Rituximab associated vasculitis flare: incidence, predictors and outcome

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Abbreviations: ANCA: Anti-Neutrophil Cytoplasmic Antibodies, HCV: Hepatitis C Virus, SLE: Systemic Erythematous Lupus, RF: Rheumatoid Factor,

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Colors should be used for figure 2.

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Objective: To report the incidence, predictor, and outcome of rituximab-associated autoimmune disease flare.

Methods: We conducted a retrospective study in a tertiary referral centre 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 rituximab.

Results: Among the 185 patients, we identified 7 (3.4%) disease flares. All were due to type II mixed cryoglobulinemia vasculitis. Vasculitis flare occurred after a median time of 8 [2; 16] days following rituximab infusion and included acute kidney insufficiency (n=7), purpura (n=7), gastrointestinal tract involvement (n=4), and myocarditis (n=1). Patients with rituximab-associated cryoglobulinemia vasculitis flare had more frequently renal involvement (p=0.008), B cell-lymphoproliferation (p=0.015), higher level of cryoglobulin (2.1 vs 0.4 g/l, p=0.004) and lower level of C4 level (0.02 vs 0.05, p=0.023) as compared to patients without flare after rituximab (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 rituximab as 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 rituximab associated vasculitis worsening highlighted the presence of rituximab, IgM, and IgG1 positive staining of endomembranous deposits and thrombi within kidney lesions.

Conclusion: Rituximab-associated involves cryoglobulinemia vasculitis and 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 rituximab.

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Introduction:

Rituximab, an anti-CD20 monoclonal antibody, is extremely often used in hematologic diseases and in autoimmune diseases, due to its capacity to target polyclonal B cells promoting auto-antibodies production. In auto-immune disorders, Rituximab is now widely used in ANCA-associated vasculitis, cryoglobulinemia vasculitis, Sjogren's syndrome and less frequently in systemic erythematous lupus (SLE). Main complications include infections, hypersensibility, serum sickness reaction (including fever and skin rash), fever and hypogammaglobulinemia. The administration of rituximab is commonly associated with general infusion reactions, including fever, chills, and rigors, 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 in relationship to the first infusion of the drug and are more frequently seen in patients with hematologic malignancies than autoimmune disease (1,2). The concurrent administration of corticosteroids and antihistamines with rituximab decreases 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 rituximab (1). Serum sickness reactions are the result of immune activation against the infused agent, and take longer (7-21 days) to mobilize. Symptoms include fever, rash and polyarthralgia or arthritis and are are through to be link to host immune mediated reaction through complement-fixing IgM and IgG antibodies directed toward an immunogenic portion of a drug. Serum sickness has been mainly reported in Sjogren's syndrome (3).

Data with respect to flare of autoimmune disease following rituximab are scarce. Few cases of severe worsening of vasculitis symptoms have been reported in patients with cryoglobulinemia vasculitis after rituximab (4). Contrasting with serum sickness where symptoms are usually self-limited, vasculitis flare is severe, frequently involves kidney, gut or heart and may be associated with a poor outcome.

In the present study conducted a study in a tertiary referral centre from 2005 to 2015 we aimed (i) to analysis of the incidence, (ii) predictors (iii) outcomes and (iiii) pathogenesis of rituximab-associated disease flare in patients with autoimmune diseases. We identified 185 patients who received rituximab for an autoimmune disease of whom seven patients (3.4%)

had a disease flare and all were due to type II mixed cryoglobulinemia vasculitis. In order to assess factors associated with cryoglobulinemia vasculitis flare following rituximab we compared the main features and outcome of these 7 patients to those of 43 cryoglobulinemia vasculitis controls without disease flare following rituximab. In addition, we provided evidence that worsening of kidney lesions are due to endocapillary proliferation due to immune complex deposition and to glomerular obstruction by cryoglobulinemia and rituximab.

Patient 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 pharmacy department, we listed all the patients with autoimmune disease treated with Rituximab, between 2005 and 2014. Pharmacovigilance reporting in our institution and systematic file analysis of the 185 patients who received rituximab for an autoimmune disease allowed us to list 7 cases with disease flare after rituximab. We provided extended follow up in four 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 rituximab. Vasculitis exacerbation should comprise more than 1 visceral manifestation (i.e kidney or gastrointestinal tract or neurological involvement, myocardial lesions) and/or histologic evidence of vasculitis.

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

In order to assess the factors associated with disease flare occurring following rituximab in cryoglobulinemia vasculitis we compared the clinical and biological features and outcome of patients to those of 43 cryoglobulinemia vasculitis controls without disease flare following rituximab. For each patient, the following data were recorded: demographic characteristics (gender, age and geographic origin), type and cause of cryoglobulin, clinical and biological features and outcome after rituximab infusions (exacerbation of the disease after rituximab 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 CNIL [(Commission Nationale de l' Informatique et des Libertés); authorisation n°1867484].

Immunohistochemistry

Detection of rituximab was performed on frozen kidney biopsy from 1 patient with vasculitis exacerbation after rituximab. Before incubation with primary antibodies, Fc receptor was blocked with normal goat serum 5%. Slides were incubated over night with monoclonal rat IgG2a anti-rituximab (dilution 1:20, AbD Serotec) or with isotype control monoclonal rat IgG2a (eBiosciences). Slides were then incubated for 2 hours 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 hours.

Statistical analysis

Baseline characteristics of patients with at least one episode of vasculitis are 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. Rituximab-injection episodes were considered as independent observations for the analysis. Their characteristics were compared according to the occurrence of a vasculitis are, using Fisher's exact test and Wilcoxon rank sum test as appropriate.

Follow-up duration was defined from the time between the date of the first rituximab injection to the date of death or date of last follow-up. Overall survival was estimated using Kaplan-Meier estimator and compared between groups using the Log Rank test.

All tests were two-sided; p-values lower than 0.05 were considered as indicating significance. Analyses were performing using R statistical platform, version 3.0.2.

Results:

Incidence and etiologies of auto-immune disease flares following rituximab

Among 185 patients with auto-immune disease treated by rituximab [cryoglobulinemia vasculitis (n=50), ANCA-associated vasculitis (n=29), immunologic thrombocytopenic purpura (n=19), systemic lupus erythematous (n=16), rheumatoid arthritis (n=13), sjogren's syndrome (n=10), and other autoimmune diseases (n=48)]. Seven patients presented flare of autoimmune disease that occurred within 4 weeks after rituximab infusions **(Figure 1)**. All cases were due to type II cryoglobulinemia vasculitis. Fifteen patients (8.1%) had minor reaction after rituximab infusion including sickness disease with 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 rituximab associated disease flare

Data are summarized in **Table 1**. Five (71.4%) patients were men and the median age was of 75 [34; 83] years. Cryoglobulin was of type II (IgM Kappa) in all cases. It was the first cure of rituximab in 75% of flares. One patient had 2 vasculitis flares after 2 injections of rituximab. Cryoglobulinemia was related to HCV in 6 patients and considered as essential in the remaining one. HCV was cured at the onset of vasculitis flare in 3 (37.5%) cases. An underlying B cell-lymphoproliferation (defined as monoclonal population in blood associated with adenopathy or splenomegaly unrelated to portal hypertension) or an overt lymphoma has been diagnosed in 4 (57%) patients.

Symptoms at time of rituximab infusion included cutaneous manifestations (purpura and ulcers) (n=7), renal involvement (n=6) [median creatinemia was of 96.5µmol/L and median albumin level was of 35g/l], peripheral neuropathy (n=6), digestive (n=2) and CNS involvement (n=1). Median level of cryoglobulinemia was of 1.35 (0.8; 1.8) g/L. All had

rheumatoid factor (RF) activity. All except one patient had hypogammaglobulinemia [median level of 2.9 (2.6; 5) g/l]. None of the patients had serum antibody against rituximab.

All patients but 1 received 4 weekly infusions of 375mg/m² of rituximab and the remaining one had 2 infusions of 1g (**Table 1**). Associated treatments at the time of rituximab 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 rituximab infusions and was severe in all patients. Symptoms included a renal flare with acute kidney insufficiency (n=7) [median creatininemia of 250 (+-139) µmol/L)], purpura and/or bullous lesions (n=7), digestive involvement (n=4) [ischemic colitis requiring surgery (n=1), severe diarrhoea (n=1) and colitis diagnosed on CT scan (n=2)] and myocarditis with pulmonary oedema (n=1).

All but one patient with acute renal injury had daily urinary proteins level higher than 1g and/or haematuria 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 one 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 rituximab revealed worsening lesions of membrano-proliferative glomerulonephritis as compared to previous kidney biopsy.

Histological analysis and evidence of rituximab staining in vasculitis kidney lesions

Histological analysis was made in 4 patients (colectomy in 1 and renal biopsy in 3) and revealed membrano-proliferative glomerulonephritis in 3 kidney biopsies and necrosis in gastro-intestinal tract secondary to ischemic lesions with mild inflammatory infiltration in the colon biopsy. Renal histological analysis was performed before and after rituximab in 3 patients and was evaluated by the same pathologist. One patient presented a dramatically

exacerbation of kidney lesions including increased endocapillary hypercellularity and the occurrence of thrombi in glomeruli **(Figure 2).** Histologic analysis of the two remaining kidney biopsies revealed extremely severe lesions without improvement after rituximab.

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

Factors associated with occurrence of rituximab associated vasculitis flare

To identify factors associated **with rituximab associated vasculitis flare** we compared patients with cryoglobulinemia vasculitis treated by rituximab according to the presence (n=7) or not (n=43) of disease flare **(Table 3)**. Patients with rituximab associated vasculitis flare had more frequently renal involvement (p=0.008), B cell-lymphoproliferation (p=0.015), a higher level of cryoglobulin (2.1 vs 0.4 g/l, p=0.004) and 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 rituximab as compared to those without flare after rituximab, respectively.

Management and outcome of patients with rituximab associated vasculitis flare

Four patients were admitted in 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 plasmapheresis per patient) One patient received cyclophosphamide, bendamustine and rituximab. 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 rituximab as compared to their negative counterpart [43% (95% CI: 18-100) vs 97% (95% CI: 92-100), p<0.001].

Discussion:

Disease flares following rituximab occur only in cryoglobulinemia vasculitis.

Rituximab is now largely used to treat autoimmune disease. The administration of rituximab is commonly associated with general infusion reactions, including fever, chills and rigors 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 receiving rituximab (1). Data with respect to flare of autoimmune disease following rituximab are scarce. Rituximab disease flare occurred in 3.8% of our series and mainly in type II IgMk cryoglobulinemia vasculitis and with rheumatoid factor activity in all cases. Except few cases report of cryoglobulinemia flare following rituximab we did not find any report of other autoimmune disease flare following rituximab in the literature (4,5). Rituximab is the mainstay of management of non-HCV cryoglobulinemia vasculitis and the prevalence of rituximab associated vasculitis flare in our series was of 14% (7 out 50 patients).

Besides vasculitis flares following rituximab, we observed general infusions reactions, fever, urticarial, arthralgia, non-severe serum-sickness disease and one exacerbation of Kaposi lesions. These findings are consistent with literature data (6,7). Karamacharya et al, in a review of, reported 33 patients from the literature with serum sickness (fever, rash and arthralgia) 7-21 days after rituximab, mainly in patients with autoimmune disease such as Sjogren's syndrome, cryoglobulinemia and idiopathic thrombopenic purpura (6). Moreover, exacerbation of Kaposi lesions have been reported in 10 out of 48 patients with Castleman disease treated with rituximab, suggesting an increased immunodeficiency leading to exacerbation of HHV8-related complications such as Kaposi lesions. (7)

Predictors and mechanisms of rituximab-associated flare

Serum sickness has been mainly described in patients with Sjogren's syndrome, cryoglobulinemia or B cell lymphoma and in individuals with concomitant

hypergammaglobulinemia and rheumatoid factor activity (6). In the present study, all patients with rituximab associated vasculitis flare had type II IgMk cryoglobulinemia with rheumatoid factor activity associated with B cell lymphoproliferation in up to 60% of cases. As compared with cryoglobulinemia vasculitis patients who did not develop flare after rituximab those with vasculitis exacerbation were 4 times more likely to have renal involvement and B cell-lymphoproliferation and had higher cryoglobulin level and lower C4 complement level.

The reasons why cryoglobulin precipitates with rituximab in some patients but not in the others are well not 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 cryoglobulinaemic humans (8). They found a specific ability of 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 also shown a crucial role of galactosylation of IgG on anti-inflammatory effect (9). Serum sickness has been mainly described in individuals with concomitant rheumatoid factor activity ,leading some to speculate about the potential pathogenic IgM rheumatoid factor-rituximab complexes (1,2,4). In cryoglobulinemia vasculitis, our group have previously shown, that sera containing Ig with RF activity such as IgM kappa mixed cryoglobulin (not IgGλ) was able to recognize and bind the rituximab IgG1k. The in vitro addition of rituximab to serum containing an RF-positive IgMk type II mixed cryoglobulin was associated with accelerated cryoprecipitation (4). In the present 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 due to immune complex deposition and to glomerular obstruction by cryoglobulinemia and rituximab. 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 complex in small vessels and their pathological consequences.

Outcome of vasculitis flare following rituximab

We have shown here that rituximab 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 2 times lower as compared to patients without rituximab-associated vasculitis flare. All rituximab-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, in order to avoid such complications, early recognition of patients with a high risk of developing vasculitis flare following rituximab is mandatory. Corticosteroids are the mainstay of management of severe of 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 where symptoms are usually self-limited rituximab associated vasculitis flares were often refractory to corticosteroids and/or plasmapheresis.

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

Table 1: Baseline characteristics of patients with flare of cryoglobulinemia vasculitis after rituximab

Table 2: Outcome of 7 patients with exacerbation of vasculitis after rituximab

Tables 3: Comparison of cryoglobulinemia vasculitis according to the presence or not of a disease flare following rituximab.

Figure legends

Figure 1. Incidence and etiologies of rituximab associated disease flares of autoimmune diseases

Figure 2. Histological findings in patients with vasculitis flare after rituximab

A. Kidney biopsy before Rituximab: Renal glomerulus with endocapillary hypercellularity (Masson's trichrome, x400)
B. Kidney biopsy after Rituximab: Renal glomerulus with increased endocapillary hypercellularity and intracapillary thrombi (Masson's trichrome, x400)
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); Rituximab positive staining of endomembranous deposits (Anti-Rituximab Ab/FITC, rat monoclonal antibody)

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Table 1: Baseline characteristics of patients with worsening MC vasculitis after rituximab

Clinical and biological characteristics											Treatments associated with rituximab				
Patient	**Sex**	**Age**	**Etiology**	**HCV cured**	**Symptoms**	**Cryo before/ after RTX (g/l)**	**Creati-nemia before/ after RTX (µmol/l)**	**γ-globulin level before RTX (g/l)**	**C4 before/ after RTX (g/l)**	**Albu-min (g/l)**	**Rituximab dose (mg)**	**First cure of RTX**	**CT (mg/day)**	**Plasma-pheresis (number)**	**Chemo-therapy**
1	M	49	HCV, lympho-prolifération	Yes	Fever, renal, cutaneous, GI tract	0.09/0.52	117/455	5.2	<0.06/ <0.01	30.4	375mg/m2 x4	No	10	–	–
2	F	78	HCV, Lupus, MZL	No	Renal, CNS	1.62/ND	76/200	4.7	0.02/ ND	27	375mg/m2x 4	Yes	–	–	–
				Yes	Renal, cutaneous, neuropathy	0.38/0.57	69/200	2.3	0.01/ 0.01	43	375mg/m2 x 4	No	–	yes (2)	Benda-mustine
3	M	78	HCV, MZL	No	Renal, cutaneous, neuropathy	2.2/0.5	125/450	2.9	ND/ <0.01	35	375mg/m2 x4	Yes	–	–	–
4	M	34	HCV	Yes	Renal, cutaneous, neuropathy	ND/1.5	138/250	ND	ND/ 0.06	ND	375mg/m2 x 4	Yes	–	–	–
5	M	59	HCV	0	Renal, cutaneous, neuropathy, GI tract	2.56/ND	58/ND	13.8	0.02/ ND	39	375mg/m2 x4	Yes	Pulse /1000	–	–
6	F	75	HCV, lympho-prolifération	0	Renal, cutaneous, neuropathy, GI tract	0.9/1.18	375/430	2.9	0.01/ 0.02	32	1g x 2	Yes	–	–	–
7	M	83	Essential	NA	cutaneous, neuropathy	1.2/ND	66/172	2.3	<0.05/ ND	ND	375mg/m2 x4	Yes	70	–	–

Abbrevations: RTX: Rituximab, M: Male, HCV: hepatitis C virus, MZL: Marginal zone lymphoma, MPGN: Membranous proliferative glomerulonephritis, GI tract: Gastro-intestinal tract, Cryo: Cryoglobulin, γ: Gammaglobulin, CT: corticosteroids, ND: Not determined, NA: Not applicable.

Table 2: Outcome of 7 patients with exacerbation of vasculitis after rituximab*

Patient	Time between RTX and exacerbation (days)	Symptoms	Histology	Urinary proteins/ Hu	ICU (days)	Mechanical ventilation	Dialysis	CT (dose)	Plasma-pheresis (number of courses)	IS	Death	Delay Between flare and deaths (months)
1	8	Fever, ARI, purpura, GI tract	Proven MPGN after RTX	0.3g/24h-Hu	Yes (10)	No	Yes	3 pulses (3g)	Yes (5)	CyC, RTX, B	Yes	3.6
2	8	Fever*, ARI, cutaneous, pulmonary oedema		3g/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 (4g)	Yes (6)	No	Yes	0.8
3	12	ARI	Worsening renal lesions	1.37g/24h-Hu	Yes (7)	No	Yes	3 pulses (3g)	Yes (5)	No	No	NA
4	13	ARI, purpura	Extremely severe lesions of MPGN	7g/l-Hu	No	No	No	Pulses (1.5g)	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 (3g)	No	No	Yes	3.8

7	4	ARI*, blebs, necrosis		NA -Hu	No	No	No	70mg	No	No	Yes	3

(Abbreviations: RTX: Rituximab, M: ARI: Acute renal injury, MPGN: Membrano-proliferative glomerulonephritis, B: Bendamustine, GI tract: Gastro-intestinal tract, Cryo: Cryoglobulin, CT: corticosteroids,CyC: Cyclophosphamide, ND: Not determined, NA: Not applicable, *new symptoms occurring at relapse

Tables 3: Comparison of cryoglobulinemia vasculitis according to the presence or not of a disease flare following rituximab.

	Patients with vasculitis flare (n=7)*	**Patients without vasculitis flare (n=43)**	
Demographic characteristics			
Gender, male	5 (71%)	21 (49%)	
Age, median [min; max]	75.0 (54.0 to 78.0) [34.0 to 83.0]	56.0 (49.0 to 67.0) [24.0 to 79.0]	0.15
Characteristics of CV			
Type II cryoglobulin	7 (100%)	34 (81%)	0.58
IgM Kappa	7 (100%)	29 (88%)	1
Cryoglobulinemic level at diagnosis (g/l), median (IQT)	2.1 (1.7 to 2.5)	0.4 (0.2 to 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 (IQT)	1.4 (0.8 to 1.8)	0.4 (0.1 to 1.3)	0.10
Creatinemia (µmol/l), median (IQT)	96.5 (68.2 to 128.2)	71.0 (63.0 to 90.8)	0.22
Gammaglobulin level (g/l), median (IQT)	2.9 (2.6 to 5.0)	10.1 (6.0 to 14.9)	**0.005**
C4 level (g/l), median (IQT)	0.02 (0.01 to 0.04)	0.05 (0.03 to 0.09)	**0.023**
Treatments associated with Rituximab			
Plasmapheresis	1 (12%)	8 (14%)	1
Corticosteroids	3 (38%)	21 (35%)	1
Pulses of corticosteroids	1 (12%)	4 (7%)	0.48
Corticosteroids at 1mg/kg	1 (12%)	6 (10%)	1

Chimiotherapy	1 (12%)	9 (15%)	1
Protocol of Rituximab			
1g	1 (12.5%)	5 (8.3%)	
375mg/m2	7 (87.5%)	55 (91.7%)	

Data are expressed as n, % or median (IQR: Interquartile range) CV: Cryoglobulinemia vasculitis.* 7 patients with 8 flares

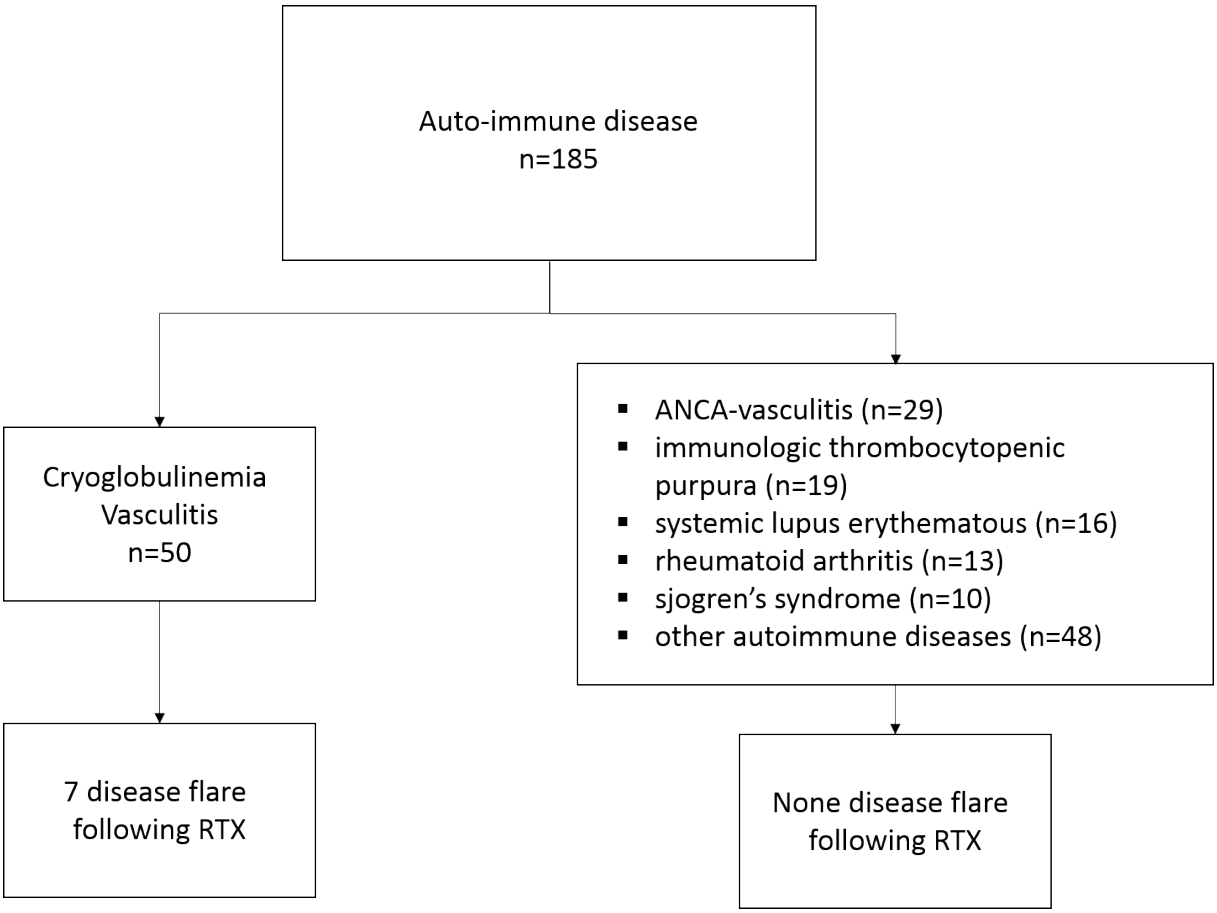

Figure 1. Incidence and etiologies of rituximab associated disease flares of autoimmune diseases

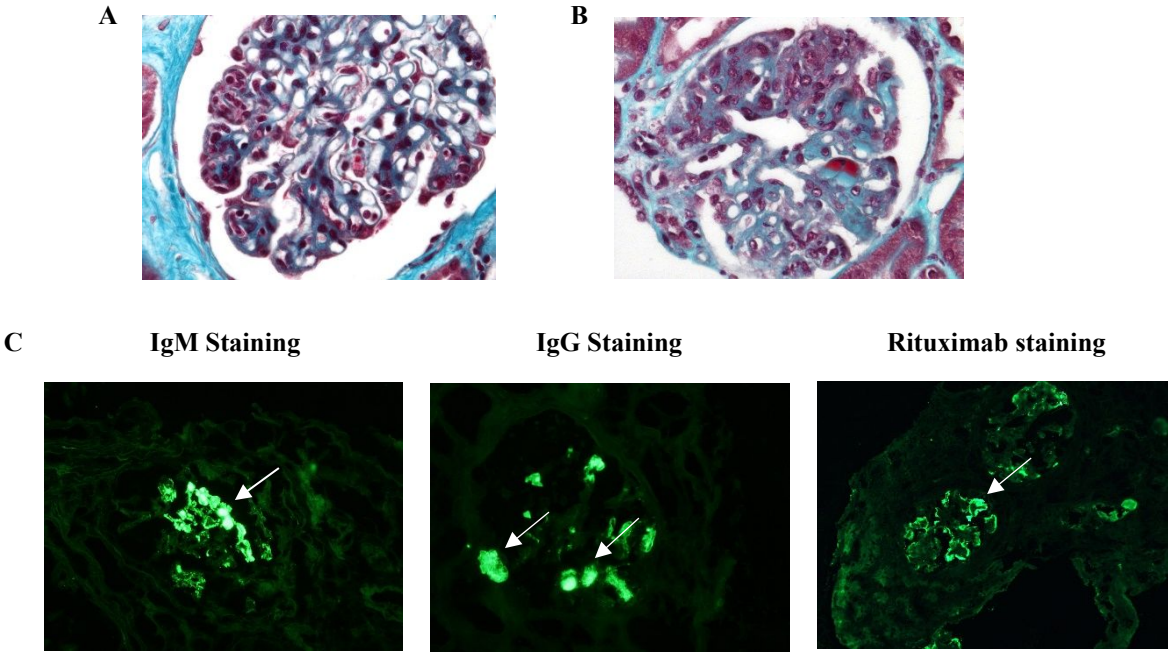

Figure 2. Histological findings in patients with vasculitis flare after Rituximab.