Rituximab-associated Vasculitis Flare: Incidence, Predictors, and Outcome

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ABSTRACT. ***Objective.*** To report the incidence, predictors, and outcome of rituximab (RTX)-associated autoimmune disease flare.

Methods. We conducted a retrospective study in a tertiary referral center from 2005 to 2015. Disease flare was defined as the onset of a new organ involvement or worsening of autoimmune disease within 4 weeks following RTX.

Results. Among the 185 patients, we identified 7 disease flares (3.4%). All were due to type II mixed cryoglobulinemia vasculitis. Vasculitis flare occurred after a median time of 8 days (range 2–16) following RTX infusion and included acute kidney insufficiency (n = 7), purpura with cutaneous (n = 7), gastrointestinal (GI) tract involvement (n = 4), and myocarditis (n = 1). Patients with RTX-associated cryoglobulinemia vasculitis flare had these conditions more frequently: renal involvement (p = 0.0008), B cell lymphoproliferation (p = 0.015), higher level of cryoglobulin (2.1 vs 0.4 g/l, p = 0.0004), and lower level of C4 (0.02 vs 0.05, p = 0.023) compared to patients without flare after RTX (n = 43). Four patients (57%) died after a median time of 3.3 months. The 1-year survival rate was poorer in patients with vasculitis flare after RTX compared to their negative counterpart [43% (95% CI 18–100) vs 97% (95% CI 92–100), p < 0.001]. Immunofluorescence analysis of kidney biopsy in patients with worsening RTX-associated vasculitis highlighted the presence of RTX-, IgM-, and IgG1-positive staining of endomembranous deposits and thrombi within kidney lesions.

Conclusion. RTX-associated cryoglobulinemia vasculitis flare is associated with high mortality rate. We provided evidence that kidney lesions are due to immune complex deposition and to glomerular obstruction by cryoglobulinemia and RTX. (First Release March 15 2020; J Rheumatol 2020; 47:896–902; doi:10.3899/jrheum.190076)

Key Indexing Terms:

RITUXIMAB	SIDE EFFECT	AUTOIMMUNITY
CRYOGLOBULINEMIA	VASCULITIS	TREATMENT OUTCOME

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Rituximab (RTX), an anti-CD20 monoclonal antibody, is quite often used to treat hematologic diseases and autoimmune diseases, because of its capacity to target polyclonal B cells promoting autoantibodies production. In autoimmune disorders, RTX is now widely used for antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), cryoglobulinemia vasculitis, and Sjögren syndrome (SS), and less frequently to treat systemic lupus erythematosus (SLE). Main complications include infections, hypersensibility, serum sickness reaction (including fever and

rash), fever, and hypogammaglobulinemia. The administration of RTX is commonly associated with general infusion reactions, including fever, chills, and stiffness, as well as allergic anaphylactoid spectrum reactions such as urticaria, angioedema, and hypotension[1,2]. Symptoms typically occur during infusions. They are more frequent and severe during the first infusion of the drug and are more frequently seen in patients with hematologic malignancies than in autoimmune disease[1,2]. The concurrent administration of corticosteroids and antihistamines with RTX reduces the occurrence of these infusion reactions, and the symptoms can sometimes be reduced with slower infusion rates. Serum sickness hypersensitivity reactions have been described less commonly in patients with autoimmune diseases receiving RTX[1]. Serum sickness reactions are the result of immune activation against the infused agent, and take longer (7–21 days) to appear. Symptoms include fever, rash, and polyarthralgia or arthritis and are linked to host immune-mediated reaction through complement-fixing IgM and IgG antibodies directed toward an immunogenic portion of the drug. Serum sickness has been reported mainly in SS[3].

Data regarding flare of autoimmune disease following RTX are scarce. Few cases of severe worsening of vasculitis symptoms have been reported in patients with cryoglobulinemia vasculitis after RTX[4]. Contrasting with serum sickness, in which symptoms are usually self-limited, vasculitis flare is severe, and frequently involves the kidney, gut, or heart and may be associated with a poor outcome.

In our present study, conducted in a tertiary referral center from 2005 to 2015, we aimed to analyze the incidence, predictors, outcomes, and pathogenesis of RTX-associated disease flare in patients with autoimmune diseases. We identified 185 patients who received RTX for an autoimmune disease, of whom 7 (3.4%) had a disease flare and all flares were due to type II mixed cryoglobulinemia vasculitis. To assess factors associated with cryoglobulinemia vasculitis flare following RTX, we compared the main features and outcome of these 7 patients to those of 43 cryoglobulinemia vasculitis controls without disease flare following RTX. In addition, we provided evidence that worsening of kidney lesions is due to endocapillary proliferation, caused by immune complex deposition and glomerular obstruction by cryoglobulinemia and RTX.

MATERIALS AND METHODS

We conducted a retrospective study, including consecutive patients followed for autoimmune disease, in the Department of Internal Medicine and Clinical Immunology, in Hôpital la Pitié-Salpétrière, Paris. By using the register of the Pharmacy Department, we listed all the patients with autoimmune disease treated with RTX between 2005 and 2014. Pharmacovigilance reporting in our institution and systematic file analysis of the 185 patients who received RTX for an autoimmune disease allowed us to list 7 cases with disease flare after RTX. We provided extended followup in 4 previously reported cases[3,4].

Disease flare was defined as the onset of a new organ involvement or worsening of the autoimmune disease within 4 weeks following RTX. Vasculitis exacerbation should comprise more than 1 visceral manifestation (i.e., kidney or GI tract or neurological involvement, of myocardial lesions) and/or histologic evidence of vasculitis.

All patients received the same protocol of premedication before RTX infusions including intravenous (IV) methylprednisolone (40 mg) and paracetamol (1 g).

To assess the factors associated with disease flare occurring following RTX in cryoglobulinemia vasculitis, we compared the clinical and biological features and outcomes of patients to those of 43 cryoglobulinemia vasculitis controls without disease flare following RTX. For each patient, the following data were recorded: demographic characteristics (sex, age, and geographic origin), type and cause of cryoglobulin, clinical and biological features, and outcome after RTX infusions (exacerbation of the disease after RTX with its clinical and biological characteristics and occurrence of death).

The study was approved by our institutional ethics review board (CPP Ile de France VI) and by the Commission Nationale de l'Informatique et des Libertés; authorization no. 1867484.

Immunohistochemistry. Detection of RTX was performed on frozen kidney biopsy from 1 patient with vasculitis exacerbation after RTX. Before incubation with primary antibodies, Fc receptor was blocked with normal goat serum 5%. Slides were incubated overnight with monoclonal rat IgG2a anti-RTX (dilution 1:20, AbD Serotec) or with isotype control monoclonal rat IgG2a (eBiosciences). Slides were then incubated for 2 h at room temperature with DyLight Alexa 488-conjugated goat anti-rat (working dilution 1:500, Dylight KPF), mounted in Mowiol, and evaluated under fluorescence microscopy. For IgM and IgG staining, slides of frozen biopsy were incubated with undiluted rabbit polyclonal anti-IgM/FITC (Daco F0203) and rabbit polyclonal anti-IgG/FITC (Daco F0202) for 2 h.

Statistical analysis. Baseline characteristics of patients with at least 1 episode of vasculitis were compared to those of patients without flare. Patients with missing files were not included in the analysis. Continuous variables are presented as median (range or interquartile range as appropriate), and were compared between groups using Wilcoxon rank-sum test. Categorical variables are presented as count (percentage) and were compared between groups using Fisher's exact test. RTX-injection episodes were considered as independent observations for the analysis. Their characteristics were compared according to the occurrence of a vasculitis flare, using Fisher's exact test and Wilcoxon rank-sum test as appropriate.

Followup duration was defined from the time between the date of the first RTX injection to the date of death or date of last followup. Overall survival was estimated using Kaplan-Meier estimator and compared between groups using the log rank test.

All tests were 2-sided; p values < 0.05 were considered significant. Analyses were performed using R statistical platform, version 3.0.2.

RESULTS

Incidence and etiologies of autoimmune disease flares following RTX. Among 185 patients with autoimmune disease treated by RTX, there were cases of cryoglobulinemia vasculitis (n = 50), AAV (n = 29), immunologic thrombocytopenic purpura (n = 19), SLE (n = 16), rheumatoid arthritis (n = 13), SS (n = 10), and other autoimmune diseases (n = 48). Seven patients presented flare of autoimmune disease that occurred within 4 weeks after RTX infusions (Figure 1). All cases were due to type II cryoglobulinemia vasculitis. Fifteen patients (8.1%) had a minor reaction after RTX infusion including fever, arthralgia, and cutaneous lesions (n = 4), cutaneous rash (n = 4), fever (n = 3), vomiting/nausea (n = 2), bronchospasm (n = 1), and worsening of cutaneous Kaposi lesions (n = 1).

Clinical features of patients with RTX-associated disease

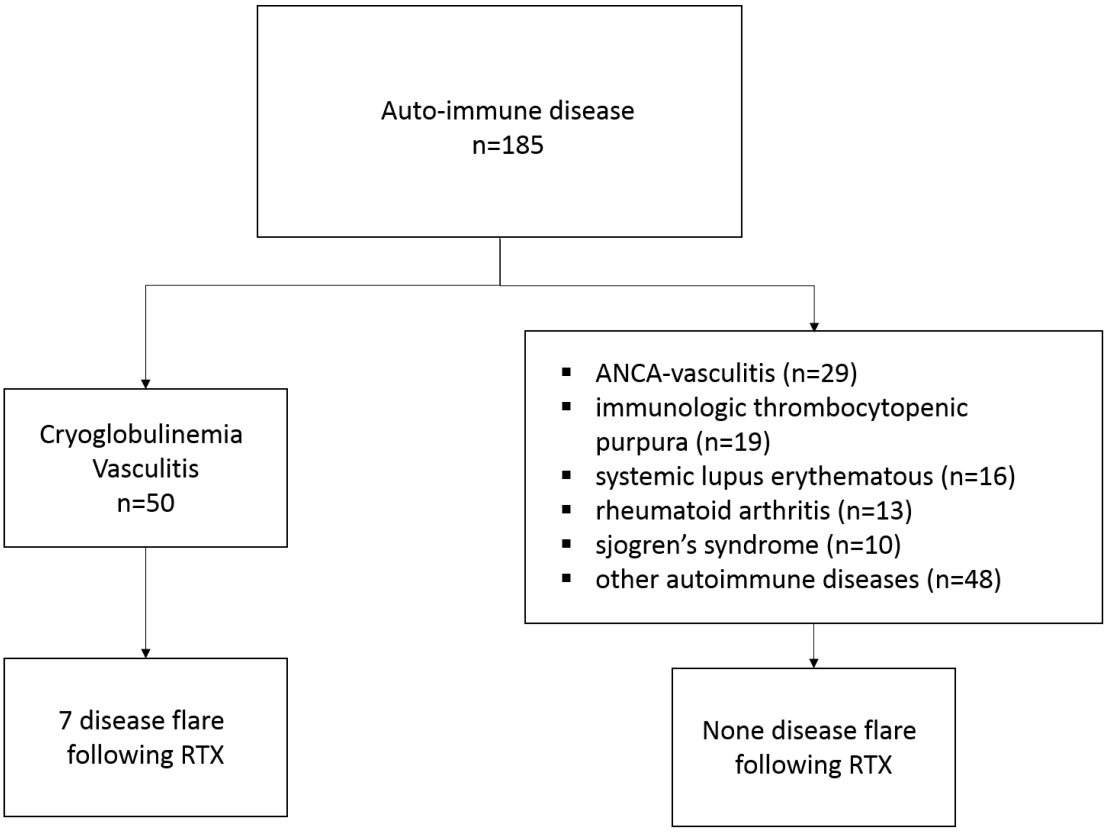

Figure 1. Incidence and etiologies of RTX-associated flares of autoimmune diseases. RTX: rituximab; ANCA: antineutrophil cytoplasmic antibodies.

flare. Data are summarized in Table 1. Five patients (71.4%) were men and the median age was 75 years (range 34-83). Cryoglobulin was of type II (IgMκ) in all cases. Flares occurred after the first cure by RTX in 75% of cases. One patient had 2 vasculitis flares after 2 injections of RTX. Cryoglobulinemia was related to hepatitis C virus (HCV) in 6 patients and considered essential in the remaining one. HCV was cured at the onset of vasculitis flare in 3 cases (37.5%). An underlying B cell lymphoproliferation (defined as monoclonal population in blood associated with

Table 1. Baseline clinical and biological characteristics of patients with worsening MC vasculitis after RTX.

Patient	Sex	Age, yrs	Etiology	HCV Cured	Symptoms	Cryo before/ after RTX, g/l	Creatinemia before/after RTX, μmol/l	Gammaglobulin Level before RTX, g/l	C4 before/ after RTX, g/l	Albumin, g/l
1	M	49	HCV, lympho-proliferation	Yes	Fever, renal, cutaneous, GI tract	0.09/0.52	117/455	5.2	< 0.06/< 0.01	30.4
2	F	78	HCV, SLE, MZL	No	Renal, CNS	1.62/ND	76/200	4.7	0.02/ND	27
				Yes	Renal, cutaneous, neuropathy	0.38/0.57	69/200	2.3	0.01/0.01	43
3	M	78	HCV, MZL	No	Renal, cutaneous, neuropathy	2.2/0.5	125/450	2.9	ND/< 0.01	35
4	M	34	HCV	Yes	Renal, cutaneous, neuropathy	ND/1.5	138/250	ND	ND/0.06	ND
5	M	59	HCV	0	Renal, cutaneous, neuropathy, GI tract	2.56/ND	58/ND	13.8	0.02/ND	39
6	F	75	HCV, lympho-proliferation	0	Renal, cutaneous, neuropathy, GI tract	0.9/1.18	375/430	2.9	0.01/0.02	32
7	M	83	Essential	NA	Cutaneous, neuropathy	1.2/ND	66/172	2.3	< 0.05/ND	ND

Note: Patient 2 had 2 disease flares. MC: mixed cryoglobulinemia; RTX: rituximab; HCV: hepatitis C virus; SLE: systemic lupus erythematosus; MZL: marginal zone lymphoma; CNS: central nervous system; GI: gastrointestinal; Cryo: cryoglobulin; ND: not determined, NA: not applicable.

adenopathy or splenomegaly unrelated to portal hypertension) or an overt lymphoma was diagnosed in 4 patients (57%).

Symptoms at the time of RTX infusion included cutaneous manifestations (purpura and ulcers; n = 7), renal involvement (n = 6; median creatinemia 96.5 μmol/l and median albumin 35 g/l), peripheral neuropathy (n = 6), digestive disorders (n = 3), and central nervous system involvement (n = 1). The median level of cryoglobulinemia was 1.35 (0.8–1.8) g/l. All had rheumatoid factor (RF) activity. All except 1 patient had hypogammaglobulinemia [median level of 2.9 (2.6–5) g/l]. None of the patients had serum antibody against RTX.

All patients but 1 received 4 weekly infusions of 375 mg/m^2 of RTX and the remaining one had 2 infusions of 1 g each (Table 2). Associated treatments at the time of RTX infusions included plasmapheresis (n = 1), bendamustine (n = 1), glucocorticosteroids pulses (n = 1), and oral glucocorticosteroids (n = 2).

Vasculitis flare occurred after a median time of 8 (2-16) days after RTX infusions and was severe in all patients (Table 3). Symptoms included a renal flare with acute kidney insufficiency [n = 7; median creatinemia of 250 (± 139) μmol/l], purpura and/or bullous lesions (n = 7), digestive involvement [n = 4; ischemic colitis requiring surgery (n = 1), severe diarrhea (n = 1), and colitis diagnosed on computed tomography scan (n = 2)], and myocarditis with pulmonary edema (n = 1).

All but 2 patients with acute renal injury had a daily urinary proteins level > 1 g and/or hematuria and/or a histologic proof of renal vasculitis. The remaining patient had anuria and renal biopsy was not performed; however, renal involvement was associated to ischemic colitis (with histologic proof) and cutaneous necrosis, suggesting that renal complications were also related to vasculitis.

All but 1 patient had exacerbation or recurrence of more than 1 vasculitis organ involvement at the time of disease flare. The remaining patient presented a renal flare with acute kidney insufficiency, and the kidney biopsy performed after RTX revealed worsening lesions of membranoproliferative glomerulonephritis as compared to the previous kidney biopsy.

Histological analysis and evidence of RTX staining in vasculitis kidney lesions. Histological analysis was made in 4 patients (colectomy in 1 and renal biopsy in 3) and revealed membranoproliferative glomerulonephritis in 3 kidney biopsies and necrosis in GI tract secondary to ischemic lesions with mild inflammatory infiltration in the colon biopsy. Renal histological analysis was performed before and after RTX in 3 patients and was evaluated by the same pathologist. One patient presented a dramatic exacerbation of kidney lesions including increased endocapillary hypercellularity and the occurrence of thrombi in glomeruli (Figure 2). Histologic analysis of the 2 remaining kidney biopsies revealed extremely severe lesions without improvement after RTX.

Immunofluorescence analysis of kidney biopsy after worsening RTX-associated vasculitis highlighted IgM-, IgG1-, and RTX-positive staining of endomembranous deposits and thrombi within kidney lesions (Figure 2).

Factors associated with occurrence of RTX-associated vasculitis flare. To identify factors associated with RTX-associated vasculitis flare, we compared patients with cryoglobulinemia vasculitis treated by RTX according to the presence (n = 7) or not (n = 43) of disease flare (Table 4). Patients with RTX-associated vasculitis flare had more frequently renal involvement (p = 0.0008), B cell lymphoproliferation (p = 0.015), a higher level of cryoglobulin (2.1 vs 0.4 g/l, p = 0.0004), and a lower level of gammaglobulin (2.9 vs 10.1 g/l, p = 0.005) and of C4 level (0.02 vs 0.05, p = 0.023) before RTX, compared to those without flare after RTX, respectively.

Management and outcome of patients with RTX-associated vasculitis flare. Four patients were admitted to the intensive care unit, 4 required dialysis, and 1 mechanical ventilation.

All flares were treated with corticosteroids (pulses for 7 with a median dose of 3 g) and 5/8 (62.5%) with plasmapheresis (median of 5 per patient). One patient received cyclophosphamide, bendamustine, and RTX. Despite this treatment, 4 patients (57%) died in a median time of 3.3 months. The 1-year survival rate was poorer in patients with vasculitis flare after RTX compared to their negative counterpart [43% (95% CI 18–100) vs 97% (95% CI 92–100), p < 0.001].

Table 2. Treatments associated with RTX.

Patient	RTX Dose, mg	First Cure of RTX	CT, mg/day	Plasmapheresis, n	Chemotherapy
1	375 mg/m^2 ×4	No	10	—	—
2	375 mg/m^2 ×4	Yes	—	—	—
	375 mg/m^2 ×4	No	—	Yes (2)	Bendamustine
3	375 mg/m^2 ×4	Yes	—	—	—
4	375 mg/m^2 ×4	Yes	—	—	—
5	375 mg/m^2 ×4	Yes	Pulse/1000	—	—
6	1 g ×2	Yes	—	—	—
7	375 mg/m^2 ×4	Yes	70	—	—

Note: Patient 2 had 2 disease flares. RTX: rituximab; CT: corticosteroids.

Table 3. Outcome of 7 patients with exacerbation of vasculitis after RTX*.

Patient	Time Between RTX and Exacerbation, days	Symptoms	Histology	Urinary Proteins/Hu	ICU, days	Mechanical Ventilation	Dialysis	CT (dose)	Plasmapheresis, no. courses	IS	Death	Delay Between Flare and Deaths, mos
1	8	Fever, ARI, purpura, GI tract	Proven MPGN after RTX	0.3 g/24 h-Hu	Yes (10)	No	Yes	3 pulses (3 g)	Yes (5)	CYC, RTX, B	Yes	3.6
2	8	Fever*, ARI, cutaneous, pulmonary edema		3 g/l	Yes (10)	No	Yes	Pulses (ND)	Yes (11)	No	No	NA
	5	Fever*, ARI, GI tract*, cutaneous necrosis, hypotension	Ischemic colitis	NA	Yes (15)	Yes	Yes	4 pulses (4 g)	Yes (6)	No	Yes	0.8
3	12	ARI	Worsening renal lesions	1.37 g/24 h-Hu	Yes (7)	No	Yes	3 pulses (3 g)	Yes (5)	No	No	NA
4	13	ARI, purpura	Extremely severe lesions of MPGN	7 g/l-Hu	No	No	No	Pulses (1.5 g)	Yes (3)	No	No	NA
5	2	Cutaneous, GI tract		NA	No	No	No	Pulses (1g)	No	No	No	NA
6	16	ARI, purpura, GI tract*, myocarditis*		0.23 g/l-Hu	No	No	No	Pulses (3 g)	No	No	Yes	3.8
7	4	ARI*, HBP, necrosis		NA-Hu	No	No	No	70 mg	No	No	Yes	3

* New symptoms occurring at relapse. RTX: rituximab; Hu: hematuria; ICU: intensive care unit; IS: immunosuppressant; ARI: acute renal injury; MPGN: membranoproliferative glomerulonephritis; B: bendamustine; GI: gastrointestinal; Cryo: cryoglobulin; CT: corticosteroids; CYC: cyclophosphamide; HBP: hemorrhagic bullous purpura; ND: not determined; NA: not applicable.

DISCUSSION

Disease flares following RTX occur only in cryoglobulinemia vasculitis. RTX is now largely used to treat autoimmune disease. The administration of RTX is commonly associated with general infusion reactions, including fever, chills, and stiffness as well as allergic anaphylactoid spectrum reactions such as urticaria, angioedema, and hypotension[1,2]. Serum sickness hypersensitivity reactions have been described less commonly in patients with autoimmune diseases who are receiving RTX[1]. Data regarding flare of autoimmune disease following RTX are scarce. RTX disease flare occurred in 3.8% of our series and mainly in type II IgMκ cryoglobulinemia vasculitis and with RF activity in all cases. Except for a few cases of cryoglobulinemia flare following RTX, we did not find any reports of other autoimmune disease flare following RTX in the literature[4,5]. RTX is the mainstay of management of non-HCV cryoglobulinemia vasculitis, and the prevalence of RTX-associated vasculitis flare in our series was 14% (7 out 50 patients).

Besides vasculitis flares following RTX, we observed general infusion reactions: fever, urticarial symptoms, arthralgia, non-severe serum sickness disease, and 1 exacerbation of Kaposi lesions. These findings are consistent with literature data[6,7]. Karmacharya, *et al*, in a review, reported 33 patients from the literature with serum sickness (fever, rash, and arthralgia) 7–21 days after RTX, mainly in patients with autoimmune diseases such as SS, cryoglobulinemia, and idiopathic thrombopenic purpura[6]. Moreover, exacerbation of Kaposi lesions has been reported in 10 out of 48 patients with Castleman disease treated with RTX, suggesting an increased immunodeficiency leading to exacerbation of human herpesvirus 8–related complications (such as Kaposi lesions)[7].

Predictors and mechanisms of RTX-associated flare. Serum sickness has been described mainly in patients with SS, cryoglobulinemia, or B cell lymphoma, and in individuals with concomitant hypergammaglobulinemia and RF activity[6]. In our present study, all patients with RTX-associated vasculitis flare had type II IgMκ cryoglobulinemia with RF activity associated with B cell lymphoproliferation in up to 60% of cases. Compared with cryoglobulinemia vasculitis patients who did not develop flare after RTX, those with vasculitis exacerbation were 4 times more likely to have renal involvement and B cell lymphoproliferation and had higher cryoglobulin levels and lower C4 complement levels.

The reasons why cryoglobulin precipitates with RTX in some patients but not in others are not well elucidated. The physical and chemical properties of the immunoglobulin might explain the formation and deposition of immune complex in vessels, particularly in the kidney. Strait, *et al* have found that IgG1-deficient mice immunized with a potent antigen developed a lethal renal disease, with immune complex precipitation in glomerular capillaries, as in cryoglobulinemic humans[8]. They found a specific ability of

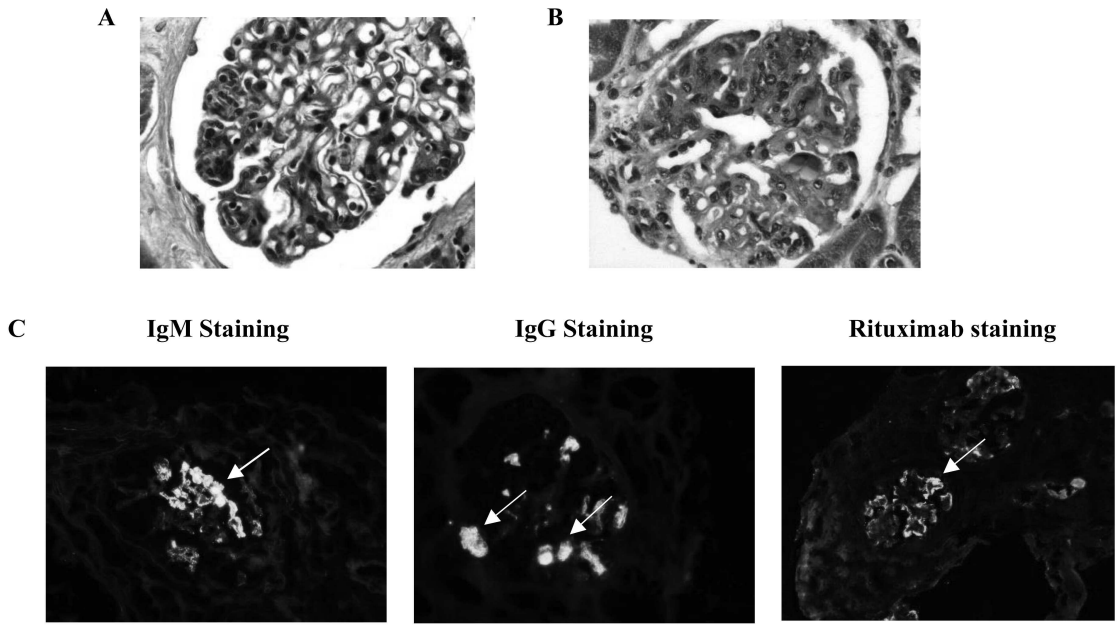

Figure 2. Histological findings in patients with vasculitis flare after RTX. A. Kidney biopsy before RTX: renal glomerulus with endocapillary hypercellularity (Masson's trichrome, ×400). B. Kidney biopsy after RTX: renal glomerulus with increased endocapillary hypercellularity and intracapillary thrombi (Masson's trichrome, ×400). C. Immunofluorescence analysis: IgM-positive staining of endomembranous deposits and thrombi (anti-IgM Ab/FITC, rabbit polyclonal antibody); IgG1-positive staining of endomembranous deposits and thrombi (anti-IgG1 Ab/FITC, rabbit polyclonal antibody); RTX-positive staining of endomembranous deposits (anti-RTX Ab/FITC, rat monoclonal antibody). RTX: rituximab.

IgG1 to inhibit IgG3-mediated renal disease by competing with IgG3 for antigen binding and/or changing immune complex solubility. Moreover, Karsten, *et al* have shown a crucial role of galactosylation of IgG on antiinflammatory effect[9]. Serum sickness has been described mainly in individuals with concomitant RF activity, leading some to speculate about the potential pathogenic IgM RF-RTX complexes[1,2,4]. In cryoglobulinemia vasculitis, our group has previously shown that sera containing Ig with RF activity such as IgMκ–mixed cryoglobulin (not IgGλ) were able to recognize and bind the RTX IgG1κ. The *in vitro* addition of RTX to serum containing an RF-positive IgMκ type II mixed cryoglobulin was associated with accelerated cryoprecipitation[4]. In our current study, we provided new insight that substantiates the immune-mediated reaction through complement-fixing IgM and IgG antibodies directed toward an immunogenic portion of a drug. Histological analysis shows that kidney lesions are due to endocapillary proliferation caused by immune complex deposition and to glomerular obstruction caused by cryoglobulinemia and RTX. Collectively, all these data suggest that physical properties of the polyclonal immunoglobulins secreted by B cells might explain in part the formation of immune complex and the deposition of such a complex in small vessels and their pathological consequences.

Outcome of vasculitis flare following RTX. We have shown here that RTX-associated vasculitis exacerbation has a poor outcome. Fifty-seven percent of patients with vasculitis flare died within 3 months after exacerbation. The 1-year survival rate was half that of patients without RTX-associated vasculitis flare. All RTX-associated flares in our study were severe and most of them included renal involvement with renal acute injury and/or digestive complications with features of ischemic colitis and/or myocarditis. Thus, to avoid such complications, early recognition of patients with a high risk of developing vasculitis flare following RTX is mandatory. Corticosteroids are the mainstay of management of severe serum sickness. In our study, all patients had high-dose corticosteroids, with IV methylprednisolone in 86% and with plasmapheresis in 57% of cases. Contrasting with mild cases of serum sickness in which symptoms are usually self-limited, RTX-associated vasculitis flares were often refractory to corticosteroids and/or plasmapheresis.

RTX-associated autoimmune disease flare is rare (3.8%) and is associated with a high mortality rate (up to 60% at 3 mos) despite aggressive management with immunosuppressants. It occurs mainly in type II IgMκ cryoglobulinemia vasculitis. Vasculitis exacerbations occur within 2 weeks after infusions and are more likely in patients with renal involvement and B cell lymphoproliferation. We provided evidence that worsening of glomerulonephritis is due to endocapillary proliferation caused by immune complex deposition and to glomerular obstruction caused by cryoglobulinemia and RTX. Because of its severity, early recognition of cryoglobulinemia patients with a high risk of RTX-associated flare remains an important challenge for clinicians.

Table 4. Comparison of cryoglobulinemia vasculitis (CV) according to the presence of a disease flare following RTX.

Variables	Patients with Vasculitis Flare, n = 7*	Patients without Vasculitis Flare, n = 43	p
Demographic characteristics			
Sex, male	5 (71)	21 (49)	
Age, median (min) [max]	75.0 (54.0–78.0) [34.0–83.0]	56.0 (49.0–67.0) [24.0–79.0]	0.15
Characteristics of CV			
Type II cryoglobulin	7 (100)	34 (81)	0.58
IgM κ	7 (100)	29 (88)	1
Cryoglobulinemic level at diagnosis, g/l, median (IQR)	2.1 (1.7–2.5)	0.4 (0.2–0.8)	0.0004
Etiology of CV			
Essential	1 (14)	4 (9)	0.55
Lymphoproliferation associated to HCV	4 (57)	5 (12)	0.015
Autoimmune disease	1 (14)	6 (14)	1
HCV infection	6 (86)	31 (72)	0.66
Comorbidities			
Cirrhosis	3 (43)	6 (14)	0.38
Hepatocellular carcinoma	1 (14.3)	2 (5)	
Clinical characteristics of CV			
Fever	1 (12)	4 (7)	0.48
Renal involvement	7 (88)	14 (23)	0.0008
Skin manifestations (purpura, ulcers)	7 (88)	36 (60)	0.24
Neuropathy	6 (75)	29 (48)	0.26
Digestive involvement	2 (25)	3 (5)	0.10
Cardiac involvement	0 (0)	2 (3)	1
Biological features			
Cryoglobulinemia level, g/l, median (IQR)	1.4 (0.8–1.8)	0.4 (0.1–1.3)	0.10
Creatinemia, μmol/l, median (IQR)	96.5 (68.2–128.2)	71.0 (63.0–90.8)	0.22
Gammaglobulin level, g/l, median (IQR)	2.9 (2.6–5.0)	10.1 (6.0–14.9)	0.005
C4 level, g/l, median (IQR)	0.02 (0.01–0.04)	0.05 (0.03–0.09)	0.023
Treatments associated with RTX			
Plasmapheresis	1 (12)	8 (14)	1
Corticosteroids	3 (38)	21 (35)	1
Pulses of corticosteroids	1 (12)	4 (7)	0.48
Corticosteroids at 1 mg/kg	1 (12)	6 (10)	1
Chemotherapy	1 (12)	9 (15)	1
Protocol of RTX			
1 g	1 (12.5)	5 (8.3)	
375 mg/m^2	7 (87.5)	55 (91.7)	

* 7 patients with 8 flares. Values are n (%) unless otherwise specified. RTX: rituximab; IQR: interquartile range; HCV: hepatitis C virus.

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