flare can occur in all types of CV, tends to arise approximately 2 days and less than 1 week after rituximab administration, and is more likely to happen in patients with an underlying B-cell lymphoproliferative disorder. It does not indicate treatment failure, and rituximab should not be abandoned altogether. AKI is a common manifestation, and mortality rate at 2 years is high.

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Cryoglobulinemia is a rare clinical entity characterized by the presence of circulating cryoglobulins. Cryoglobulins are serum Igs that self-aggregate and precipitate at low temperatures (below 37°C) and dissolve on rewarming.[1–5] Although many patients with cryoglobulinemia may be asymptomatic, many develop symptoms of multiorgan vasculitis, which is referred to as cryoglobulinemic syndrome or cryoglobulinemic vasculitis (CV).[1,2] CV can be diagnosed clinically, serologically, and/or pathologically.[1] CV tends to typically involve the small vessels, resulting in heterogeneous clinical features like purpura, necrotic ulcers, arthralgia, peripheral neuropathy, and kidney disease. Serologic features can include detectable cryoglobulin (CG) levels and evidence of complement activation like low serum complement 4 (C4). Histologically, leukocytoclastic vasculitis has been described in these patients.[2]

Rituximab is a humanized mouse chimeric anti-CD20 monoclonal antibody linked to human IgG1 and kappa constant regions.[6] It is an important therapeutic option in the management of a variety of both hematologic and autoimmune disorders, including CV, and targets B cells. Case reports of a CV flare phenomenon with a rise in serum CG level (i.e., IgM) following rituximab administration have been described in patients with Waldenstrom macroglobulinemia (WM).[6–9] Desbois *et al.*[10] described rituximab-associated autoimmune disease flare in 7 of 185 patients diagnosed with an autoimmune disease (3.8%), all of whom also had type II CV. The pathogenesis is not well understood, but hypotheses include rituximab-associated immune complex formation and deposition in multiple body

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sites, including skin and kidneys, and rapid Ig production because of increased interleukin 6 production.[11–14] This flare phenomenon can be a diagnostic challenge for many clinicians and could possibly be misinterpreted as a failure of response to treatment.[6,7,9] Although rituximab is the most widely used anti-CD20 monoclonal antibody, new-generation anti-CD20 monoclonal antibodies, such as obinutuzumab, have been developed to try to overcome mechanisms of resistance to rituximab and to enhance therapeutic response.[15,16] One such mechanism of resistance is the development of anti-rituximab antibodies.[16] De Fremont *et al.*[17] recently described a patient with Sjogren's syndrome–associated refractory CV who was found to have anti-rituximab antibodies and developed disease flare after treatment with obinutuzumab.

The objective of our study was to assess and evaluate the cohort of patients with CV treated with rituximab in our center and describe the prevalence, clinical characteristics, predisposing factors, and outcomes of rituximab-associated flare of CV.

MATERIALS AND METHODS

After approval of the protocol and waiver of informed consent by the Mayo Clinic Institutional Review Board (ID: 20–003504), we used the Advanced Cohort Explorer database available at Mayo Clinic and obtained a list of all patients who had a positive result on CG testing until March 25, 2020. We cross-referenced this list with patients who had received rituximab therapy and had 70 unique patients, excluding those without research authorization. Six patients were further excluded because they had remotely received rituximab therapy before developing CV, had insufficient information, and had no follow-up. In total, we identified 64 patients with known CV who received rituximab therapy.

We defined disease flare as any clinical deterioration within 2 weeks following rituximab administration, including onset of a new organ involvement or worsening of the underlying autoimmune disease not clearly explained by disease progression alone, with or without laboratory evidence. A total of 14 patients fulfilled the criteria of disease flare. We also defined AKI as $\geq$1.5 times increase from the baseline creatinine.

To identify potential predisposing factors associated with the occurrence of disease flare following rituximab administration, we compared the clinical features of the 14 patients who had a disease flare (case) with those of the remaining 50 patients who did not have a disease flare (control). The following data were collected: demographic characteristics (age, sex, and race), comorbidities, type of CV, etiology of CV, symptoms of CV, laboratory findings before rituximab treatment (creatinine, CG, and C4), and other treatment modalities used before and together with current rituximab treatment (chemotherapy, corticosteroids, and plasmapheresis).

Statistical Analyses

Continuous variables were summarized as median (range) and were compared between patients with and without disease flare using Wilcoxon's rank sum test. Categorical variables were summarized as frequency (percentages) and were compared between patients with and without disease flare using χ^2 test. Two-sided P value of <0.05 was considered statistically significant. Statistical analyses were performed using JMP statistical software (version 14; SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics, Clinical Features, Laboratory Findings, and Treatments of All Patients With CV

Data are summarized in Table 1. We have a total of 64 patients with known CV who received rituximab therapy. The median age was 63 years (range: 24–91 years); 45% were men and 95% were Caucasians. The most common comorbidities were malignancy (75%), hypertension (53%), and chronic kidney disease (41%). Twenty-four patients (37.5%) had biopsy-proven membranoproliferative glomerulonephritis as the etiology of their chronic kidney disease. Most of the patients (69%) had type II CV followed by type I (27%) and type III (5%). In 50% of patients, their CV was ascribed to an underlying B-cell lymphoproliferative disorder. The most common organ systems involved were the skin (69%), kidneys (42%), and peripheral nerves (19%). Before rituximab treatment, the median creatinine was 1.0 mg/dl (interquartile range: 0.8–1.4 mg/dl), the median CG level was 7%, and the median C4 level was 6.5 mg/dl (reference range: 14–40 mg/dl). Together with rituximab, 55%, 45%, and 6% of patients also received corticosteroids, chemotherapy, and plasmapheresis, respectively.

Prevalence and Baseline Characteristics of Patients With Rituximab-Associated Flare of CV

Among 64 patients who were diagnosed with CV and were treated with rituximab, 14 of them (22%) developed a disease flare, including one who had 2 disease flares. Data are summarized in Tables 1 and 2. The

Table 1. Comparison of patients with cryoglobulinemic vasculitis according to the presence of a disease flare following rituximab

Variables	All patients with vasculitis, $n = 64$	Patients with vasculitis flare, $n = 14$	Patients without vasculitis flare, $n = 50$	*P* value
Demographic characteristics				
• Age, median (range)	63 (24–91)	67.5 (38–91)	63 (24–83)	0.9
• Sex, male	29 (45)	7 (50)	22 (44)	0.5
• Race, white	61 (95)	14 (100)	47 (94)	0.4
Comorbidities				
• Hypertension	34 (53)	8 (57)	26 (52)	0.9
• Diabetes	5 (8)	1 (7)	4 (8)	0.9
• CKD or ESKD	26 (41)	4 (29)	22 (44)	0.3
• Autoimmune disease	24 (38)	5 (36)	19 (38)	0.7
• Malignancy	48 (75)	12 (86)	36 (72)	0.1
• HCV infection	14 (22)	1 (7)	13 (26)	0.1
Type of cryoglobulinemic vasculitis				0.2
• I	17 (27)	6 (43)	11 (22)	
• II	44 (69)	7 (50)	37 (74)	
• III	3 (5)	1 (7)	2 (4)	
Etiology of cryoglobulinemic vasculitis				*0.03*
• HCV infection	12 (19)	1 (7)	11 (22)	
• B-cell lymphoproliferative disorder	32 (50)	12 (86)	20 (40)	
• Autoimmune disease	14 (22)	1 (7)	13 (26)	
• Unclear	6 (9)	0	6 (12)	
Organ involvement				
• Kidneys	27 (42)	6 (43)	22 (44)	0.7
• Skin	44 (69)	10 (71)	34 (68)	0.7
• Joints	3 (5)	1 (7)	2 (4)	0.6
• Nerves	12 (19)	3 (21)	9 (18)	0.8
• GI	6 (9)	1 (7)	5 (10)	0.7
• CNS	1 (2)	1 (7)	0	0.06
• Lungs	2 (4)	0	2 (4)	0.4
• Heart	1 (2)	0	1 (2)	0.6
Laboratory findings before RTX treatment				
• Cr (mg/dl), median (IQR)	(0.8–1.4)	0.8 (0.7–1.25)	1.05 (0.8–1.625)	*0.01*
• Cryo (%), median (IQR)	7 (4–16)	12 (4–67)	8 (4–13.75)	0.7
• C4 (mg/dl), median (IQR)	5 (3–7)	6.5 (3–7.5)	5 (3–7)	0.6
Treatments prior to current RTX				
• Chemotherapy	23 (36)	8 (57)	15 (30)	0.06
• Corticosteroids	26 (41)	5 (36)	21 (42)	0.7
• Plasmapheresis	11 (17)	2 (14)	9 (18)	0.7
Concurrent treatments with RTX				
• Chemotherapy	29 (45)	9 (64)	20 (40)	0.4
• Corticosteroids	35 (55)	9 (64)	26 (52)	0.4
• Plasmapheresis	4 (6)	3 (21)	1 (2)	*0.03*

C4, complement C4; CKD, chronic kidney disease; CNS, central nervous system; Cr, creatinine; cryo, cryoglobulin; ESKD, end-stage kidney disease; GI, gastrointestinal; HCV, hepatitis C virus; IQR, interquartile range; RTX, rituximab.
Values are n (%) unless otherwise indicated.
Italic indicates *P* values that are <0.05 are considered statistically significant.

median age was 67.5 years (range: 38–91 years); 50% were men and all were Caucasians. Twelve patients (86%) had an underlying malignancy as the cause of their CV, all of which were a B-cell lymphoproliferative disorder, with 4 of them having either concomitant skin cancer or sarcoma. Six had WM, 3 had non-Hodgkin's lymphoma, 2 had chronic lymphocytic leukemia, and 1 had a nonspecific low-grade B-cell lymphoproliferative disorder. Hypertension ($n = 8$), autoimmune disease ($n = 5$), and chronic kidney disease ($n = 4$) were the other comorbidities present in our cohort, with the etiology of chronic kidney disease being membranoproliferative glomerulonephritis secondary to CV in all patients. One patient had chronic hepatitis C virus (HCV) infection, and another had Sjogren's syndrome causing the CV.

Half of the patients ($n = 7$) had type II CV, and the other half had either type I ($n = 6$) or type III ($n = 1$). The Ig involved in most of the patients was IgM kappa ($n = 11$), and the rest were IgG lambda ($n = 1$), IgM lambda ($n = 1$), and IgA kappa ($n = 1$). The median follow-up duration (defined as the time between the date of disease flare and either the date of last follow-up

Table 2. Baseline demographic and clinical characteristics of patients with rituximab-associated flare of cryoglobulinemic vasculitis

Patient	Age	Sex	Race	HTN	DM	CKD	Cause of CKD	AD	Malignancy	Hepatitis	Type of hepatitis	Type of Ig	Type of cryo	Cause of cryo
1	58	M	W	Y	N	Y	MPGN secondary to cryoglobulinemic vasculitis	N	Y	N		IgM kappa	II	Waldenstrom macroglobulinemia
2	80	F	W	N	N	N		N	Y	N		IgM kappa	I	B-cell non-Hodgkin's lymphoma
3	38	M	W	N	N	N		Y	N	Y	C	IgM kappa	II	Hepatitis C
4	62	M	W	N	Y	N		N	Y	N		IgM kappa	I	Low-grade B-cell lymphoproliferative disorder
5	62	F	W	N	N	N		N	Y	N		IgM lambda	I	Waldenstrom macroglobulinemia
6	57	F	W	Y	N	Y	MPGN secondary to cryoglobulinemic vasculitis	Y	Y	N		IgM kappa	I	Waldenstrom macroglobulinemia
7	73	F	W	N	N	N		Y	Y	N		IgG lambda	I	CLL
8	88	F	W	N	N	N		N	Y	N		IgM kappa	III	Splenic marginal zone lymphoma
9	56	M	W	Y	N	Y	MPGN secondary to cryoglobulinemic vasculitis	N	Y	N		IgM kappa	II	IgM monoclonal gammopathy suspicious for Waldenstrom macroglobulinemia
10	74	F	M	N	N	N		Y	Y	N		IgA kappa	II	B-cell non-Hodgkin's lymphoma
11	91	F	W	Y	N	N		Y	Y	N		IgM kappa	II	Sjogren's syndrome
12	73	M	W	Y	N	N		Y	Y	N		IgM kappa	II	Waldenstrom macroglobulinemia
13	80	M	W	Y	N	N		N	Y	N		IgM kappa	I	Waldenstrom macroglobulinemia
14	57	M	W	Y	N	Y	MPGN secondary to cryoglobulinemic vasculitis	N	Y	Y	B	IgM kappa	II	CLL

AD, autoimmune disease; CKD, chronic kidney disease; CLL, chronic lymphocytic leukemia; cryo, cryoglobulinemia; DM, type 2 diabetes mellitus; F, female; HTN, hypertension; Ig, immunoglobulin; M, male; MPGN, membranoproliferative glomerulonephritis; N, no; W, white; Y, yes.

or the date of death) after disease flare was 32 months (range: 6–137 months).

Clinical Features and Treatments of Patients With Rituximab-Associated Flare of CV

Eleven of the 14 patients developed a disease flare during their first cycle of rituximab treatment, and 6 patients had the flare after the first dose. One patient had a flare after both his first and second doses. Most of them received weekly rituximab dosed at 375 mg/m^2 and 1 patient received a dose of 1 g. Data are summarized in Table 3.

The median time to disease flare (defined as the time between the date of first rituximab infusion and the date of first symptom manifestation) was 5.5 days (range: 2–8 days). The following organ systems were involved: skin ($n = 10$), kidneys ($n = 6$), peripheral nerves ($n = 3$), joints ($n = 1$), gastrointestinal tract ($n = 1$), and central nervous system ($n = 1$). Symptoms were heterogeneous and included petechial and purpuric skin rash, leg ulcers, symptoms of volume overload resulting from AKI, peripheral neuropathy, arthralgia, weight loss, dysphagia, abdominal pain, headache, and confusion. Two patients had a minor infusion reaction.

Other treatments administered concurrently with rituximab were chemotherapy ($n = 9$), corticosteroids ($n = 9$), and plasmapheresis ($n = 3$). The most used chemotherapeutic agent was cyclophosphamide ($n = 6$), and the rest included vincristine, cladribine, methotrexate, chlorambucil, and bendamustine.

Kidney Manifestations of Patients With Rituximab-Associated Flare of CV

Before rituximab treatment, the median creatinine was 0.8 mg/dl (interquartile range: 0.7–1.25 mg/dl). During disease flare, 5 patients (patients #2, #6, #9, #13, and #14) developed AKI with subsequent improvement in their kidney function after the episode (Figure 1). One patient (patient #14) had 2 disease flares and had AKI in both instances. Three of them (patients #6, #13, and #14) presented with hematuria and proteinuria of more than 1 g, and 1 patient (patient #2) had only proteinuria as his presenting feature. The median predicted 24-hour urine total protein was 5.9 g (range: 1.9–9.6 g).

The 3 patients who had both hematuria and proteinuria during disease flare underwent kidney biopsy, and the results were consistent with cryoglobulinemic glomerulonephritis with a membranoproliferative pattern of injury with IgM kappa deposits (Figure 2a–d).

Table 3. Clinical features and treatments of patients with rituximab-associated flare of cryoglobulinemic vasculitis

Patient	RTX cycle and dose	Time to disease flare (d)	Organ involvement	Infusion reaction	RTX dose (mg)	Other treatments received	
						Prior to current RTX	Concurrently with RTX
1	1st, 4th	4	Skin, GI, and kidneys	N	375 mg/m^2	None	CYC, vincristine, and CS
2	1st, 1st	5	CNS, nerves, and kidneys	N	NA	Fludarabine	CYC, cladribine, and intrathecal methotrexate and CS
3	1st, 1st	7	Skin	N	375 mg/m^2	CS	CS
4	3rd, 2nd	2	Skin and joints	Y	375 mg/m^2	None	None
5	1st, 3rd	3	Nerves	N	375 mg/m^2	None	None
6	2nd, 2nd	5	Kidneys	N	375 mg/m^2	CYC, MM, CS, and RTX	CYC and PLEX
7	1st, 1st	7	Skin	N	NA	Obinutuzumab	CYC and CS
8	1st, 3rd	7	Skin	Y	375 mg/m^2	Chlorambucil	Chlorambucil
9	2nd, 1st	Unclear	Skin and kidneys	N	NA	Methotrexate, CS, RTX, and PLEX	CS
10	1st, 2nd	Unclear	Skin	N	NA	RTX	CYC and CS
11	1st, 1st	2	Skin and nerves	N	1000	CS	CS
12	1st, 3rd	6	Skin	N	375 mg/m^2	Bortezomib, CS, and PLEX	Bendamustine
13	1st, 3rd	8	Kidneys	N	NA	None	Chlorambucil, CS, and PLEX
14	1st, 1st and 2nd	Few	Skin and kidneys	N	375 mg/m^2	CYC	CYC, CS, and PLEX
	2nd, 3rd	7	Skin and kidneys	N	375 mg/m^2	None	CS

CNS, central nervous system; CS, corticosteroid; CYC, cyclophosphamide; GI, gastrointestinal; MM, mycophenolate mofetil; N, no; NA, not available; PLEX, plasma exchange; RTX, rituximab; Y, yes.
Note: Patient 14 had 2 disease flares.

Laboratory Findings of Patients With Rituximab-Associated Flare of CV

Before rituximab treatment, the median CG level was 12% and the median C4 level was 6.5 mg/dl (reference range: 14–40 mg/dl). Almost half of the patients had missing data for either parameter. Three patients also had elevated rheumatoid factor (RF) (range: 99–1430 IU/ml; reference range: <15 IU/ml), all of whom had either type II or type III CV. Most of the patients did not have their CG, C4, and RF checked during disease flare. There were a few exceptions: 1 patient (patient #13) did have a CG detected at a level of 63% and an elevated RF at 92 in his serum during disease flare, and 2 patients (patients #6 and #14) had low C4 levels at <3 mg/dl.

Interventions During and Outcomes of Patients With Rituximab-Associated Flare of CV

In addition to initiation of chemotherapy, corticosteroids, and/or plasmapheresis, some patients were either switched from oral to i.v. corticosteroids ($n = 1$; 7%) or

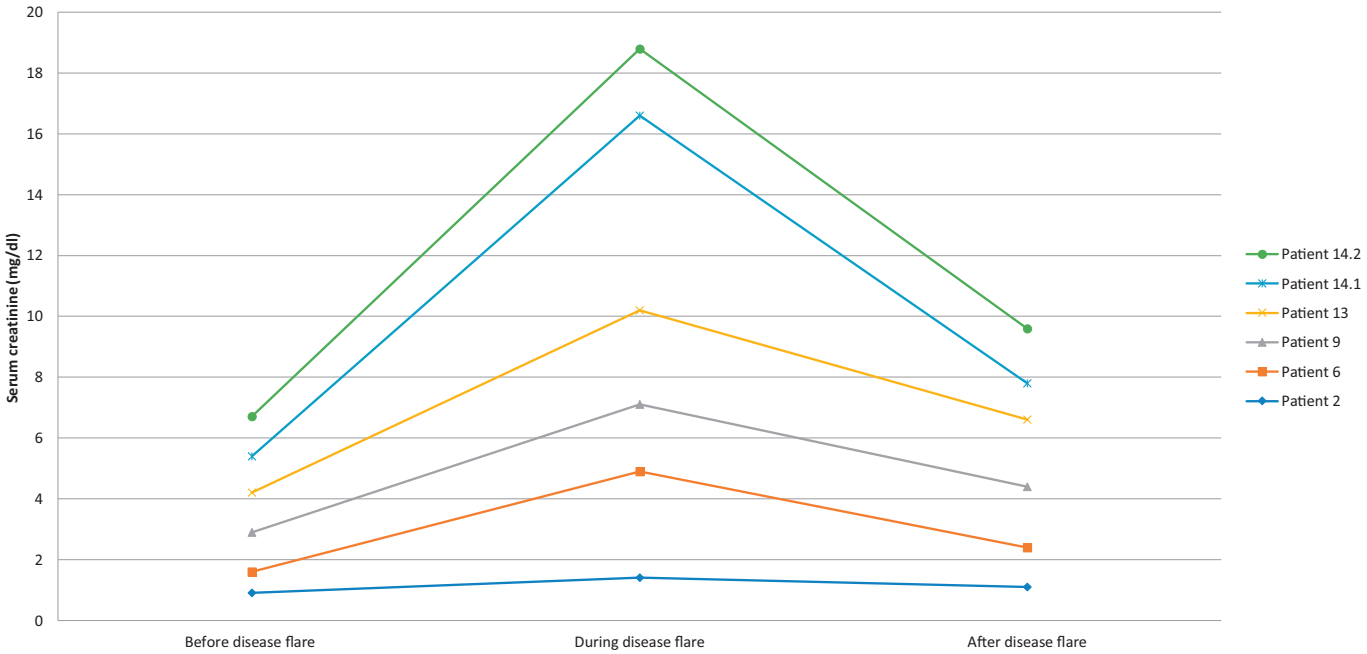

Figure 1. Serum creatinine trend of patients who developed AKI during disease flare.

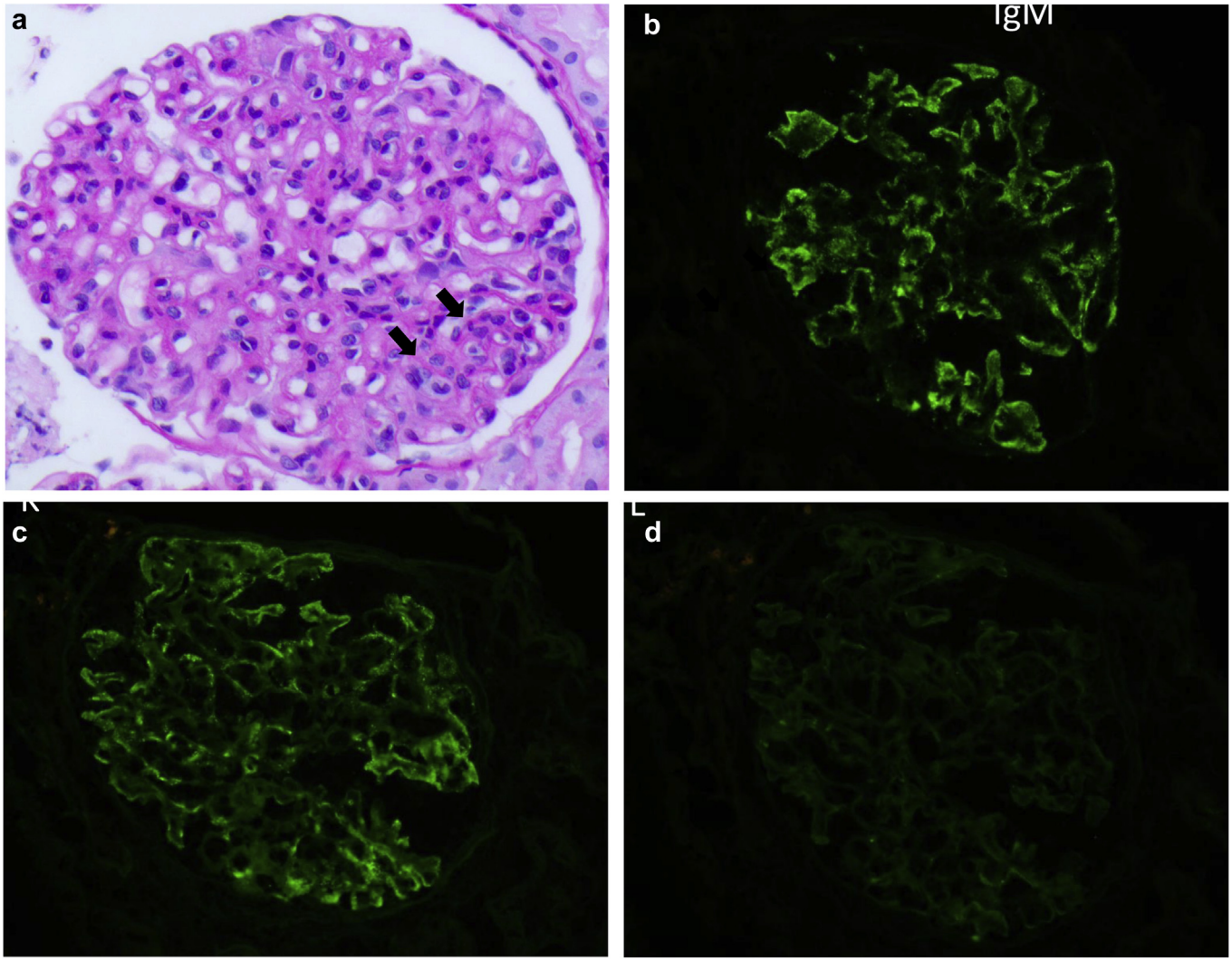

Figure 2. (a) Light microscopy, periodic acid-Schiff stain: glomerulus with moderate mesangial hypercellularity and segmental endocapillary proliferative changes with marginating mononuclear cells (black arrows). Magnification is 60×. (b–d) Immunofluorescence studies: granular mesangial and capillary loop staining with 2+ IgM (b), 2+ kappa light chain (c), and negative lambda light chain (d).

had the doses of their oral corticosteroids increased ($n = 2$; 14%) to better control the disease flare. Some patients ($n = 3$; 21%) were also managed symptomatically with diuretics for edema and gabapentin for peripheral neuropathy. All 6 patients recovered from their disease flare uneventfully. One patient (patient #14) developed complications of AKI, including recurrent hyperkalemia and volume overload, and required kidney replacement therapy in the form of intermittent hemodialysis, as well as admission to the intensive care unit. None of the patients required mechanical ventilation. Data are summarized in Table 4.

Eleven of the 14 patients continued treatment after the flare with chemotherapy, corticosteroids, and/or plasmapheresis. Rituximab was continued in 10 of these patients with the addition of cyclophosphamide, bortezomib, chlorambucil, or fludarabine for those with WM and bendamustine and/or lenalidomide for those with non-Hodgkin's lymphoma. On the other hand, it was discontinued in 1 patient in the setting of a very minimal B-cell clonal population on repeat bone marrow aspirate and biopsy, and he was started on ibrutinib instead.

Overall, 8 patients died, with 1 patient's cause of death being cryoglobulinemic crisis. Three patients died from non–CV-related problems, and 4 patients died from unclear causes.

Predisposing Factors Related to Rituximab-Associated Flare of CV

Patients who developed disease flare were more likely to have B-cell lymphoproliferative disorder as the underlying etiology of their CV ($P = 0.03$), had lower creatinine levels before rituximab treatment (0.8 vs. 1.05 mg/dl, $P = 0.01$), and received more treatments with plasmapheresis together with rituximab ($P =$

Table 4. Interventions during and outcomes of patients with rituximab-associated flare of cryoglobulinemic vasculitis

Patient	Intervention (in addition to concurrent treatment)	KRT	Type of KRT	ICU stay	Follow-up duration after flare (mo)	Subsequent treatments after flare	Death	Cause of death
1	Increased dose of oral CS	N		N	108	RTX and CS	N	
2	SxT	N		N	36	RTX and bendamustine	N	
3	None	N		N	6	CS	Y	Unclear
4	SxT	N		N	26	RTX, CS, and PLEX	Y	Cryoglobulinemic crisis
5	None	N		N	28	RTX, fludarabine, and CS	Y	Septic shock, acute MI, and acute intracranial hemorrhage
6	None	N		N	91	RTX, CYC, and CS	N	
7	None	N		N	56	None	N	
8	None	N		N	18	None	Y	Metastatic colorectal cancer progression
9	SxT	N		N	6	RTX, CYC, and CS	N	
10	None	N		N	74	RTX, bendamustine, lenalidomide, and PLEX	Y	Unclear
11	Switched to i.v. CS	N		N	23	RTX and CS	Y	Unclear
12	Increased dose of oral CS	N		N	26	None	N	
13	None	N		N	133	RTX, CYC, CS, chlorambucil, and bortezomib	Y	Unclear
14	None	Y	iHD	Y			N	
	None	N		N	137	CS, PLEX, and ibrutinib	Y	Acute cholecystitis complications

CS, corticosteroid; ICU, intensive care unit; iHD, intermittent hemodialysis; KRT, kidney replacement therapy; MI, myocardial infarction; N, no; PLEX, plasma exchange; RTX, rituximab; SxT, symptomatic treatment; Y, yes.
Note: Patient 14 had 2 disease flares.

0.03) compared with those who did not develop disease flare (Table 1). Other variables, including demographic characteristics, comorbidities, type of CV, symptoms of CV, CG level, C4 level, and treatments received in the past, did not influence the occurrence of rituximab-associated flare of CV.

Subgroup Analysis of Patients With CV Secondary to a B-Cell Lymphoproliferative Disorder Based on IgM Level and Serum Viscosity

Fifty percent of the patients had CV secondary to a B-cell lymphoproliferative disorder, 12 of whom had a disease flare, whereas 20 of whom did not. Among these 12 patients, however, only 10 of them had an IgM protein (71%), and 2 of them had IgG and IgA proteins respectively. As shown in Table 5, compared with those who did not develop disease flare but also had CV secondary to a B-cell lymphoproliferative disorder, their median IgM level was 129 mg/dl (vs. 1570 mg/dl, $P = 0.1$), and their median serum viscosity was 1.6 centipoises (vs. 1.7 centipoises, $P = 0.8$) before rituximab treatment.

DISCUSSION

In our retrospective study, we found that 14 of the 64 patients (22%) who received rituximab developed a CV flare. In comparison with other reported studies, our patient cohort had varying types of underlying cryoglobulinemia with type I, type II, and type III in 6 (42%), 7 (50%), and 1 (7.1%) patient(s), respectively. Five of these 14 patients (36%) had AKI during flare, 3 of whom presented with nephritic syndrome, with kidney biopsy showing cryoglobulinemic glomerulonephritis.

CGs are classified according to Brouet's classification into 3 subtypes based on the clonality of the Ig, as outlined in Table 6. Many hypotheses have been proposed to explain their formation, including genetic susceptibility, sustained antigenic stimulation, monoclonal or polyclonal activation of B-lymphocytes, aberrant autoantibody production, reduced clearance of immune complexes by the liver, and possible cross-reactivity with other antigens.[1–5] The reversible cryoprecipitability of CGs is influenced by several environmental factors like temperature, pH, structure, salt and protein concentrations, and amino acid composition.[18] That is why not all Igs are cryoprecipitable. Type I CGs typically tend to self-associate, resulting in

Table 5. Subgroup analysis of patients with cryoglobulinemic vasculitis secondary to a B-cell lymphoproliferative disorder according to the presence of a disease flare following rituximab based on IgM level and serum viscosity

Variables	Patients with BCLPD with vasculitis flare, $n = 10$[a]	Patients with BCLPD without vasculitis flare, $n = 20$	P value
IgM (mg/dl), median (IQR) Range: 37–286 mg/dl	129 (80–1690)	1570 (261–3603)	0.1
Serum viscosity (centipoise), median (IQR) Range: 0–1.5 centipoise/s	1.6 (1.35–1.68)	1.7 (1.6–2.1)	0.8

[a]Although there were 12 patients who had cryoglobulinemic vasculitis secondary to a B-cell lymphoproliferative disorder and developed a disease flare, only 10 of them had IgM protein and 2 of them had IgG and IgA proteins respectively.
BCLPD, B-cell lymphoproliferative disorder; IQR, interquartile range.

Table 6. Brouet's classification of cryoglobulins with relative incident frequency among patients with cryoglobulinemia and commonly associated diseases

Type of cryoglobulin	Ig	Incidence, %	Commonly associated diseases
Type I (1)	Isolated monoclonal Ig (most commonly IgM, can be IgG but very rarely IgA)	10–15	Hematologic disorders like Waldenstrom macroglobulinemia, multiple myeloma, and chronic lymphocytic leukemia
Type II (2)	Monoclonal Igs (typically IgM but rarely can be IgG or IgA) with rheumatoid factor activity (against a polyclonal IgG) AND polyclonal IgG	50–60	Infections like chronic hepatitis C virus, hematologic disorders, and autoimmune diseases like Sjogren's syndrome and systemic lupus erythematosus
Type III (3)	Polyclonal Igs of various types (like IgM and IgG)	20–30	Infections like chronic hepatitis C virus, hematologic disorders, and autoimmune diseases like Sjogren's syndrome and systemic lupus erythematosus

aggregates and do not form immune complexes, so clinically, these patients manifest more commonly with signs of vascular obstruction rather than of vasculitis.[19] These aggregates are frequently amorphous material but can present as crystals, which can cause more severe manifestations of vessel occlusion and necrosis like in cryoglobulinemic glomerulonephritis.[18,20] In these patients, highly concentrated type I CG precipitates in the relatively "warmer" glomeruli as a result of the high protein content. Type II and III CGs typically form immune complexes and form a precipitate in the vasculature resulting in an inflammatory response. The cryoprecipitation of type II and III CGs are likely in part related to the Fc-Fc interaction. In patients with HCV infection, the immune complexes are formed by IgM with RF activity that are linked to IgG with anti-HCV reactivity.[19]

As a B-cell depleting agent, rituximab has been at the forefront of immunosuppressive management in both HCV and non–HCV-associated CV. A clinical flare phenomenon following rituximab administration has been described since the early 2000s, and all cases involved WM leading to either type I or type II CV.[6–9] Ghobrial *et al*.[7] described a patient with WM resulting in type II CV with predominant skin manifestations and a CG level of 3%, which, after receiving weekly 375 mg/m^2 rituximab therapy, increased to 9.7% during the first week without any clinical changes before showing improvement. The same authors did a follow-up study looking at their cohort of patients with WM and showed that there was a rise in IgM levels in 54% of their patients after rituximab therapy, most of whom eventually had a decrease in their IgM levels within 4 months after administration of medication.[9] In addition, Treon *et al*.,[8] in their series of 11 patients with WM, showed that the IgM levels increased by more than 25% in most of their patients after rituximab therapy, with one of them developing a subdural hemorrhage before showing improvement. This phenomenon is also referred to as IgM flare, which is characterized by a transient increase in IgM level and can occur within hours to days following initiation of rituximab therapy.[6–9] When the IgM level exceeds 5000 mg/dl or when serum viscosity exceeds 4.0 centipoises, signs and symptoms of hyperviscosity, such as headache, dizziness, visual impairment, coma, and seizures, may occur.[21,22]

In our study, we included patients with both clinical and laboratory evidence of a disease flare. The prevalence of such a flare was relatively high at 22% (14 of 64 patients) with AKI in 5 patients. The incidence of AKI in cryoglobulinemia is reported to be approximately 10% to 17%, with 20% to 50% of patients presenting with nephrotic syndrome and 20% to 30% of patients with nephritic syndrome.[23] Histologically, membranoproliferative glomerulonephritis is the most described pattern of injury.[23] Without a kidney biopsy, this diagnosis is very difficult to make considering that these patients likely have hemodynamic insults and/or concurrent use of nephrotoxic agents that may also explain their AKI. Our patients developed AKI after they had started treatment for their underlying disease, making the diagnosis and management challenging. We speculated if the overall Ig burden before rituximab therapy could be a contributing factor to a flare; however, many of our patients had received cytoreductive measures like cyclophosphamide either before or concurrently with rituximab.

The mechanism of rituximab-associated CV flare remains poorly understood. Because type II CGs consist of a combination of monoclonal IgM with RF activity and polyclonal IgG, rituximab-associated CV flares have typically been seen in type II CV because of the hypothesized immune-mediated reaction between an antigenic portion of rituximab and complement-fixing IgM and IgG antibodies.[10,11] Potential pathogenic complexes consisting of IgM with RF activity and rituximab have previously been postulated, wherein these Igs were said to have the ability to recognize and bind the IgG1 portion of rituximab.[10,11,24,25] This theory was further supported by the histological presence of IgM-, IgG1-, and rituximab-positive staining of

endomembranous deposits and thrombi within kidney lesions on immunofluorescence analysis performed in one study.[10] The kidney lesions could ultimately be the result of endocapillary proliferation secondary to immune complex deposition and glomerular obstruction by cryoglobulinemia and rituximab. Another hypothesis is that rituximab stimulates interleukin-6 production by bystander immune cells, including monocytes and B cells, resulting in the rapid production of Igs.[13,14]

Several important conclusions can be drawn from our study. Not all cases of rituximab-associated CV flare occur after the first dose of the medication. In our experience, most of the patients had the flare after their second or subsequent dose. The flare also occurred more than once in a treatment cycle. Clinicians therefore need to be aware of this possibility. Furthermore, disease flare occurred after a median time of 5.5 days following rituximab administration. In addition, compared with patients who did not have a CV flare after being treated with rituximab, those who had a flare were more likely to have a B-cell lymphoproliferative disorder as the underlying etiology of their CV. It is important to note that most of these patients had IgM protein, rendering them susceptible to IgM flare. Based on our study, however, it appears that the presence of CGs is independent of IgM level and serum viscosity in determining IgM flare. They received more treatments with plasmapheresis because of the flare, all of whom had kidney involvement. They were also incidentally found to have a lower creatinine level before rituximab treatment. We do not have a clear explanation for this finding, which, in a larger study, possibly may not be statistically significant. In the study by Debois *et al.*,[10] exacerbations were noted to occur more likely in patients with kidney involvement, a higher CG level, and a lower C4 level, which were not present in our study.

In terms of management, almost all our patients received chemotherapy, corticosteroids, and/or plasmapheresis during their disease flare and continued to require these treatments, which were directed against their underlying diseases, even after it resolved. Although 3 of the 14 patients had their corticosteroid regimen intensified during disease flare, it is difficult to interpret if this change in therapy resulted in any impact to the patients' overall clinical trajectory. It is important to note that this flare phenomenon does not indicate failure of response to treatment, and rituximab should not be abandoned altogether. In addition, approximately 60% of our patients who experienced rituximab-associated flare of their underlying CV died within a median period of 27 months after exacerbation but with only 1 confirmed case of cryoglobulinemic crisis being the cause of death.

Our study has several limitations. In our initial search strategy, we may have missed patients with CV who did not undergo CG testing. Most of the patients who had a disease flare did not have pertinent laboratory parameters trended, including CG, C4, and RF, either immediately before, during, or subsequent to resolution of the flare, so these missing data were not included in the statistical analyses. In some of the patients who had their CG checked, the results were read as "trace," and these results were also not included. The disease flares were at times documented by the treating clinician, but in many instances, these flares had to be interpreted in retrospect based on the consensus of the authors, hence possibly leading to bias.

CONCLUSION

Rituximab-associated flare of CV is not uncommon (22%) and can occur in any type of CV. Disease flares tend to arise after 2 days and less than 1 week after rituximab administration and are more likely to occur in patients with a B-cell lymphoproliferative disorder as the underlying etiology of their CV. Five (36%) patients developed AKI during disease flare, with kidney biopsy showing cryoglobulinemic glomerulonephritis. Management involves continuing treatment of the underlying disease while providing supportive care during disease flare with possibly intensified corticosteroid regimen and/or plasmapheresis. Mortality rate at 2 years is high at 60% in these patients. Because it is an underrecognized clinical entity with potentially dire consequences, clinicians should be cognizant of its existence and have a high index of suspicion, particularly in patients who are at high risk of developing disease flare. It is not indicative of treatment failure, so rituximab should be resumed or continued appropriately and as necessary.

DISCLOSURE

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PATIENT CONSENT

The authors declare that waiver of informed consent was approved by the Mayo Clinic Institutional Review Board.

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