Nephrol Dial Transplant (2019) 1–3
doi: 10.1093/ndt/gfz041

ndt
Nephrology Dialysis Transplantation

Rituximab bioavailability in primary membranous nephropathy

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Rituximab is a chimeric monoclonal antibody with human IgG1 constant regions and a murine monoclonal anti-human cluster of differentiation 20 (CD20) variable region, which can deplete $CD20^+$ B cells [1]. Rituximab was first developed for the treatment of non-Hodgkin's lymphoma [2], but is now used to treat many immune-mediated diseases [3–8], including membranous nephropathy (MN) [3–7], with an excellent efficacy, tolerability and safety profile in comparison with conventional treatment regimens [8]. In several non-randomized studies, rituximab induced clinical remission in 60–80% of patients with primary MN [3–6]. Its efficacy was established in a recent controlled study after long follow-up. However, rituximab at two 375 mg/m^2 infusions (i.e. cumulative dose of rituximab in GEMRITUX was 1.45 g) failed to demonstrate its efficacy at Month 6 versus placebo [7].

As described for other autoimmune diseases, many factors modify rituximab response [9, 10], especially large inter-individual variability, related to either disease or genetic factors, which affect B-cell depletion and treatment response [11, 12]. Residual rituximab serum levels were detected at 6–9 months after the first infusion due to recycling from endothelial cells via FcRn receptors [12]. However, rituximab may be lost in the urine of nephrotic patients [13]. In MN patients, rituximab half-life was 11.5 days as compared with 18.0 days in patients with rheumatoid arthritis, and no differences in serum rituximab levels between responders and non-responders were detected at any time point until Day 15 post-dose [11].

The aim of this study was to analyse rituximab bioavailability in a cohort of primary MN patients.

A total of 43 patients with primary MN treated with two infusions of 1 g rituximab at a 2-week interval were enrolled and followed for a median time of 39 months: 35 (81%) had anti-phospholipase A2 receptor 1 (PLA2R1) antibodies, 2 (5%) had anti-thrombospondin containing 7 domains A (THSD7A) antibodies and 6 patients (14%) were double negative. All patients received angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. Residual serum rituximab levels were high at 1 month after infusion and still detected at Month 3, but became undetectable at Month 6 (Figure 1A and B). Residual rituximab levels at Month 3 were significantly lower in MN patients compared to myastenia gravis patients with no proteinuria matched for age, gender and weight treated with a similar treatment regimen [2.27 (0.19–7.5) µg/mL versus 12.7 (7.45–27.84); $P < 0.0001$] (Figure 1B). Residual rituximab was only detectable until Month 6 in myasthenia gravis patients. After 2 weeks, rituximab could be detected in the urine of nephrotic patients. Residual rituximab levels at Month 3 were not correlated with baseline characteristics except for anti-PLA2R1 titre [$r = -0.39$ (-0.66 to -0.04); $P = 0.03$], suggesting that patients with higher titre of anti-PLA2R1 antibodies might benefit from higher doses of rituximab. Low residual rituximab levels at Month 3 correlated with poor B-cell depletion at Months 3 and 6 with high anti-PLA2R1 titre at Months 3, 6 and 12, and with proteinuria at Months 3, 6 and 12.

Twenty-six out of 43 patients (60%) achieved complete or partial remission before any treatment modification. There were no significant differences for baseline characteristics between patients who received only one course of rituximab compared with those who required a second course for resistant MN (i.e. persistent active disease and anti-PLA2R1 activity for anti-PLA2R1-related MN after 1 year of follow-up) or relapse (i.e. active disease after complete or partial remission and positive anti-PLA2R1 activity for anti-PLA2R1-related MN) (Figure 1C). The patients who required a second course of rituximab had significantly lower residual serum rituximab levels at Month 3 [0.20 (0.00–3.59) versus 3.05 (1.67–11.70); $P = 0.004$] (Figure 1D). Using receiver operating characteristics (ROC) curve, we determined a threshold of residual serum rituximab level at Month 3 of 1 µg/mL that was associated with long-term response to only one course of rituximab with a sensitivity of 66.7% (38.4–88.2%) and a specificity of 84.0% (63.9–95.5%) (air under curve (AUC) = 0.77; $P = 0.005$). In patients with residual rituximab level <1 µg/mL, B cells re-emerged more quickly (slope = 5.23 versus 22.45; $P = 0.0465$) (Figure 1E).

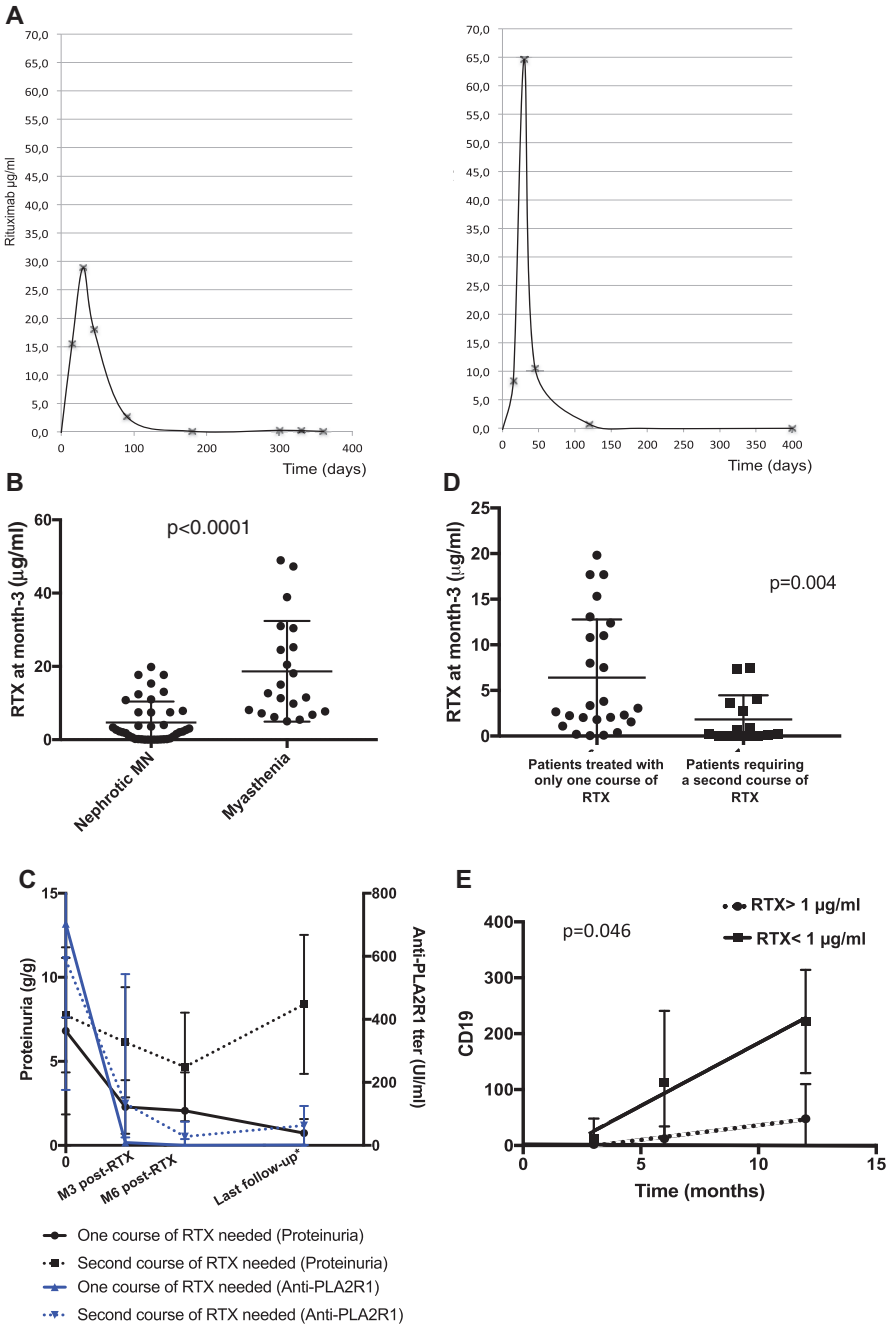

FIGURE 1: Bioavailability of rituximab in idiopathic MN. (**A**) Residual-free rituximab level measured by ELISA (LISA-TRACKER Duo Rituximab, Theradiag©Croissy Beaubourg, France) in two MN patients matched for age, weight and proteinuria. The limit of detection for rituximab defined by the manufacturer was 2 µg/mL with an intra-run variability being at 7.6% and inter-run variability at 9.8%. Under 2 µg/mL, we performed a dilution at 1:50 (instead of 1:100 as recommended by the manufacturer) validated on a cohort of 20 controls. (**B**) Residual rituximab level at Months 3 in the MN cohort compared with a cohort of myasthenia gravis patients with no proteinuria. Patients with nephrotic syndrome have lower residual rituximab level at Month 3: 12.7 (7.45–27.84) versus 2.27 (0.19–7.5) µg/mL, P <0.0001. (**C**) Clinical and immunological outcome for MN patients according to the need or not of a second course of RTX: patients who required a second RTX course exhibited an increase in proteinuria and anti-PLA2R1 antibodies titre before re-treatment. M3: 3 months after rituximab; M6: 6 months after rituximab; last follow-up: last value timepoint of follow-up for patients treated with only one course of rituximab and for patients who required a second course, values before re-treatment. (**D**) Residual rituximab serum level at Month 3 in MN patients treated with only one course of rituximab versus those requiring a second course after resistant MN or relapse: 0.20 (0.00–3.59) versus 3.05 (1.67–11.70), P = 0.004. (**E**) Count of CD19 at Months 3, 6 and 12 in two groups of MN patients according to their residual rituximab level at Month 3. In patients with low residual rituximab level (<1 µg/mL), B cells re-emerged more quickly (slope = 5.23 versus 22.45, P = 0.0465). RTX: rituximab.

We found a large inter-individual variability among MN patients treated with the same schedule of rituximab that was independent of age, weight and proteinuria. Residual rituximab serum levels may depend on the efficacy of recycling by endothelial cells via FcRn [14], possibly due to the polymorphism of FcRn [15, 16]. Rituximab recycling allows long time exposure to rituximab that could be associated with a good therapeutic response, while early measurements of rituximab levels do not correlate with the clinical outcome [4, 11]. However, despite rituximab recycling, nephrotic patients have a shorter exposure to rituximab compared with a population with no proteinuria, due to rituximab wasting in the urine. Internalization and destruction of rituximab by target B cells could also contribute to low residual rituximab levels [11, 12].

We observed that higher residual serum rituximab concentrations at Month 3 significantly correlated with higher B-cell depletion. Whether undetectable serum rituximab at Month 3 might be a useful biomarker for patients with persistently high anti-PLA2R1 activity, epitope spreading [17] and active disease, which can predict resistance to rituximab and correlate with clinical outcomes, it needs to be tested in prospective studies.

FUNDING

This study was funded by a grant from the Centre Hospitalier Universitaire de Nice: 14-AOI-05.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Maloney DG. Mechanism of action of rituximab. *Anticancer Drugs* 2001; 12 (Suppl 2): S1–S4
2. Lim SH, Beers SA, French RR *et al.* Anti-CD20 monoclonal antibodies: historical and future perspectives. *Haematologica* 2010; 95: 135–143
3. Remuzzi G, Chiurchiu C, Abbate M *et al.* Rituximab for idiopathic membranous nephropathy. *Lancet* 2002; 360: 923–924
4. Fervenza FC, Cosio FG, Erickson SB *et al.* Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int* 2008; 73: 117–125
5. Fervenza FC, Abraham RS, Erickson SB *et al.* Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. *Clin J Am Soc Nephrol* 2010; 5: 2188–2198
6. Beck LH, Fervenza FC, Beck DM *et al.* Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol* 2011; 22: 1543–1550
7. Dahan K, Debiec H, Plaisier E *et al.* Rituximab for severe membranous nephropathy: A 6-month trial with extended follow-up. *J Am Soc Nephrol* 2017; 28: 348–358
8. van den Brand JAJG, Ruggenenti P, Chianca A *et al.* Safety of rituximab compared with steroids and cyclophosphamide for idiopathic membranous nephropathy. *J Am Soc Nephrol* 2017; 28: 2729–2737
9. Albert D, Dunham J, Khan S *et al.* Variability in the biological response to anti-CD20 B cell depletion in systemic lupus erythaematosus. *Ann Rheum Dis* 2008; 67: 1724–1731
10. Reddy V, Croca S, Gerona D *et al.* Serum rituximab levels and efficiency of B cell depletion: differences between patients with rheumatoid arthritis and systemic lupus erythematosus. *Rheumatology* 2013; 52: 951–952
11. Thurlings RM, Teng O, Vos K *et al.* Clinical response, pharmacokinetics, development of human anti-chimaeric antibodies, and synovial tissue response to rituximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2010; 69: 409–412
12. Cartron G, Trappe RU, Solal-Céligny P *et al.* Interindividual variability of response to rituximab: from biological origins to individualized therapies. *Clin Cancer Res* 2011; 17: 19–30
13. Jacobs R, Langer-Jacobus T, Duong M *et al.* Detection and quantification of rituximab in the human urine. *J Immunol Methods* 2017; 451: 118–121
14. Boye J, Elter T, Engert A. An overview of the current clinical use of the anti-CD20 monoclonal antibody rituximab. *Ann Oncol* 2003; 14: 520–535
15. Colombat P, Salles G, Brousse N *et al.* Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. *Blood* 2001; 97: 101–106
16. Cartron G, Dacheux L, Salles G *et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* 2002; 99: 754–758
17. Seitz-Polski B, Debiec H, Rousseau A *et al.* Phospholipase A2 Receptor 1 epitope Spreading at baseline predicts reduced likelihood of remission of membranous nephropathy. *J Am Soc Nephrol* 2018; 29: 401–408

Received: 13.12.2018; Editorial decision: 24.1.2019