

Pharmacokinetic Characterization of Rituximab in Patients with Glomerular Diseases

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Background: Rituximab is commonly used to treat patients with primary glomerular diseases; however, its pharmacokinetics in this population have not been fully described yet.

Materials and methods: This single-center, open-label, uncontrolled clinical trial included adult patients with glomerular diseases who required rituximab treatment (NEFRTX; EudraCT: 2020-000484-23). Patients received 1 or 0.5 g of rituximab on day 1 (and d14 in some cases). Blood and urine samples were collected at days 1, 7, 14, 28, and 45 to measure biochemical parameters (proteinuria, albuminemia, plasma immunoglobulin, and urine immunoglobulin), rituximab, and antidrug antibody concentrations. The gene encoding the neonatal fragment-crystallizable receptor was also characterized. Linear regression and Win-Nonlin 1.1 were used for pharmacokinetic analysis.

Results: Thirty-five cases (30 patients) were included in this study. Pharmacokinetic parameters were expressed as mean (SD): maximum plasma concentration, 179.4 (71.8) mcg/mL; volume of distribution, 78.9 (31.4) mL/kg; clearance, 0.30 (0.27) mL/h/kg; half-life ($t_{1/2}$), 11.6 (5.8) d; elimination rate constant, 0.0036 (0.0030) hour⁻¹; and area under the curve, 117,756.1 (88,228.1) mcg·h/mL. Antidrug antibody was detected on d1 in 3 cases (8.6%) and was negative by d28. Rituximab $t_{1/2}$ was represented by the formula: $t_{1/2} = A \cdot B \cdot \log(\text{Proteinuria}) + C \cdot \text{Albuminemia}$, where $A = 515.1$ (128.8–901.3), $B = 182.1$ (–108.6 to –35.4), and $C = 39.5$ (–10.9 to 89.9). There were significant differences in rituximab $t_{1/2}$ based on diagnosis ($P = 0.025$), early treatment ($P = 0.008$), proteinuria $>2.4\text{g}/24\text{h}$ ($P < 0.001$), plasma immunoglobulin $<650\text{ mg}/\text{dL}$ ($P = 0.048$), and detectable urine immunoglobulin ($P = 0.018$).

Conclusions: Albuminemia and proteinuria affect rituximab $t_{1/2}$ and drug exposure in patients with glomerular diseases. Patients with proteinuria $>2.4\text{g}/24\text{h}$ may require higher frequent dosing for adequate rituximab exposure. Establishing an optimal dosing regimen in this population remains warranted.

Key Words: rituximab, nephropathies, monoclonal antibodies, therapeutic drug monitoring, pharmacokinetics

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INTRODUCTION

Rituximab is a chimeric monoclonal antibody that binds to the CD20 protein expressed on the surface of B cells, leading to apoptosis by activating the complement cascade and inducing antibody-mediated cellular cytotoxicity.^{1,2} Rituximab, the first of several marketed anti-CD20 antibodies, was initially approved for the treatment of non-Hodgkin lymphoma; however, owing to its ability to deplete B cells and prevent the production of new autoantibodies, its use has expanded to other indications, including autoimmune diseases, such as rheumatoid arthritis, lupus, multiple sclerosis, and glomerular diseases.³

Various immune-mediated diseases can cause kidney damage through diverse mechanisms, including the production of autoantibodies targeting different components in cells in the glomerulus, among others. Based on their specific pathophysiology, they are categorized as idiopathic nephrotic syndromes (INS), which include minimal change disease, focal segmental glomerulosclerosis, membranous nephropathy (MN), and

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This study was conducted in accordance with the ethical guidelines and approved by the Spanish Agency of Medicines and Medical Products and the Vall d'Hebron Clinical Research Ethics Committee. Informed consent was obtained from all the participants.

The data supporting the findings of this study are available from the corresponding author upon request. Some data may not be available due to privacy or ethical restrictions.

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antineutrophil cytoplasmic antibody-associated vasculitis (AAV). These can cause excessive loss of protein in the urine, potentially developing nephrotic syndrome (NS), which includes hypoalbuminemia, edema, and hyperlipidemia.^{4,5}

Rituximab is a first-line treatment strategy for certain glomerular diseases and vasculitis owing to its efficacy and safety profile compared to other treatment options, such as cyclosporine or cyclophosphamide.^{6–10} Dosages and frequencies of rituximab have been extrapolated from other indications; the most accepted approach is 2 doses of 1 g of intravenous rituximab administered 2 weeks apart. Another scheme of 375 mg/m² weekly for 1–4 weeks has been suggested; therefore, consensus on the optimal dose remains lacking.⁴ Several studies have attempted to identify a target rituximab concentration range associated with a sustained response to treatment and an optimal dose schedule to reach it in different clinical contexts, including glomerular diseases.^{11–20}

Indeed, rituximab pharmacokinetics show significant interindividual and intraindividual variability, which is influenced by various factors, including anthropometric variables, clinical factors (B-cell count), and antidrug antibody (ADA) formation.^{21–23} Previous studies have also identified certain genetic variants associated with altered rituximab and monoclonal antibodies' pharmacokinetics, including the variable number of tandem repeats (VNTR) in the promoter region of the *FCGRT* gene, which encodes the neonatal fragment crystallizable receptor (FcRn) involved in antibody recycling.^{24–27} Specifically, studies involving patients with glomerular diseases have reported altered pharmacokinetics in this population, possibly due to aberrant drug elimination in urine through glomerular filtration. This could potentially limit exposure to rituximab and compromise treatment success.^{28–30} Further research remains necessary to fully elucidate rituximab pharmacokinetics in patients with certain primary glomerular diseases to ensure optimal doses are used in this population.

Therefore, this study aimed to characterize rituximab pharmacokinetics in a population of patients with glomerular diseases, identify factors affecting rituximab behavior, and quantify their effects, especially those differentially involved in glomerular diseases, such as proteinuria, to predict rituximab behavior and facilitate effective dose individualization.

MATERIALS AND METHODS

Study Design

This single-center, open-label, uncontrolled clinical trial included patients with glomerular disease diagnosed after a renal biopsy who required rituximab. The study (NEFRTX, EudraCT: 2020-000484-23) was approved by the Spanish Agency of Medicines and Medical Products and the Vall d'Hebron Clinical Research Ethics Committee in July 2020. The patients were recruited between September 2020 and May 2023.

Participants

Eligible subjects were adults affected by glomerular diseases (INS, MN, and AAV) who required rituximab

treatment according to international guidelines and nephrologist criteria approved by the Pharmacy & Therapeutics Committee of the Vall d'Hebron University Hospital. Informed consent was obtained from all participants.

The exclusion criteria included receiving rituximab in the past 6 months, an estimated life expectancy of <6 months, potentially requiring hemodialysis in the following 3 months, being enrolled in another clinical trial, and unable to provide informed consent or commit to a 1-year follow-up at the Vall d'Hebron University Hospital.

Patients were considered evaluable per protocol when at least three blood samples were collected for rituximab concentration determination 45 days after rituximab administration. Patients were enrolled if received rituximab more than 6 months apart during the study period, which was considered sufficient for rituximab to be completely washed out.

Procedures

Patients received rituximab according to clinical practice guidelines. One dose of rituximab (0.5 or 1 g) was administered on day 1, and it could be administered again on day 14 based on the clinician's criteria. Serological status was determined for hepatitis and tuberculosis screening, and oral prophylaxis with trimethoprim-sulfamethoxazole was prescribed before the initiation of rituximab.

Rituximab (Rixathon) was prepared by the Hospital Pharmacy Service following the Summary of Drug Characteristics and Good Manufacturing Practice. The drug was administered on the hospital day by nurses according to standard practice: 1 g of paracetamol, 40 mg of methylprednisolone, and 5 mg of dexchlorpheniramine were administered before rituximab infusion, and the rate of rituximab infusion was individualized considering patients' weight and prior exposure to the drug. Weight, blood pressure, and incidence of infusion reactions were recorded on the day of rituximab administration.

Blood and 24-hour urine samples were collected on days 1 (prerituximab and postrituximab administration), 7, 14 (prerituximab and postrituximab administration), 28, and 45.

Data Collection

Patient data, including weight, height, body surface area (BSA), and body mass index (BMI), were recorded, along with other clinical characteristics, such as the type of disease and concomitant immunosuppressive treatment, including tacrolimus, mycophenolate, and corticosteroids. Patients were considered for early treatment when rituximab was administered within 3 months of diagnosis.

Biochemical analyses of the collected blood samples included albumin, cholesterol, C-reactive protein, plasma immunoglobulin G (pIgG), rheumatoid factor, 24-hour proteinuria, antineutrophil cytoplasmic antibody, and antiphospholipase A2 receptor (anti-PLA2R) antibodies. Creatinine clearance was estimated using the Cockcroft–Gault formula.³¹ Urine was analyzed to measure total protein and urine immunoglobulin G (uIgG); uIgG was measured through turbidimetry using the IgG Low Optilite (The Binding Site Group Ltd,

Birmingham, the United Kingdom) kit according to the manufacturer's instructions and was considered detectable when uIgG concentration was >0.859 mg/dL.

NS and chronic kidney disease (CKD) risk were assessed according to the KDIGO guidelines.^{4,32} Obesity was defined as BMI >30 kg/m² and overweight as BMI within 25–30 kg/m².

Rituximab and ADA Plasma Concentration Measures

Rituximab and ADA levels were measured using an ELISA kit, Lisa-Tracker Rituximab (Theradiag, Croissy-Beaubourg, France) and an ELISA kit: Lisa-Tracker anti-Rituximab (Theradiag, Croissy-Beaubourg, France), respectively, according to the manufacturer's instructions in serum samples. When the rituximab concentration was >50 ng/mL, the plasma was diluted 1:10.

FCGRT Gene Characterization

Genotyping of the *FCGRT* VNTR polymorphism was performed using polymerase chain reaction (PCR), followed by agarose gel electrophoresis. Aliquots of 40 ng of DNA obtained from whole blood were amplified using the FIREPol DNA Polymerase (Solis Biodyne). Primers (forward: 5'-TGGCTGGGGGTCTCGACAC-3'; reverse: 5'-CAATGCCCTCCTCGTACCTGAAG-3') encompassing the VNTR region of *FCGRT* were used. The PCR conditions were as follows: heating the reaction at 95°C for 5 minutes was followed by 35 cycles of 95°C for 30 seconds (denaturation), 64°C for 30 seconds (annealing) and 72°C for 1 minute (extension), and a final extension at 72°C for 5 minutes. The reaction products were resolved on 1.8% agarose gels. Sanger sequencing was performed on five patients to validate the PCR and gel electrophoresis techniques.

Lymphocytes Count

Blood was collected in vacutainer tubes containing ethylenediaminetetraacetic acid (BD-Plymouth, PL6 7BP, the United Kingdom) and processed within 4 hours of collection.

The absolute counts and relative numbers of peripheral blood lymphocytes were determined for all participants using Tetra CHROME Tube 1 (CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5) and Tetra CHROME Tube 2 (CD45-FITC/CD56-PE/CD19-ECD/CD3-PC5) (Beckman Coulter, Brea, CA) according to the manufacturer's instructions.

CD3CD20 staining was performed using standard procedures and the following antibodies: CD45-KrO, CD3-APC-AF750, and CD20-ECD. Samples were analyzed using a NAVIOS EX (BC) flow cytometer, and flow cytometry data were analyzed using Kaluza Software (Beckman Coulter Inc.).

Pharmacokinetic Analysis

For each patient, the concentration–time curves of rituximab were analyzed in a multiple-dosing regimen after a compartmental method, assuming an open one-compartment model. The basic pharmacokinetic parameters of the one-compartment model—biological half-life ($t_{1/2}$),

volume of distribution, clearance (Cl), maximum plasma concentration, area under the curve (AUC), and elimination rate constant (K_{el})—were estimated via linear regression. Similarly, the parameters were estimated using a nonlinear regression iteration method with the software package WinNonlin 1.1 (Pharsight Corporation, mountain View, CA).

Statistical Analysis

Data from pharmacokinetic analyses, demographic variables, and laboratory tests are expressed as frequencies, means, and SD. The paired Student *t* test was used to compare means (Mann–Whitney *U* test, when appropriate).

Multiple linear regression was performed to evaluate the association between pharmacokinetic parameters and proteinuria. Using a backward nested multiple regression model, the influence of other factors, including demographic, analytical, and clinical measures (such as urinary protein excretion, CD19⁺ B-cell counts, *FCGRT* VNTR polymorphism, ADA, total serum cholesterol, and albumin), was evaluated.

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS, Chicago, IL). For single-outcome comparisons, linear and nonlinear regression, the observed effect was considered significant at *P* values of ≤ 0.05 .

RESULTS

Basal Characteristics

A total of 65 cases were screened, yielding 48 enrollments. Some patients were enrolled more than once since they received rituximab more than 6 months apart; overall, 35 cases (30 patients) were finally included in the analysis (see **Figure, Supplemental Digital Content 1**, <http://links.lww.com/TDM/A855>). A total of 13 cases were excluded due to degradation of the plasma samples owing to accidental defrosting in 7 cases and insufficient sample collection during the first 45 days after rituximab administration in 6.

The baseline characteristics of the cases included in the study are presented in Table 1 (patients' characteristics are described in **Supplemental Digital Content 2**, see **Table**, <http://links.lww.com/TDM/A856>).

All cases were adults (57.1% were men) with a mean age of 63.4 (17.6) years. Most cases (21, 60%) were older adults (older than 65 years); four cases (11.4%) were older than 85 years. Most cases were overweight (54.2%); 5 (14.3%) had obesity. VNTR 3/3 was the most common *FCGRT* genotype in 82.9% of the cases, followed by VNTR 3/2, with only 1 case each of VNTR 3/1 and VNTR 2/2. The most frequent indication for rituximab was MN (40.0%), followed by AAV (34.3%); INS was the least common diagnosis. Potential differences in baseline characteristics among the different diagnoses were analyzed (see **Table, Supplemental Digital Content 3**, <http://links.lww.com/TDM/A857>).

Most cases received immunosuppressants in addition to rituximab (57.1%), with corticosteroids being the most common (34.3%), followed by tacrolimus (17.1%) and mycophenolate (5.7%). Six cases (17.1%) received early

TABLE 1. Cases Baseline Characteristics

Demographic and Clinical Characteristics of Cases Included (N = 35)	
Anthropometric parameters	
Age (yrs)	63.4 (17.6)
Sex (male)	20 (57.1%)
Weight (kg)	70.9 (13.9)
Height (cm)	164.6 (9.9)
BMI (kg/m ²)	26.7 (4.0)
BSA (m ²)	1.8 (0.2)
FCGR2 (FcRn) genotype (%)	
VNTR 3/3	29 (82.9)
VNTR 3/2	4 (11.4)
VNTR 3/1	1 (2.9)
VNTR 2/2	1 (2.9)
Diagnosis (%)	
MN	14 (40.0)
AAV	12 (34.3)
INS	9 (25.7)
Treatment (%)	
Previous exposure to rituximab	24 (68.6)
Early rituximab treatment	6 (17.1)
Use of immunosuppressants (%)	—
None	15 (42.9)
Corticosteroids	12 (34.3)
Tacrolimus	6 (17.1)
Mycophenolate	2 (5.7)
Baseline laboratory values (%)	
Creatinine (mg/dL)	1.28 (0.54)
Creatinine clearance (mL/min)	68.0 (36.3)
Albumin (g/dL)	3.30 (0.96)
Cholesterol (mg/mL)	245.5 (80.2)
CRP (mg/dL)	0.31 (0.29)
Proteinuria (g/24 h)	6.139 (8.270)
uACR (mg/g creatinine)	3372 (3408)
pIgG (mg/dL)	673.9 (297.6)
uIgG (%)*	
Detectable	10 (47.6)
Indetectable	11 (52.4)
Lymphocyte count (cells·10 ⁹ /L)	2.02 (1.29)
CD19 ⁺ count (cells·10 ⁹ /L)	0.17 (0.31)
CD20 ⁺ count (cells·10 ⁹ /L)	0.21 (0.37)
CD3 ⁺ /CD20 ⁺ count (cells·10 ⁹ /L)	0.05 (0.09)
NK Count (cells·10 ⁹ /L)	0.239 (0.172)

Data are expressed as mean (SD) or n (%), as appropriate.

*The percentages of detectable uIgG calculated considering cases where urine samples were available, 21 in total.

NK, natural killer cells.

rituximab treatment, and 68.6% had been previously exposed to rituximab, all of whom had undetectable rituximab plasma concentrations before rituximab administration.

Proteinuria was present in 34 of the 35 cases (97.1%); the mean protein level in the urine was 6.139 (8.270) g/24 h; 14 cases (40.0%) had NS. Moreover, uIgG was detectable in 47.6% of cases. The mean creatinine clearance was 68.0 (36.3) mL/min; all cases had CKD, 2 (5.7%) had moderately

increased risk, 15 (42.9%) had high risk, and 18 (51.4%) had very high-risk CKD.³²

Baseline lymphocyte counts were normal in virtually all cases; only 2 cases (5.7%) had $<1.0 \times 10^9$ lymphocytes/L, of whom 1 was diagnosed with folic acid deficiency-related lymphopenia. No other cases were previously diagnosed with conditions related to hematological diseases affecting lymphocytes. The potential differences in lymphocyte counts between naïve cases and those previously treated with rituximab were evaluated and found to be comparable (see **Table, Supplemental Digital Content 4**, <http://links.lww.com/TDM/A858>).

Rituximab Treatment and Pharmacokinetics

The most frequent rituximab dose was 1 g (26 cases, 74.3%); the 0.5 g dose was administered in 9 cases, and the second dose was administered on day 14 in 20 cases (57.1%). When the second dose was administered, the amount of rituximab was identical to that of the initial dose.

Pharmacokinetic parameters were calculated considering a one-compartment model using linear and nonlinear regression (Table 2), which allowed for an adequate fit to the data ($R^2 = 0.509$, $P < 0.001$ and $R^2 = 0.415$, $P < 0.001$, respectively); thus, linear regression was chosen. The mean (SD) values for pharmacokinetic parameters were as follows: $t_{1/2}$, 11.6 (5.8) days; volume of distribution, 78.9 (31.4) mL/kg; Cl, 0.30 (0.27) mL/h/kg; maximum plasma concentration, 179.4 (71.8) mcg/mL; AUC 117,756.1 (88,228.1) mcg·h/mL; and Kel, 0.0036 (0.0030) hour⁻¹ (Table 2). **Supplemental Digital Content 5** (see **Figure**, <http://links.lww.com/TDM/A859>) illustrates how rituximab concentrations evolved.

Rituximab was well tolerated, and no infusion-related or other adverse reactions or severe infectious disease were detected during the study period.

ADA was detected before rituximab administration on day 1 in 3 cases that had received rituximab more than 6 months before; the concentration was 16.1 (5.6) mcg/mL. ADA was not detected in plasma by day 28 (after rituximab administration on days 1 and 15).

Factors Influencing Rituximab Pharmacokinetics

The effects of different parameters that could potentially affect rituximab pharmacokinetics on rituximab $t_{1/2}$ were analyzed using linear regression (see **Table, Supplemental Digital Content 6**, <http://links.lww.com/TDM/A860>). Proteinuria and serum albumin levels were the primary factors significantly affecting rituximab $t_{1/2}$ and were included in the multiple regression model (Table 3, Fig. 1). Proteinuria affected rituximab elimination rate ($R = 0.733$; determination coefficient = 0.509), accounting for 48.5% and 39.4% of $t_{1/2}$ and Cl variability, respectively ($P < 0.001$). By contrast, albuminuria accounted for 38.8% and 39.2% of $t_{1/2}$ and Cl variability, respectively ($P < 0.001$) (see **Figure, Supplemental Digital Content 7**, <http://links.lww.com/TDM/A861>).

TABLE 2. Rituximab Pharmacokinetic Parameters

	1-Compartment, Linear Regression, N = 35	1-Compartment, Nonlinear Regression, N = 35	P
Cmax (μg/mL)	179.4 (71.8)	228.8 (74.4)	0.006
Vd (mL/kg)	78.9 (31.4)	58.4 (15.1)	0.001
Cl (mL/h/kg)	0.30 (0.27)	0.40 (0.45)	0.242
t _{1/2} (d)	11.6 (5.8)	7.1 (4.1)	0.001
Kel (h ⁻¹)	0.0036 (0.0030)	0.0067 (0.0073)	0.023
AUC (μg·h/mL)	117,756.1 (88,228.1)*	52,451.3 (29,227.6)†	NC

Data are expressed as mean (SD).
*AUC for both doses.
†AUC for the first dose.
NC, not comparable.

Differences were observed in the pharmacokinetic parameters within specific subpopulations defined by the clinical situation (Table 4). The rituximab $t_{1/2}$ varied across populations with different underlying diseases, with those diagnosed with MN exhibiting a significantly shorter exposure ($P = 0.025$). Furthermore, patients with 24-hour proteinuria levels exceeding 2.4 g, early rituximab initiation, pIgG levels below 650 mg/dL, and detectable uIgG presented with a significantly reduced rituximab $t_{1/2}$. Among these factors, only severe proteinuria was associated with a significant decrease in rituximab exposure ($P = 0.047$).

DISCUSSION

This study analyzed rituximab pharmacokinetics in patients with glomerular diseases and demonstrated that rituximab $t_{1/2}$ was significantly decreased in the presence of proteinuria. Furthermore, albuminemia and proteinuria were the primary factors influencing the variability in rituximab $t_{1/2}$. Heavy proteinuria (over 2.4 g/24 h) correlated with significant changes in rituximab pharmacokinetics, including reduced $t_{1/2}$ and exposure (AUC) and increased Kel and Cl; moreover, the effect of proteinuria on rituximab was proportional to the amount of protein in urine collected over 24 hours. Consistently, populations with clinical features related to heavy proteinuria, such as being affected by INS or MN, decreased pIgG (<650 mg/mL), detectable uIgG, and requiring early treatment following diagnosis, showed a significantly shorter rituximab $t_{1/2}$. Other parameters, including anthropometric, biochemical, and genetic variables, did not significantly affect rituximab pharmacokinetics.

The rituximab $t_{1/2}$ equation was solved based on the pharmacokinetic parameters obtained using linear regression. Both linear and nonlinear regressions fit the data similarly in

the multiple regression analysis; therefore, the simplest method was selected. Different variables might have been included in the model to describe the urine protein loss.

Both 24-hour proteinuria and urine albumin-creatinine ratio showed significant association with rituximab $t_{1/2}$ ($R^2 = 0.485$, $P < 0.001$ and $R^2 = 0.345$, $P < 0.001$, respectively).

The 24-hour proteinuria was selected since it is the gold standard to represent protein loss in urine and better fits the equation; however, since urine albumin-creatinine ratio allows to obtain urine sample at random times (making sample collection more feasible in the real-world setting) and accounts for dynamic changes in proteinuria, it can be an appropriate variable to measure proteinuria to facilitate the translation of results into clinical practice. Plasma albumin concentration was included in the model since it increased the explained variance by 2.5% (0.509 instead of 0.485), indicating an improvement in the model fit.

The pharmacokinetic parameters of rituximab described in patients with glomerular diseases differ from those in patients with other conditions. Specifically, rituximab $t_{1/2}$ in this study was shorter than that described in the information sheet for patients with non-Hodgkin lymphoma and rheumatoid arthritis.¹ This is consistent with previous studies evaluating patients with MN.^{29,30} Interestingly, the rituximab $t_{1/2}$ in patients with AAV in this study was shorter than that previously reported for this population, which could be attributed to proteinuria being more common in the patients in this study.³³

Several factors have been suggested as potential covariates affecting rituximab pharmacokinetics; however, consistency in the literature remains lacking, suggesting that differences among different populations studied could jeopardize the ability to identify those factors.²⁹ For instance, rituximab Cl decreases over time when used for non-Hodgkin lymphoma, indicating that tumor burden and antigen availability are related to rituximab consumption.³⁴ Similarly, Cornec et al¹⁷ reported that baseline B-cell count negatively correlated with rituximab plasma concentration in AAV. By contrast, Bensalem et al found that rituximab-target complex

TABLE 3. Multiple Regression Adjusted Final Equation, Including Variables in the Model

	Coefficient (95% CI)
Constant	515.0 (128.8–901.3)
Proteinuria (Log, g/24 h)	−108.6 (−182.1 to −35.4)
Albumin (g/dL)	39.5 (−10.9 to 89.9)

Covariates included in the multiple regression analysis.

$$t_{1/2} = 515.0 - 108,6 * \log PROT_u + 39.5 * ALB_p$$

FIGURE 1. Pharmacokinetic model of rituximab in patients with glomerular disease. ALBp, albumin concentration in plasma (g/dL); PROT_u, Proteinuria (g/24 h).

TABLE 4. Pharmacokinetic Parameters Differences Among Clinically Relevant Subpopulations

	Vd (mL/kg)	Cl (mL/h/kg)	t _{1/2} (d)	C _{max} (μg/mL)	K _{el} (h ⁻¹)	AUC (μg·h/mL)
Diagnosis						
INS	83.6 (24.1)	0.33 (0.24)	10.7 (6.1)	196.6 (69.1)	0.008 (0.006)	148,527.6 (107,166.9)
MN	85.4 (36.9)	0.40 (0.35)	9.1 (4.8)	181.0 (80.0)	0.009 (0.010)	86,862.9 (70,642.6)
AAV	67.8 (28.5)	0.16 (0.11)	15.1 (5.4)	164.5 (66.5)	0.004 (0.002)	130,719.6 (87,391.3)
<i>P</i>	0.325	0.079	0.025	0.608	0.182	0.220
Early rituximab treatment						
No	76.6 (28.4)	0.22 (0.14)	12.7 (5.5)	171.4 (58.4)	0.003 (0.002)	123,080.4 (89,186.7)
Yes	90.0 (43.7)	0.65 (0.46)	6.0 (4.3)	217.8 (117.7)	0.007 (0.005)	92,022.2 (86,194.6)
<i>P</i>	0.350	<0.001	0.008	0.152	0.001	0.441
NS						
No	74.0 (29.9)	0.21 (0.15)	13.0 (5.8)	167.5 (66.9)	0.005 (0.004)	123,071.5 (92,982.3)
Yes	86.2 (33.3)	0.42 (0.37)	9.4 (5.4)	197.3 (77.6)	0.009 (0.010)	109,783.0 (83,327.5)
<i>P</i>	0.266	0.024	0.077	0.235	0.133	0.669
Proteinuria						
<2.4 g/24 h	73.4 (29.8)	0.17 (0.11)	14.9 (4.9)	165.7 (60.1)	0.004 (0.002)	146,282.6 (97,158.5)
≥2.4 g/24 h	84.7 (33.0)	0.43 (0.33)	8.0 (4.5)	193.9 (81.8)	0.010 (0.010)	87,551.6 (68,020.8)
<i>P</i>	0.295	0.003	<0.001	0.252	0.014	0.047
pIgG						
>650 mg/dL	82.39 (34.52)	0.23 (0.13)	12.54 (5.2)	166.4 (52.9)	0.003 (0.002)	117,974.3 (88,586.1)
<650 mg/dL	76.28 (29.52)	0.35 (0.34)	10.8 (6.3)	189.1 (83.3)	0.004 (0.004)	117,592.5 (90,261.7)
<i>P</i>	0.577	0.185	0.048	0.362	0.170	0.990
uIgG						
Indetectable	79.3 (36.3)	0.19 (0.12)	7.95 (3.58)	196.5 (70.5)	0.002 (0.001)	159,923.1 (93,155.2)
Detectable	94.2 (35.5)	0.40 (0.32)	4.51 (2.31)	154.9 (54.3)	0.004 (0.002)	87,566.7 (74,081.6)
<i>P</i>	0.355	0.067	0.018	0.149	0.058	0.065

N = 35; results expressed as mean (SD). *P* values are shown in bold.

elimination rate was lower in nonmalignant diseases than hematological disorders¹⁶; consistent with the findings of this study, the authors found no association between basal B-cell count and rituximab-enhanced Cl in patients with AAV.

Proteinuria can enhance rituximab Cl.^{28,35,36} Other studies including patients with MN have reported a significant increase in Cl in this situation.^{18,28} Interestingly, this study established an association between proteinuria and rituximab t_{1/2} (*P* < 0.001, 48.5% variance explained), showing that the increased rituximab elimination rate in glomerular diseases was mainly explained by proteinuria. In addition, proteinuria had a direct impact on the total exposure to rituximab, as measured using AUC.

Rituximab was previously detected in the urine by the authors of this study and other groups,^{35,37,38} demonstrating that it can be aberrantly excreted through this route in cases of heavy proteinuria. Owing to the insufficient sensitivity and accuracy of rituximab concentration measurements in urine, the authors of this study used uIgG as a proxy variable for the presence of rituximab in urine. The results showed that IgG was aberrantly excreted in the urine of patients with heavy proteinuria; more interestingly, uIgG correlated with decreased rituximab t_{1/2}. Collectively, these findings indicate that rituximab elimination increased in patients with heavy proteinuria due to urinary loss.

Consistent with the results of Hartinger et al,²⁸ this study found differences in rituximab pharmacokinetics among

different types of glomerular diseases. Hartinger et al suggested that diagnosis was a primary factor in rituximab elimination rather than proteinuria, due to the differential level and selectivity of proteinuria in MN, INS, and AAV. Therefore, the authors of this study explored uIgG excretion and found no statistically significant differences in uIgG levels among the different diagnoses. Considering the results of this study, the authors suggest that diagnosis could act as a confounder and that proteinuria is the primary factor to consider when designing dosing approaches for rituximab in glomerular diseases.

Extensive loss of protein in the urine leads to hypoalbuminemia, a risk factor for rituximab treatment failure.^{18,20} In this sense, hypoalbuminemia is a covariate related to rituximab Cl and could be related to insufficient rituximab exposure. Moreover, it has been hypothesized that inflammation could lead to higher drug consumption, and C-reactive protein (CRP) has been suggested as a biomarker for increased drug Cl for monoclonal antibodies³⁹; however, this study detected no association between CRP and rituximab t_{1/2}. By contrast, immunosuppressants have been shown to alter certain monoclonal antibody pharmacokinetics,^{12,40} but this effect was not identified in this study, consistent with a previous study that analyzed rituximab in combination with methotrexate and cyclophosphamide.⁴¹

The authors of this study also found no relationship between anthropometric parameters and rituximab t_{1/2},

whereas others reported that BSA and/or weight were negatively correlated with rituximab plasma concentrations.^{17,20,24,28,42} These data should be interpreted cautiously since rituximab doses were calculated based on BSA in some studies, while others, including this study, used a fixed dose. Cornec et al¹⁷ identified being a male and a recent diagnosis as covariates defining lower rituximab plasma levels. In this article, early rituximab administration after being diagnosed correlated with a lower $t_{1/2}$ ($P = 0.008$); however, the authors of this study considered that the actual reason in these patients was their high proteinuria and low albuminemia.

ADA formation is a known factor that alters monoclonal antibody CI, including rituximab.⁴³ Hartinger et al found that ADA significantly enhanced rituximab elimination²⁸; however, this study found no association, possibly due to the small sample size.

The binding of antibodies to FcRn increases their $t_{1/2}$. It has been hypothesized that circulating pIgG in the plasma can reduce monoclonal antibody $t_{1/2}$ due to FcRn binding competition⁴⁴; moreover, different *FCGRT* variants have been linked to higher monoclonal antibody $t_{1/2}$.^{26,27} In this article, no association was found between pIgG or *FCGRT* polymorphisms and rituximab $t_{1/2}$; similarly, other studies found no genetic variants linked to the risk of relapse.¹¹

No adverse events were reported during the study period. The absence of infections may be related to the short follow-up period (45 days) and use of prophylaxis. Premedication prevented infusion reactions.

Based on the results of this study, it may be necessary to individualize and intensify rituximab dosage in certain populations, including AAV,^{11,14–16} INS, and MN.^{18–20} Repeating rituximab courses more frequently is a potentially interesting approach, especially in the case of heavy proteinuria. Hartinger et al²⁸ developed individualized rituximab dosing schedules according to the type of autoimmune glomerular diseases (MN, AAV, minimal change disease, focal segmental glomerulosclerosis, and lupus), which imply more frequent rituximab administration in all cases than standard treatment. Liang et al¹⁹ concluded that a “mini-dose” schedule was the best option for patients with MN to ensure an adequate and sustained CD20 B-cell depletion cost-effectively. Artificial intelligence has been used to identify an ideal treatment schedule for MN patients; previous studies developed an algorithm that could predict the optimal individualized dosage. However, this algorithm has yet to be evaluated in a clinical trial.²⁰ Further research remains warranted to set a rituximab concentration target and optimal individualized treatment schedule.

This study has some limitations. Ideally, one-compartment and two-compartment models could have been tested; however, the authors of this study decided to test only the one-compartment models to minimize the number of samples, similar to a previous study.²⁸ Since this was a low-intervention clinical study conducted in a real-world setting, the priority was to reduce the burden on patients by collecting the fewest samples and accounting for the potential loss of samples due to patients' lack of adherence, while respecting the predictive ability of the model. Conversely, despite this

study demonstrating that proteinuria and albumin concentrations in plasma affect rituximab $t_{1/2}$, it did not account for the expected pharmacological impact of rituximab on improving those parameters. Therefore, in future studies, the authors of this study will use a nonlinear mixed-effects model to address this limitation. Finally, this was an exploratory study with a limited number of patients; future studies should confirm these findings and explore the association between rituximab pharmacokinetics and its effect on clinical outcomes in a real-world setting.

In conclusion, this study confirmed that proteinuria and albuminemia strongly define the rituximab $t_{1/2}$ and drug exposure in patients with immune-mediated diseases that cause glomerular damage. Proteinuria >2.4 g/24 h was associated with significant rituximab pharmacokinetic alterations, including an increase in CI and Kel and a decrease in $t_{1/2}$ and AUC. Therefore, more frequent rituximab administration may be required in cases of severe proteinuria to ensure adequate rituximab exposure. Future studies should establish an optimal rituximab plasma concentration target for patients with primary glomerular diseases and assess the clinical relevance of dose individualization in this population.

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