Rituximab for the treatment of adult-onset IgA vasculitis (Henoch-Schönlein purpura)

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Rituximab for the treatment of adult-onset IgA vasculitis (Henoch-Schönlein purpura)

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ABSTRACT

Objective. Adult-onset IgA vasculitis (IgAV, formerly Henoch-Schönlein purpura) is a rare systemic vasculitis characterised by IgA1-dominant deposits. The treatment of adult-onset IgAV is controversial and is based on the combination of glucocorticoids and immunosuppressive agents, but many patients have refractory or relapsing disease despite treatment. Rituximab is a B cell-depleting antibody of proven efficacy in anti-neutrophil cytoplasmic antibody-associated vasculitis. We tested the efficacy and safety of rituximab in a multicentre cohort of patients with adult-onset IgAV.

Methods. In this multicentre, observational study we included patients with adult-onset IgAV who had received rituximab for either refractory/relapsing disease or because they had contraindications to conventional glucocorticoid/immunosuppressive therapy. We analysed the rates of remission (defined on the basis of the Birmingham Vasculitis Activity Score, BVAS) and relapse, and the variations over time in estimated glomerular filtration rate (eGFR), proteinuria, C-reactive protein (CRP) levels, BVAS and prednisone dose.

Results. Twenty-two patients were included; their median follow-up was 24 months (interquartile range, 18-48). Sixteen patients received rituximab as add-on therapy and six as monotherapy. Twenty patients (90.9%) achieved remission and seven (35%) subsequently relapsed. There was a significant reduction in 24h-proteinuria ($p<0.0001$), C-reactive protein ($p=0.0005$), BVAS ($p<0.0001$), and prednisone dose ($p<0.0001$) from rituximab initiation through last follow-up; eGFR remained stable. Rituximab was generally well tolerated. One patient died after 60 months of follow-up.

Conclusions. Our data suggest that rituximab is an effective and safe therapeutic option for adult-onset IgAV.

INTRODUCTION

Immunoglobulin A (IgA) vasculitis (IgAV), formerly Henoch-Schönlein purpura, is a systemic small-vessel vasculitis characterised by IgA1-dominant immune deposits. Clinical manifestations include purpura, gastrointestinal involvement, arthritis, and glomerulonephritis.[1] IgAV is the most common vasculitis in children, whereas it is rare in adults. Childhood-onset IgAV usually has a benign course only requiring supportive care. In adults, its prognosis is worse, particularly because of the frequency and severity of renal involvement; indeed, adult-onset IgAV leads to advanced chronic kidney disease (CKD) or end-stage renal disease (ESRD) in 10-30% of the patients with renal involvement.[2]

The treatment of adult-onset IgAV is still a matter of debate. Glucocorticoids and immunosuppressive drugs are routinely used especially in patients with organ- or life-threatening manifestations.[3] However, the efficacy of these treatments is controversial. In the only randomised trial performed in adult-onset IgAV, the addition of cyclophosphamide to glucocorticoids failed to provide any clear benefit.[4] Other immunosuppressive agents such as azathioprine, mycophenolate mofetil or cyclosporine have only been reported in anecdotal cases.[3,5] Therefore, effective therapies are needed in adult-onset IgAV.

The anti-CD20 monoclonal antibody rituximab (RTX) has become a standard treatment for remission-induction and remission-maintenance in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.[6,7] RTX induces B-cell depletion and thus dampens the expansion of plasma cells that produce pathogenic autoantibodies. RTX can also impair antigen-presentation by B-cells or reduce B cell-mediated stimulation of T-cells and other inflammatory cell types.[6] Recent reports have shown that RTX may be effective for adult-onset IgAV, but they were based on single cases or small case series.[8,9] In this multicentre, observational study, we analysed the effectiveness and safety of RTX in adult-onset IgAV patients who were either relapsing/refractory or had contraindications to conventional glucocorticoid/immunosuppressive therapy.

METHODS

Patients and treatment

We reviewed the clinical records of all patients with adult-onset IgAV treated with RTX at nine different Vasculitis Centres in Europe. The diagnosis of IgAV was based on the 1990 American College of Rheumatology criteria[10] and the 2012 revised Chapel Hill Consensus Conference nomenclature.[1] All patients had to have a biopsy-proven diagnosis of IgAV. The patients were included if: i) their age at IgAV diagnosis was >18 years; ii) they had severe involvement of at least one organ (including biopsy-proven IgAV-related nephritis class 3-4;[2] gastrointestinal involvement with haemorrhage, ischemia, perforation and/or abdominal pain unresponsive to common analgesics and lasting for >24h; pulmonary haemorrhage, episcleritis, cardiac and central nervous system involvement); iii) other systemic autoimmune or neoplastic diseases were excluded; iv) RTX was given for the treatment of relapsing or refractory disease or because there were contraindications to the use of standard-dose glucocorticoids and/or conventional immunosuppressants. Allowed RTX schedules included 375 mg/m^2/week for four consecutive weeks or 1000 mgx2 given two weeks apart; RTX could be given alone or on top of other immunosuppressive therapies. Glucocorticoids and other immunosuppressants were tapered or withdrawn according to local practice. RTX was obtained by local hospital pharmacies for off-label use; all patients signed an informed consent. The study was performed in accordance with the declaration of Helsinki and received ethical approval by Parma University Institutional Review Board.

Data collection and disease activity assessment

We retrieved data regarding patient demographics, clinical manifestations, disease duration and treatments prior to RTX therapy and during the post-RTX follow-up, and reviewed all the pathology reports of diagnostic biopsies. With regards to the post-treatment follow-up, we collected clinical and serological data at specific time-points (months 1, 6, 12, and last follow-up). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology collaboration) formula. Information regarding any type of

adverse events was also collected; adverse events were graded using the Common Terminology Criteria for Adverse Events version 4.0.[11] Disease activity was evaluated using the Birmingham Vasculitis Activity Score (BVAS), version 3.[12] This score refers to new or worsening symptoms due to active vasculitis, with higher scores indicating a more active disease. Remission was defined as BVAS=0, or BVAS≤5 if all scores were due to persistent haematuria or proteinuria in the presence of stable or improving renal function. Relapses were defined as an increase in disease activity requiring the reinstitution of glucocorticoids or other immunosuppressants, or a significant increase in glucocorticoid dose (>50% for prednisone doses ≥15 mg/day or >100% for doses ≤12.5 mg/day). The disease was defined as refractory in case of lack of response to standard-dose glucocorticoids, alone or combined with other immunosuppressive therapies.

Tissue biopsies

Renal biopsy findings compatible with a diagnosis of IgAV-related nephritis were classified as follows according to Pillebout *et al.*[2] class 1, “mesangiopathic” glomerulonephritis; class 2, focal and segmental glomerulonephritis (segmental endo- and/or extra-capillary proliferation involving <50% of the glomeruli); class 3, diffuse and severe endocapillary proliferative glomerulonephritis; class 4, endo- and extra-capillary glomerulonephritis; class 5, global glomerular sclerosis involving >90% of the glomeruli.

In order to investigate which inflammatory cell types are more abundant in the affected organs of patients with IgAV, we performed immunohistochemical analyses of three renal biopsies and three skin biopsies from patients included in this study. In addition to routine stainings (*e.g.*, haematoxylin and eosin, periodic acid Schiff) and immunofluorescence (to detect immunoglobulin and complement fraction deposition), we also performed immunohistochemistry using monoclonal antibodies directed to the B-cell marker CD20, the T-cell marker CD3, and the macrophage marker CD163.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR), while categorical variables as n(%). Variations in continuous variables between two time-points were assessed using the Wilcoxon's test. Variations in continuous variables across different time-points (baseline, month 1, month 6, month 12, last follow-up) were assessed using Friedman's test. P values were corrected using Dunn's test for multiple comparisons. Corrected p values <0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 5.

RESULTS

Twenty-two patients were included in this study. Six cases were described in previous papers;[8,9] the data presented here regarding these six patients are based on a longer follow-up than that originally reported. Of the 22 patients, 12 were men; their median age at diagnosis was 37.5 years (IQR 22.8–49.8). At diagnosis, the most frequent clinical manifestation was purpura (21 patients, 95.5%); it predominantly affected the lower limbs and was necrotic or bullous in six cases (27.3%). Gastrointestinal involvement was found in 18 (81.8%); all experienced abdominal pain and seven (39%) gastrointestinal haemorrhage. Twenty patients (90.9%) had kidney involvement. The median eGFR was 76 ml/min/1.73m^2 (IQR 65–104), and the median 24h-proteinuria 1900 mg (IQR 580–3275) *(table 1)*. One patient was dialysis-dependent at diagnosis but became dialysis-free after initial therapy (before RTX). Fifteen (68.2%) underwent kidney biopsy, which confirmed IgAV in all cases. A diffuse endo- and/or extra-capillary IgAV-glomerulonephritis (classes 3-4) was observed in nine cases (60%). At diagnosis, disease activity assessment showed a median BVAS of 16.5 (IQR 13.0–23.8) (*table 1*).

Sixteen patients (73%) had received other treatments prior to RTX: all of them had received glucocorticoids, alone or combined with cyclophosphamide, azathioprine, mycophenolate mofetil or other drugs (*table 1*). These 16 patients received RTX (only one as monotherapy) for refractory or relapsing disease. The remaining six received RTX as first-line therapy (five

as monotherapy and one together with low-dose glucocorticoids) because they had contraindications to standard-dose glucocorticoids or other immunosuppressive agents. Overall, 16 patients received RTX as add-on therapy and six as monotherapy.

At RTX initiation, 18 patients (81.8%) had skin manifestations, 15 (68.2%) gastrointestinal involvement, 18 (81.8%) kidney involvement, and 16 (72.7%) arthritis or arthralgia. The median BVAS was 15.0 (IQR 9.5–19.8); the median eGFR was 82 ml/min/1.73m^2 (IQR 65-101) and the median 24h-proteinuria 1700 mg (IQR 750-2375) (*table 2*).

One month after RTX, 10 patients (45.5%) had achieved remission and BVAS significantly decreased (median, 6 at month 1 vs. 15 at baseline, p=0.0005) (*figure 1*). At month 6, 16 patients (72.7%) were in remission and six (27.3%) had active disease (three because of persisting disease and three because of relapse after remission) (*table 2*); as compared with baseline, BVAS and prednisone dose decreased (p=0.0008 and p=0.03, respectively). At month 12, 16 patients (72.7%) were in remission and six (27.3%) had active disease (persistent disease in five and relapse in one); reductions vs. baseline were observed for BVAS (p=0.0005), proteinuria (p=0.001), C-reactive protein (CRP) (p=0.0007), and prednisone dose (p=0.01) (*figure 1*). Thus, during the first 12 months after RTX, remission was achieved by 20 patients (cumulative incidence of remission 90.9%), four of whom relapsed. After remission, no patient received RTX as maintenance therapy. Of the seven patients who were taking immunosuppressants at the time of RTX treatment, three stopped them (*table 2*). Eight patients were taking ACE-inhibitors and/or angiotensin receptor blockers at the time of RTX treatment; four stopped them during the first 12 months after RTX (*table 2*), but resumed them afterwards, usually for hypertension, persistent proteinuria or non-specific nephroprotection.

We analysed the outcomes at months 6 and 12 of the six patients who received RTX alone vs. those of the 16 patients who received RTX and glucocorticoids/immunosuppressants; no significant differences emerged (*supplementary table*). No differences in terms of remission and relapse rates were found between patients treated with either of the two different RTX

regimens and between patients who received RTX as first-line therapy vs those who received RTX for refractory/relapsing disease (data not shown).

The median follow-up from the start of RTX was 24 months (IQR 18-48). At last follow-up, 18 patients were in remission, three had active disease, and one was deceased (month 60). Of the patients in remission, three were taking low-dose prednisone (2.5-5 mg/day), and two low-dose prednisone and immunosuppressants. Reductions from RTX initiation through last follow-up were observed for BVAS ($p<0.0001$), 24h-proteinuria ($p<0.0001$), CRP ($p=0.0005$), and prednisone dose ($p<0.0001$) (*figure 1*). eGFR did not change significantly (median 77 ml/min/1.73 m^2 at last follow-up vs 82 at baseline). At last follow-up, one patient had reached ESRD requiring hemodialysis (month 13 after RTX), one had stage 4 CKD, four had 24h-proteinuria>1g; eight were taking ACE-inhibitors or angiotensin-receptor blockers. Microhematuria persisted in eight patients.

When considering the entire follow-up, we recorded relapses in seven of the 20 patients (35%) who had achieved remission. Relapses occurred a median of 12 months (IQR 6.5-15) after RTX; they involved the kidney in four cases, the gastrointestinal tract in one and the skin in two. Of the four patients with kidney relapses, three were re-treated with RTX and all achieved remission, one only received ACE-inhibitors and progressed to ESRD. The two patients with skin relapses were successfully treated with either sulfasalazine (one case) or low-dose glucocorticoids (one case); the patient with gastrointestinal relapse also responded to resumption of low-dose glucocorticoids.

Circulating $CD19^+$ B-cell levels were not systematically tested and were only available for 11 patients, all of whom achieved B-cell depletion ($<0.01x10^9$ cells/L) after RTX; given the paucity of data, we could not establish any association between B-cell return and relapse.

RTX therapy was generally well tolerated. Two patients experienced infusion reactions: one developed bullous urticaria (grade 3) that responded to oral steroids, while the other developed mild and self-limited dyspnoea (grade 2). In the former patient, the reaction precluded subsequent drug infusions. One patient died because of advanced liver cirrhosis

(of undetermined aetiology) and pneumonia (month 60 after RTX), but this fatal event was probably unrelated to RTX, given the long period of time elapsed since RTX therapy.

DISCUSSION

Adult-onset IgAV is a rare, often severe, small-vessel vasculitis; if left untreated, it usually has an adverse prognosis.[2] Current treatments, including high-dose glucocorticoids and cyclophosphamide, are only partially effective and their toxicity may be high.[3,4] Our results identify RTX as an effective therapeutic option for adult-onset IgAV. In our study, RTX was given for refractory/relapsing disease or as first-line therapy when conventional immunosuppressive agents were contraindicated. The remission rate after RTX was 91%, with most patients achieving remission within six months of therapy. Remission was defined based on the BVAS, a widely used score for disease activity assessment in systemic vasculitides. However, other parameters such as CRP levels and prednisone dose also declined after RTX. Renal outcomes were good: although eGFR remained stable, the reduction in proteinuria was marked (approximately 5-fold at end of follow-up vs. baseline). Only one patient reached ESRD and one developed stage 4 CKD. Interestingly, the outcomes of the patients treated with RTX-monotherapy appeared to be similar to those of the patients receiving combination therapies.

Relapses were common (relapse rate 35%) after RTX-induced remission; their clinical presentation was variable, and in severe cases re-treatment with RTX proved effective. Overall, RTX was well tolerated; no major treatment-related adverse events were recorded.

The potential mechanisms of action of RTX in IgAV are only speculative. As in IgA nephropathy,[13] also in IgAV immune-complexes containing galactose-deficient IgA1 (Gd-IgA1) are thought to accumulate in target organs and initiate tissue damage. We could neither measure serum Gd-IgA1 nor Gd-IgA1-containing immune-complexes; total serum IgA levels, available in 17/22 patients, showed a slight, progressive reduction after RTX (p=0.03) (*supplementary figure 1*). However, the slow kinetics and the minor degree of IgA reduction

did not seem to mirror the clinical activity of RTX. We also performed immunohistochemistry on renal and skin biopsies of IgAV patients (*supplementary figure 2*) to investigate which cell types infiltrate target tissues: in all cases, $CD20^+$ B-cells were either absent or scarce, whereas most infiltrating cells were $CD3^+$ T-cells or $CD163^+$ macrophages. Thus, although it cannot be excluded that RTX in IgAV reduces the expansion of IgA-producing plasma cells or depletes tissue-infiltrating B-cells, its therapeutic efficacy most likely results from the impairment of other B-cell functions such as antigen-presentation and T-cell co-stimulation, as already postulated in ANCA-associated vasculitis and other autoimmune conditions.[6]

RTX has been recently investigated in a randomised trial of patients with IgA nephropathy. In this trial, which enrolled patients at high risk of progression (persistent proteinuria >1g/day despite ACE-inhibitors or angiotensin-receptor blockers), RTX failed to reduce proteinuria at 12 months; serum levels of Gd-IgA1 or antibodies against Gd-IgA1 were also unaffected.[14] The apparent contrast between our positive findings and the lack of efficacy of RTX in the IgA nephropathy trial can be due to several reasons. The chronic and advanced stage of IgA nephropathy of the patients enrolled in the trial may have impaired their response to RTX.[14] Additionally, it must be acknowledged that IgAV and IgA nephropathy are clinically distinct, with IgAV being certainly more “inflammatory”.[15] Their genetic associations are also different: although they are both associated with HLA class II variants, other associations with polymorphisms of genes involved in innate immune responses (*e.g.*, *ITGAM*, *CARD9*) were only demonstrated for IgA nephropathy.[13,15]

Our study has limitations, mainly related to its retrospective nature and the small sample size. Also, it cannot be excluded that concomitant treatments such as other immunosuppressants, ACE-inhibitors or angiotensin-receptor blockers, although limited to a minority of patients, influenced patients’ responses. Nevertheless, this study represents the largest reported experience on the use of RTX in adult-onset IgAV. We observed that RTX effectively induced disease remission, allowed glucocorticoid tapering and was well tolerated. Larger, controlled studies are warranted to clarify its role in the management of this rare condition.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

FM and AV designed the study, collected data, analysed results and wrote the manuscript. MN, GT and MI performed histological and immunohistochemical analysis. MG performed statistical analysis. RF, EP, GE, MLU, RR, ES, AJM, DJ, PE, MS, PN, HH and DR contributed clinical and serologic data of the patients included in the study. All authors revised the manuscript critically for important intellectual content and approved the final version.

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Table 1. Main characteristics of the 22 patients with IgA vasculitis

	n=22
Male gender, n (%)	12 (54.5)
Age at diagnosis, median (IQR)- *years*	37.5 (22.8-49.8)
Organ involvement at diagnosis, n (%)	
Skin	21 (95.5)
Gastro-intestinal	18 (81.8)
Kidney	20 (90.9)
Joint	17 (77.3)
Other sites*	1 (4.5)
BVAS, median (IQR)	16.5 (13.0-23.8)
eGFR (CKD-EPI), median (IQR)- *ml/min/1.73m^2*	76.0 (65.0-104.0)
Proteinuria, median (IQR)- *mg/24h*	1900 (580-3275)
Kidney biopsy¶, n (%)	15 (68.2)
Class I	4/15 (26.7)
Class II	2/15 (13.3)
Class III	6/15 (40.0)
Class IV	3/15 (20.0)
Treatments before RTX, n (%)	
Glucocorticoids	16 (72.7)
Cyclophosphamide	7 (31.8)
Azathioprine	7 (31.8)
Mycophenolate mofetil	9 (40.9)
Other	7 (31.8)
Disease duration at the time of RTX therapy, median (IQR)- *months*	13.5 (0.8-25.8)
Indication for RTX therapy, n (%)	
Refractory disease	8 (36.4)
Relapsing disease	8 (36.4)
Contraindications to conventional steroid/IS therapy	6 (27.3)
RTX schedule, n (%)	
375 mg/m^2/week x4	7 (31.8)
1 g x2 (two weeks apart)	15 (68.2)

Data are presented as n(%) or median (interquartile range, IQR)
¶Kidney biopsy findings are classified according to Pillebout *et al.* (ref #2)
* one patient had pancreatic involvement

Abbreviations used in the table. IQR: interquartile range; BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate; CKD-EPI: chronic kidney disease epidemiology collaboration; RTX: Rituximab; IS: immunosuppressive

Table 2. Frequencies of organ involvement and main disease parameters at different time-points

	Time after RTX treatment			
	Baseline n=22	**Month 1 n=22**	**Month 6 n=22**	**Month 12 n=22**
Active organ involvement, n (%)				
Skin	18 (81.8)	5 (22.7)	1 (4.5)	3 (13.6)
Gastro-intestinal	15 (68.2)	0	2 (9.1)	1 (4.5)
Kidney	18 (81.8)	13 (59.1)	3 (13.6)	3 (13.6)
Joint (arthritis/arthralgia)	16 (72.7)	4 (18.2)	2 (9.1)	1 (4.5)
BVAS, median (IQR)	15.0 (9.5–19.8)	6.0 (4.5-8.5)	5.0 (4.5-10.5)	5.0 (1.5-8.0)
eGFR (CKD-EPI), median (IQR)- *ml/min/1.73m^2*	82.0 (65.0–101.0)	92.0 (54.0-98.5)	78.0 (66.2-90.7)	77.0 (60.0-106.0)
Proteinuria, median (IQR)- *mg/24h*	1700 (750 – 2375)	1730 (618-3708)	479 (150-3000)	493 (100-1000)
Concomitant immunosuppressive treatments, n (%)				
Glucocorticoids and/or immunosuppressants	16 (72.7)	11 (50.0)	6 (27.3)	4 (18.2)
Glucocorticoids	15 (68.2)	11 (50.0)	6 (27.3)	4 (18.2)
Immunosuppressants[§]	7 (31.8)	5 (22.7)	4 (18.2)	4 (18.2)
Patients taking ACEi and/or ARB, n (%)	8 (36.4)	5 (22.7)	4 (18.2)	4 (18.2)
Patients in remission, n (%)	-	10 (45.5)	16 (72.7)	16 (72.7)

Abbreviations used in the table. RTX: Rituximab; BVAS: Birmingham Vasculitis Activity Score; IQR: interquartile range; eGFR: estimated glomerular filtration rate; CKD-EPI: chronic kidney disease epidemiology collaboration; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers

[§]At baseline, four patients were taking azathioprine, one mycophenolate mofetil, one sulphasalazine, and one leflunomide; at month 1, two patients had stopped azathioprine; at month 6, an additional patient had stopped azathioprine

FIGURE LEGENDS

Figure 1. *Variations in Birmingham Vasculitis Activity Score (BVAS), C-reactive protein (CRP) levels, prednisone dose, estimated glomerular filtration rate (eGFR) and proteinuria throughout the study period*. Significant reductions in BVAS ($p<0.0001$), CRP ($p=0.0005$), prednisone dose ($p<0.0001$) and 24h-proteinuria ($p<0.0001$) were observed after RTX therapy. No significant change of eGFR was found ($p=0.59$). These variations were calculated using Friedman's test and corrected using Dunn's test for multiple comparisons.

In the plots, the boxes indicate the 25^{th}-75^{th} percentile, the bar inside the boxes indicates the median, the whiskers indicate the 10^{th}-90^{th} percentile, and the dots the outliers.

Abbreviations: BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate. t0, t1, t6, t12, last-fu denote respectively the time of Rituximab therapy, month 1, month 6, month 12 and last follow-up.

Supplementary figure 1. *Variations in total IgA serum levels throughout the study period*. Serum IgA levels slightly but significantly declined over time ($p=0.03$ using Friedman's test)

Supplementary figure 2. *Immunohistochemical characterisation of inflammatory cell types in renal and skin IgA vasculitis lesions*. The images show representative findings obtained from 3 renal biopsies and 3 skin biopsies of adult-onset IgA vasculitis patients. Renal biopsy findings are shown in figures A, C, E, G and I, and skin biopsy findings in figures B, D, F, H and J. A and C show endo- and extra-capillary glomerulonephritis (A) with dominant IgA deposits on immunofluorescence (C). Immunohistochemically, the main infiltrating cells are $CD163^+$ macrophages (E), and $CD3^+$ T-cells (G), whereas $CD20^+$ B-cells are scarce (I). B and D show leukocytoclastic skin vasculitis (B) with dominant IgA deposits on immunofluorescence (D). On immunohistochemistry, the main infiltrating cells are $CD163^+$ macrophages (F), and $CD3^+$ T-cells (H); $CD20^+$ B-cells are absent (J).

A,B: haematoxylin and eosin, original magnification x20, bar 200μm. C,D: immunofluorescence staining using anti-IgA antibodies; original magnifications x40 (C), x20 (D), bar 75 μm (C), 150 μm (D). E, F: immunohistochemical staining using an anti-CD163 antibody, original magnification x20. G, H: immunohistochemical staining using an anti-CD3 antibody, original magnification x20. J: immunohistochemical staining using an anti-CD20 antibody, original magnification x20. E-J: the scale (no bar is shown) is the same as in A, B.

Rituximab for the treatment of adult-onset IgA vasculitis (Henoch-Schönlein purpura)

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ABSTRACT

Objective. Adult-onset IgA vasculitis (IgAV, formerly Henoch-Schönlein purpura) is a rare systemic vasculitis characterised by IgA1-dominant deposits. The treatment of adult-onset IgAV is controversial and is based on the combination of glucocorticoids and immunosuppressive agents, but many patients have refractory or relapsing disease despite treatment. Rituximab is a B cell-depleting antibody of proven efficacy in anti-neutrophil cytoplasmic antibody-associated vasculitis. We tested the efficacy and safety of rituximab in a multicentre cohort of patients with adult-onset IgAV.

Methods. In this multicentre, observational study we included patients with adult-onset IgAV who had received rituximab for either refractory/relapsing disease or because they had contraindications to conventional glucocorticoid/immunosuppressive therapy. We analysed the rates of remission (defined on the basis of the Birmingham Vasculitis Activity Score, BVAS) and relapse, and the variations over time in estimated glomerular filtration rate (eGFR), proteinuria, C-reactive protein (CRP) levels, BVAS and prednisone dose.

Results. Twenty-two patients were included; their median follow-up was 24 months (interquartile range, 18-48). Sixteen patients received rituximab as add-on therapy and six as monotherapy. Twenty patients (90.9%) achieved remission and seven (35%) subsequently relapsed. There was a significant reduction in 24h-proteinuria ($p<0.0001$), C-reactive protein ($p=0.0005$), BVAS ($p<0.0001$), and prednisone dose ($p<0.0001$) from rituximab initiation through last follow-up; eGFR remained stable. Rituximab was generally well tolerated. One patient died after 60 months of follow-up.

Conclusions. Our data suggest that rituximab is an effective and safe therapeutic option for adult-onset IgAV.

INTRODUCTION

Immunoglobulin A (IgA) vasculitis (IgAV), formerly Henoch-Schönlein purpura, is a systemic small-vessel vasculitis characterised by IgA1-dominant immune deposits. Clinical manifestations include purpura, gastrointestinal involvement, arthritis, and glomerulonephritis.[1] IgAV is the most common vasculitis in children, whereas it is rare in adults. Childhood-onset IgAV usually has a benign course only requiring supportive care. In adults, its prognosis is worse, particularly because of the frequency and severity of renal involvement; indeed, adult-onset IgAV leads to advanced chronic kidney disease (CKD) or end-stage renal disease (ESRD) in 10-30% of the patients with renal involvement.[2]

The treatment of adult-onset IgAV is still a matter of debate. Glucocorticoids and immunosuppressive drugs are routinely used especially in patients with organ- or life-threatening manifestations.[3] However, the efficacy of these treatments is controversial. In the only randomised trial performed in adult-onset IgAV, the addition of cyclophosphamide to glucocorticoids failed to provide any clear benefit.[4] Other immunosuppressive agents such as azathioprine, mycophenolate mofetil or cyclosporine have only been reported in anecdotal cases.[3,5] Therefore, effective therapies are needed in adult-onset IgAV.

The anti-CD20 monoclonal antibody rituximab (RTX) has become a standard treatment for remission-induction and remission-maintenance in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.[6,7] RTX induces B-cell depletion and thus dampens the expansion of plasma cells that produce pathogenic autoantibodies. RTX can also impair antigen-presentation by B-cells or reduce B cell-mediated stimulation of T-cells and other inflammatory cell types.[6] Recent reports have shown that RTX may be effective for adult-onset IgAV, but they were based on single cases or small case series.[8,9] In this multicentre, observational study, we analysed the effectiveness and safety of RTX in adult-onset IgAV patients who were either relapsing/refractory or had contraindications to conventional glucocorticoid/immunosuppressive therapy.

METHODS

Patients and treatment

We reviewed the clinical records of all patients with adult-onset IgAV treated with RTX at nine different Vasculitis Centres in Europe. The diagnosis of IgAV was based on the 1990 American College of Rheumatology criteria[10] and the 2012 revised Chapel Hill Consensus Conference nomenclature.[1] All patients had to have a biopsy-proven diagnosis of IgAV. The patients were included if: i) their age at IgAV diagnosis was >18 years; ii) they had severe involvement of at least one organ (including biopsy-proven IgAV-related nephritis class 3-4;[2] gastrointestinal involvement with haemorrhage, ischemia, perforation and/or abdominal pain unresponsive to common analgesics and lasting for >24h; pulmonary haemorrhage, episcleritis, cardiac and central nervous system involvement); iii) other systemic autoimmune or neoplastic diseases were excluded; iv) RTX was given for the treatment of relapsing or refractory disease or because there were contraindications to the use of standard-dose glucocorticoids and/or conventional immunosuppressants. Allowed RTX schedules included 375 mg/m^2/week for four consecutive weeks or 1000 mgx2 given two weeks apart; RTX could be given alone or on top of other immunosuppressive therapies. Glucocorticoids and other immunosuppressants were tapered or withdrawn according to local practice. RTX was obtained by local hospital pharmacies for off-label use; all patients signed an informed consent. The study was performed in accordance with the declaration of Helsinki and received ethical approval by Parma University Institutional Review Board.

Data collection and disease activity assessment

We retrieved data regarding patient demographics, clinical manifestations, disease duration and treatments prior to RTX therapy and during the post-RTX follow-up, and reviewed all the pathology reports of diagnostic biopsies. With regards to the post-treatment follow-up, we collected clinical and serological data at specific time-points (months 1, 6, 12, and last follow-up). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology collaboration) formula. Information regarding any type of

adverse events was also collected; adverse events were graded using the Common Terminology Criteria for Adverse Events version 4.0.[11] Disease activity was evaluated using the Birmingham Vasculitis Activity Score (BVAS), version 3.[12] This score refers to new or worsening symptoms due to active vasculitis, with higher scores indicating a more active disease. Remission was defined as BVAS=0, or BVAS≤5 if all scores were due to persistent haematuria or proteinuria in the presence of stable or improving renal function. Relapses were defined as an increase in disease activity requiring the reinstitution of glucocorticoids or other immunosuppressants, or a significant increase in glucocorticoid dose (>50% for prednisone doses ≥15 mg/day or >100% for doses ≤12.5 mg/day). The disease was defined as refractory in case of lack of response to standard-dose glucocorticoids, alone or combined with other immunosuppressive therapies.

Tissue biopsies

Renal biopsy findings compatible with a diagnosis of IgAV-related nephritis were classified as follows according to Pillebout *et al.*[2] class 1, “mesangiopathic” glomerulonephritis; class 2, focal and segmental glomerulonephritis (segmental endo- and/or extra-capillary proliferation involving <50% of the glomeruli); class 3, diffuse and severe endocapillary proliferative glomerulonephritis; class 4, endo- and extra-capillary glomerulonephritis; class 5, global glomerular sclerosis involving >90% of the glomeruli.

In order to investigate which inflammatory cell types are more abundant in the affected organs of patients with IgAV, we performed immunohistochemical analyses of three renal biopsies and three skin biopsies from patients included in this study. In addition to routine stainings (*e.g.*, haematoxylin and eosin, periodic acid Schiff) and immunofluorescence (to detect immunoglobulin and complement fraction deposition), we also performed immunohistochemistry using monoclonal antibodies directed to the B-cell marker CD20, the T-cell marker CD3, and the macrophage marker CD163.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR), while categorical variables as n(%). Variations in continuous variables between two time-points were assessed using the Wilcoxon's test. Variations in continuous variables across different time-points (baseline, month 1, month 6, month 12, last follow-up) were assessed using Friedman's test. P values were corrected using Dunn's test for multiple comparisons. Corrected p values <0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 5.

RESULTS

Twenty-two patients were included in this study. Six cases were described in previous papers;[8,9] the data presented here regarding these six patients are based on a longer follow-up than that originally reported. Of the 22 patients, 12 were men; their median age at diagnosis was 37.5 years (IQR 22.8–49.8). At diagnosis, the most frequent clinical manifestation was purpura (21 patients, 95.5%); it predominantly affected the lower limbs and was necrotic or bullous in six cases (27.3%). Gastrointestinal involvement was found in 18 (81.8%); all experienced abdominal pain and seven (39%) gastrointestinal haemorrhage. Twenty patients (90.9%) had kidney involvement. The median eGFR was 76 ml/min/1.73m^2 (IQR 65–104), and the median 24h-proteinuria 1900 mg (IQR 580–3275) *(table 1)*. One patient was dialysis-dependent at diagnosis but became dialysis-free after initial therapy (before RTX). Fifteen (68.2%) underwent kidney biopsy, which confirmed IgAV in all cases. A diffuse endo- and/or extra-capillary IgAV-glomerulonephritis (classes 3-4) was observed in nine cases (60%). At diagnosis, disease activity assessment showed a median BVAS of 16.5 (IQR 13.0–23.8) (*table 1*).

Sixteen patients (73%) had received other treatments prior to RTX: all of them had received glucocorticoids, alone or combined with cyclophosphamide, azathioprine, mycophenolate mofetil or other drugs (*table 1*). These 16 patients received RTX (only one as monotherapy) for refractory or relapsing disease. The remaining six received RTX as first-line therapy (five

as monotherapy and one together with low-dose glucocorticoids) because they had contraindications to standard-dose glucocorticoids or other immunosuppressive agents. Overall, 16 patients received RTX as add-on therapy and six as monotherapy.

At RTX initiation, 18 patients (81.8%) had skin manifestations, 15 (68.2%) gastrointestinal involvement, 18 (81.8%) kidney involvement, and 16 (72.7%) arthritis or arthralgia. The median BVAS was 15.0 (IQR 9.5–19.8); the median eGFR was 82 ml/min/1.73m^2 (IQR 65-101) and the median 24h-proteinuria 1700 mg (IQR 750-2375) (*table 2*).

One month after RTX, 10 patients (45.5%) had achieved remission and BVAS significantly decreased (median, 6 at month 1 vs. 15 at baseline, p=0.0005) (*figure 1*). At month 6, 16 patients (72.7%) were in remission and six (27.3%) had active disease (three because of persisting disease and three because of relapse after remission) (*table 2*); as compared with baseline, BVAS and prednisone dose decreased (p=0.0008 and p=0.03, respectively). At month 12, 16 patients (72.7%) were in remission and six (27.3%) had active disease (persistent disease in five and relapse in one); reductions vs. baseline were observed for BVAS (p=0.0005), proteinuria (p=0.001), C-reactive protein (CRP) (p=0.0007), and prednisone dose (p=0.01) (*figure 1*). Thus, during the first 12 months after RTX, remission was achieved by 20 patients (cumulative incidence of remission 90.9%), four of whom relapsed. After remission, no patient received RTX as maintenance therapy. Of the seven patients who were taking immunosuppressants at the time of RTX treatment, three stopped them (*table 2*). Eight patients were taking ACE-inhibitors and/or angiotensin receptor blockers at the time of RTX treatment; four stopped them during the first 12 months after RTX (*table 2*), but resumed them afterwards, usually for hypertension, persistent proteinuria or non-specific nephroprotection.

We analysed the outcomes at months 6 and 12 of the six patients who received RTX alone vs. those of the 16 patients who received RTX and glucocorticoids/immunosuppressants; no significant differences emerged (*supplementary table*). No differences in terms of remission and relapse rates were found between patients treated with either of the two different RTX

regimens and between patients who received RTX as first-line therapy vs those who received RTX for refractory/relapsing disease (data not shown).

The median follow-up from the start of RTX was 24 months (IQR 18-48). At last follow-up, 18 patients were in remission, three had active disease, and one was deceased (month 60). Of the patients in remission, three were taking low-dose prednisone (2.5-5 mg/day), and two low-dose prednisone and immunosuppressants. Reductions from RTX initiation through last follow-up were observed for BVAS ($p<0.0001$), 24h-proteinuria ($p<0.0001$), CRP ($p=0.0005$), and prednisone dose ($p<0.0001$) (*figure 1*). eGFR did not change significantly (median 77 ml/min/1.73 m^2 at last follow-up vs 82 at baseline). At last follow-up, one patient had reached ESRD requiring hemodialysis (month 13 after RTX), one had stage 4 CKD, four had 24h-proteinuria>1g; eight were taking ACE-inhibitors or angiotensin-receptor blockers. Microhematuria persisted in eight patients.

When considering the entire follow-up, we recorded relapses in seven of the 20 patients (35%) who had achieved remission. Relapses occurred a median of 12 months (IQR 6.5-15) after RTX; they involved the kidney in four cases, the gastrointestinal tract in one and the skin in two. Of the four patients with kidney relapses, three were re-treated with RTX and all achieved remission, one only received ACE-inhibitors and progressed to ESRD. The two patients with skin relapses were successfully treated with either sulfasalazine (one case) or low-dose glucocorticoids (one case); the patient with gastrointestinal relapse also responded to resumption of low-dose glucocorticoids.

Circulating $CD19^+$ B-cell levels were not systematically tested and were only available for 11 patients, all of whom achieved B-cell depletion ($<0.01x10^9$ cells/L) after RTX; given the paucity of data, we could not establish any association between B-cell return and relapse.

RTX therapy was generally well tolerated. Two patients experienced infusion reactions: one developed bullous urticaria (grade 3) that responded to oral steroids, while the other developed mild and self-limited dyspnoea (grade 2). In the former patient, the reaction precluded subsequent drug infusions. One patient died because of advanced liver cirrhosis

(of undetermined aetiology) and pneumonia (month 60 after RTX), but this fatal event was probably unrelated to RTX, given the long period of time elapsed since RTX therapy.

DISCUSSION

Adult-onset IgAV is a rare, often severe, small-vessel vasculitis; if left untreated, it usually has an adverse prognosis.[2] Current treatments, including high-dose glucocorticoids and cyclophosphamide, are only partially effective and their toxicity may be high.[3,4] Our results identify RTX as an effective therapeutic option for adult-onset IgAV. In our study, RTX was given for refractory/relapsing disease or as first-line therapy when conventional immunosuppressive agents were contraindicated. The remission rate after RTX was 91%, with most patients achieving remission within six months of therapy. Remission was defined based on the BVAS, a widely used score for disease activity assessment in systemic vasculitides. However, other parameters such as CRP levels and prednisone dose also declined after RTX. Renal outcomes were good: although eGFR remained stable, the reduction in proteinuria was marked (approximately 5-fold at end of follow-up vs. baseline). Only one patient reached ESRD and one developed stage 4 CKD. Interestingly, the outcomes of the patients treated with RTX-monotherapy appeared to be similar to those of the patients receiving combination therapies.

Relapses were common (relapse rate 35%) after RTX-induced remission; their clinical presentation was variable, and in severe cases re-treatment with RTX proved effective. Overall, RTX was well tolerated; no major treatment-related adverse events were recorded.

The potential mechanisms of action of RTX in IgAV are only speculative. As in IgA nephropathy,[13] also in IgAV immune-complexes containing galactose-deficient IgA1 (Gd-IgA1) are thought to accumulate in target organs and initiate tissue damage. We could neither measure serum Gd-IgA1 nor Gd-IgA1-containing immune-complexes; total serum IgA levels, available in 17/22 patients, showed a slight, progressive reduction after RTX (p=0.03) (*supplementary figure 1*). However, the slow kinetics and the minor degree of IgA reduction

did not seem to mirror the clinical activity of RTX. We also performed immunohistochemistry on renal and skin biopsies of IgAV patients (*supplementary figure 2*) to investigate which cell types infiltrate target tissues: in all cases, $CD20^+$ B-cells were either absent or scarce, whereas most infiltrating cells were $CD3^+$ T-cells or $CD163^+$ macrophages. Thus, although it cannot be excluded that RTX in IgAV reduces the expansion of IgA-producing plasma cells or depletes tissue-infiltrating B-cells, its therapeutic efficacy most likely results from the impairment of other B-cell functions such as antigen-presentation and T-cell co-stimulation, as already postulated in ANCA-associated vasculitis and other autoimmune conditions.[6]

RTX has been recently investigated in a randomised trial of patients with IgA nephropathy. In this trial, which enrolled patients at high risk of progression (persistent proteinuria >1g/day despite ACE-inhibitors or angiotensin-receptor blockers), RTX failed to reduce proteinuria at 12 months; serum levels of Gd-IgA1 or antibodies against Gd-IgA1 were also unaffected.[14] The apparent contrast between our positive findings and the lack of efficacy of RTX in the IgA nephropathy trial can be due to several reasons. The chronic and advanced stage of IgA nephropathy of the patients enrolled in the trial may have impaired their response to RTX.[14] Additionally, it must be acknowledged that IgAV and IgA nephropathy are clinically distinct, with IgAV being certainly more “inflammatory”.[15] Their genetic associations are also different: although they are both associated with HLA class II variants, other associations with polymorphisms of genes involved in innate immune responses (*e.g.*, *ITGAM*, *CARD9*) were only demonstrated for IgA nephropathy.[13,15]

Our study has limitations, mainly related to its retrospective nature and the small sample size. Also, it cannot be excluded that concomitant treatments such as other immunosuppressants, ACE-inhibitors or angiotensin-receptor blockers, although limited to a minority of patients, influenced patients’ responses. Nevertheless, this study represents the largest reported experience on the use of RTX in adult-onset IgAV. We observed that RTX effectively induced disease remission, allowed glucocorticoid tapering and was well tolerated. Larger, controlled studies are warranted to clarify its role in the management of this rare condition.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

FM and AV designed the study, collected data, analysed results and wrote the manuscript. MN, GT and MI performed histological and immunohistochemical analysis. MG performed statistical analysis. RF, EP, GE, MLU, RR, ES, AJM, DJ, PE, MS, PN, HH and DR contributed clinical and serologic data of the patients included in the study. All authors revised the manuscript critically for important intellectual content and approved the final version.

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Table 1. Main characteristics of the 22 patients with IgA vasculitis

	n=22
Male gender, n (%)	12 (54.5)
Age at diagnosis, median (IQR)- *years*	37.5 (22.8-49.8)
Organ involvement at diagnosis, n (%)	
Skin	21 (95.5)
Gastro-intestinal	18 (81.8)
Kidney	20 (90.9)
Joint	17 (77.3)
Other sites*	1 (4.5)
BVAS, median (IQR)	16.5 (13.0-23.8)
eGFR (CKD-EPI), median (IQR)- *ml/min/1.73m^2*	76.0 (65.0-104.0)
Proteinuria, median (IQR)- *mg/24h*	1900 (580-3275)
Kidney biopsy¶, n (%)	15 (68.2)
Class I	4/15 (26.7)
Class II	2/15 (13.3)
Class III	6/15 (40.0)
Class IV	3/15 (20.0)
Treatments before RTX, n (%)	
Glucocorticoids	16 (72.7)
Cyclophosphamide	7 (31.8)
Azathioprine	7 (31.8)
Mycophenolate mofetil	9 (40.9)
Other	7 (31.8)
Disease duration at the time of RTX therapy, median (IQR)- *months*	13.5 (0.8-25.8)
Indication for RTX therapy, n (%)	
Refractory disease	8 (36.4)
Relapsing disease	8 (36.4)
Contraindications to conventional steroid/IS therapy	6 (27.3)
RTX schedule, n (%)	
375 mg/m^2/week x4	7 (31.8)
1 g x2 (two weeks apart)	15 (68.2)

Data are presented as n(%) or median (interquartile range, IQR)
¶Kidney biopsy findings are classified according to Pillebout *et al.* (ref #2)
* one patient had pancreatic involvement

Abbreviations used in the table. IQR: interquartile range; BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate; CKD-EPI: chronic kidney disease epidemiology collaboration; RTX: Rituximab; IS: immunosuppressive

Table 2. Frequencies of organ involvement and main disease parameters at different time-points

	Time after RTX treatment			
	Baseline n=22	**Month 1 n=22**	**Month 6 n=22**	**Month 12 n=22**
Active organ involvement, n (%)				
Skin	18 (81.8)	5 (22.7)	1 (4.5)	3 (13.6)
Gastro-intestinal	15 (68.2)	0	2 (9.1)	1 (4.5)
Kidney	18 (81.8)	13 (59.1)	3 (13.6)	3 (13.6)
Joint (arthritis/arthralgia)	16 (72.7)	4 (18.2)	2 (9.1)	1 (4.5)
BVAS, median (IQR)	15.0 (9.5–19.8)	6.0 (4.5-8.5)	5.0 (4.5-10.5)	5.0 (1.5-8.0)
eGFR (CKD-EPI), median (IQR)- *ml/min/1.73m^2*	82.0 (65.0–101.0)	92.0 (54.0-98.5)	78.0 (66.2-90.7)	77.0 (60.0-106.0)
Proteinuria, median (IQR)- *mg/24h*	1700 (750 – 2375)	1730 (618-3708)	479 (150-3000)	493 (100-1000)
Concomitant immunosuppressive treatments, n (%)				
Glucocorticoids and/or immunosuppressants	16 (72.7)	11 (50.0)	6 (27.3)	4 (18.2)
Glucocorticoids	15 (68.2)	11 (50.0)	6 (27.3)	4 (18.2)
Immunosuppressants[§]	7 (31.8)	5 (22.7)	4 (18.2)	4 (18.2)
Patients taking ACEi and/or ARB, n (%)	8 (36.4)	5 (22.7)	4 (18.2)	4 (18.2)
Patients in remission, n (%)	-	10 (45.5)	16 (72.7)	16 (72.7)

Abbreviations used in the table. RTX: Rituximab; BVAS: Birmingham Vasculitis Activity Score; IQR: interquartile range; eGFR: estimated glomerular filtration rate; CKD-EPI: chronic kidney disease epidemiology collaboration; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers

[§]At baseline, four patients were taking azathioprine, one mycophenolate mofetil, one sulphasalazine, and one leflunomide; at month 1, two patients had stopped azathioprine; at month 6, an additional patient had stopped azathioprine

FIGURE LEGENDS

Figure 1. *Variations in Birmingham Vasculitis Activity Score (BVAS), C-reactive protein (CRP) levels, prednisone dose, estimated glomerular filtration rate (eGFR) and proteinuria throughout the study period*. Significant reductions in BVAS ($p<0.0001$), CRP ($p=0.0005$), prednisone dose ($p<0.0001$) and 24h-proteinuria ($p<0.0001$) were observed after RTX therapy. No significant change of eGFR was found ($p=0.59$). These variations were calculated using Friedman's test and corrected using Dunn's test for multiple comparisons.

In the plots, the boxes indicate the 25^{th}-75^{th} percentile, the bar inside the boxes indicates the median, the whiskers indicate the 10^{th}-90^{th} percentile, and the dots the outliers.

Abbreviations: BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate. t0, t1, t6, t12, last-fu denote respectively the time of Rituximab therapy, month 1, month 6, month 12 and last follow-up.

Supplementary figure 1. *Variations in total IgA serum levels throughout the study period*. Serum IgA levels slightly but significantly declined over time ($p=0.03$ using Friedman's test)

Supplementary figure 2. *Immunohistochemical characterisation of inflammatory cell types in renal and skin IgA vasculitis lesions*. The images show representative findings obtained from 3 renal biopsies and 3 skin biopsies of adult-onset IgA vasculitis patients. Renal biopsy findings are shown in figures A, C, E, G and I, and skin biopsy findings in figures B, D, F, H and J. A and C show endo- and extra-capillary glomerulonephritis (A) with dominant IgA deposits on immunofluorescence (C). Immunohistochemically, the main infiltrating cells are $CD163^+$ macrophages (E), and $CD3^+$ T-cells (G), whereas $CD20^+$ B-cells are scarce (I). B and D show leukocytoclastic skin vasculitis (B) with dominant IgA deposits on immunofluorescence (D). On immunohistochemistry, the main infiltrating cells are $CD163^+$ macrophages (F), and $CD3^+$ T-cells (H); $CD20^+$ B-cells are absent (J).

A,B: haematoxylin and eosin, original magnification x20, bar 200μm. C,D: immunofluorescence staining using anti-IgA antibodies; original magnifications x40 (C), x20 (D), bar 75 μm (C), 150 μm (D). E, F: immunohistochemical staining using an anti-CD163 antibody, original magnification x20. G, H: immunohistochemical staining using an anti-CD3 antibody, original magnification x20. J: immunohistochemical staining using an anti-CD20 antibody, original magnification x20. E-J: the scale (no bar is shown) is the same as in A, B.

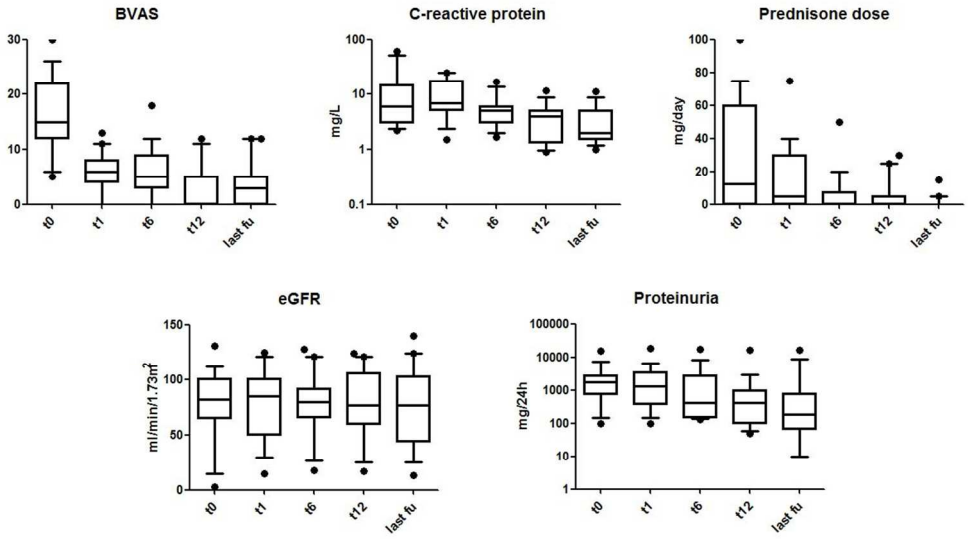

Figure 1. Variations in Birmingham Vasculitis Activity Score (BVAS), C-reactive protein (CRP) levels, prednisone dose, estimated glomerular filtration rate (eGFR) and proteinuria throughout the study period. Significant reductions in BVAS ($p<0.0001$), CRP ($p=0.0005$), prednisone dose ($p<0.0001$) and 24h-proteinuria ($p<0.0001$) were observed after RTX therapy. No significant change of eGFR was found ($p=0.59$). These variations were calculated using Friedman's test and corrected using Dunn's test for multiple comparisons.
In the plots, the boxes indicate the 25th-75th percentile, the bar inside the boxes indicates the median, the whiskers indicate the 10th-90th percentile, and the dots the outliers.
Abbreviations: BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate. t0, t1, t6, t12, last-fu denote respectively the time of Rituximab therapy, month 1, month 6, month 12 and last follow-up.

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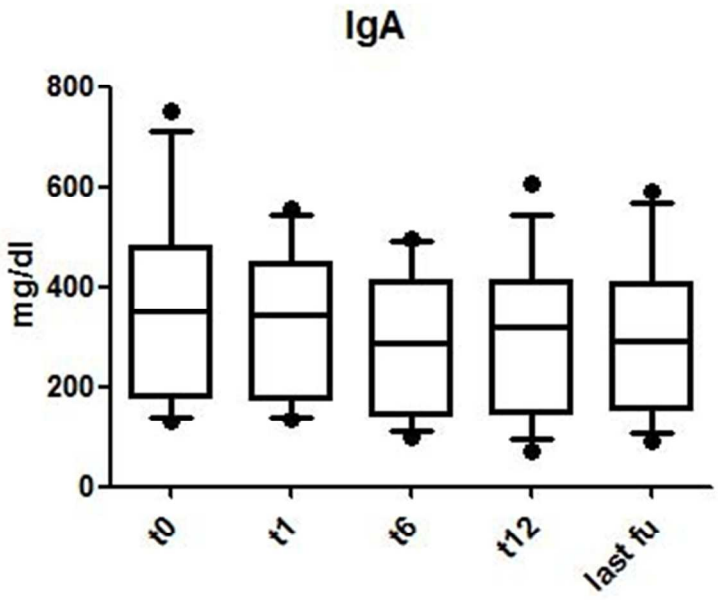
Supplementary table. Clinical characteristics and outcomes of the 6 IgAV patients treated with Rituximab alone versus those of the 16 patients treated with Rituximab plus other immunosuppressive drugs

	RTX alone (n=6)	RTX+GCs or other IS (n=16)	P value[§]
Baseline (time of RTX treatment)			
Age (at diagnosis), median (IQR)	45.5 (37.5-53.5)	29.5 (21-49.3)	0.23
Active organ involvement, n (%)			
Skin	5 (83.3)	13 (81.3)	1.00
Gastro-intestinal	5 (83.3)	10 (62.5)	0.61
Kidney	6 (100)	12 (75.0)	0.54
Joint	4 (66.7)	12 (75.0)	1.00
BVAS, median (IQR)	21.5 (14.3-25.8)	14 (11.5-19.0)	0.25
eGFR (CKD-EPI), median (IQR)- *ml/min/1.73m^2*	73.5 (65.0-117.3)	82.0 (74.0-101.0)	1.00
Proteinuria, median (IQR)- *mg/24h*	2200 (1725-3200)	1000 (375-2210)	0.16
Kidney biopsy[¶], n (%)			
Class I-II	2 (33.3)	4/9 (44.4)	1.00
Class III-IV	4 (66.7)	5/9 (55.6)	1.00
Month 6 after RTX			
Active organ involvement, n (%)			
Skin	0	1 (6.3)	1.00
Gastro-intestinal	0	2 (12.6)	1.00
Kidney	2 (33.3)	1 (6.3)	0.17
Joint	0	2 (12.6)	1.00
eGFR (CKD-EPI), median (IQR)- ml/min/1.73m2	72.0 (72.0-80.0)	82.0 (66.0-92.0)	0.92
Proteinuria, median (IQR)- mg/24h	200 (150-4000)	679.5 (187.5-2475)	0.63
BVAS, median (IQR)	5 (3-5)	5 (4-9)	0.24
Patients in remission, n (%)	4 (66,7)	12 (75)	1.00
Month 12 after RTX			
Active organ involvement, n (%)			
Skin	0	3 (18.9)	0.53
Gastro-intestinal	0	1 (6.3)	1.00
Kidney	0	3 (18.9)	0.53
Joint	0	1 (6.3)	1.00
eGFR (CKD-EPI), median (IQR)- ml/min/1.73m2	82 (60-112)	76.5 (63.3-91.8)	0.56
Proteinuria, median (IQR)- mg/24h	140 (100-493)	675 (175-1075)	0.32
BVAS, median (IQR)	3 (0-3)	5 (2-5)	0.22
Patients in remission, n (%)	6 (100)	10 (62.5)	0.13

Abbreviations used in the table. RTX: rituximab; GCs: glucocorticoids; IS: immunosuppressants; IQR: interquartile range; BVAS: Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate (using the Chronic Kidney Disease Epidemiology collaboration -CKD-EPI- formula)

[¶]Renal biopsy findings were graded according to Pillebout's classification (see text for details)

[§]P values were calculated using Mann-Whitney U test for continuous variables or Fisher's exact test for categorical variables



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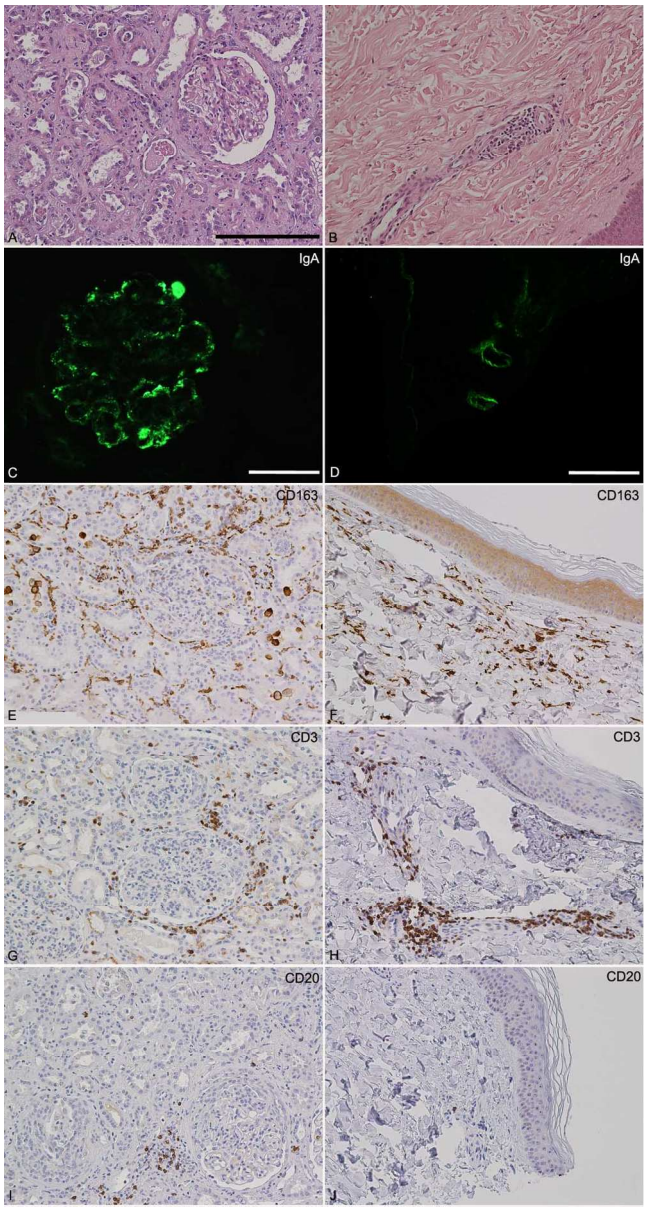

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