KI REPORTS
KIReports.org



# Circulating Anti-Rituximab Antibodies Do Not Affect Response to Rituximab in Steroid-Dependent Nephrotic Syndrome



Andrea Angeletti[1,2], Maurizio Bruschi[2], Manuela Colucci[3], Xhuliana Kajana[2], Edoardo La Porta[1], Gianluca Caridi[2], Francesca Lugani[2], Pietro Ravani[4], Marina Vivarelli[5], Paolo Cravedi[6] and Gian Marco Ghiggeri[1,2]

[1]Division of Nephrology, Dialysis, and Transplantation, IRCCS Istituto Giannina Gaslini, Genoa, Italy; [2]Laboratory on Molecular Nephrology, IRCCS Istituto Giannina Gaslini, Genoa, Italy; [3]Renal Diseases Research Unit, Genetics and Rare Diseases Division, Bambino Gesù Children's Hospital IRCCS, Rome, Italy; [4]Division of Nephrology, University of Calgary, Calgary, Alberta, Canada; [5]Division of Nephrology, Bambino Gesù Children's Hospital IRCCS, Rome, Italy; and [6]Translational Transplant Research Center, Renal Division, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA

**Correspondence**: Andrea Angeletti, Division of Nephrology, Dialysis, and Transplantation, IRCCS Istituto Giannina Gaslini, Via Gaslini 5, Genoa 40147, Italy. E-mail: andreaangeletti@gaslini.org

**Received 12 July 2022; revised 8 August 2022; accepted 15 August 2022; published online 20 August 2022**

*Kidney Int Rep* (2022) **7,** 2509–2512; https://doi.org/10.1016/j.ekir.2022.08.006
KEYWORDS: anti-rituximab antibodies; focal segmental glomerulosclerosis; monoclonal anti-CD20 antibodies; nephrotic syndrome; rituximab; steroid-dependent nephrotic syndrome


## INTRODUCTION

Nephrotic syndrome (NS) is the most common cause of proteinuria in children and young adults.[1] Steroids still represent the cornerstone of therapy for pediatric NS, achieving remission in 80% to 90% of the cases.[S1] However, half of the patients develop relapses and require chronic steroid therapy to maintain remission (steroid-dependent NS, [SDNS]).[S2]

Rituximab is a mouse-human chimeric monoclonal antibody targeting the CD20 antigen expressed on B cells[S3] and represents first-line steroid-sparing agent in complicated NS.[2]

Owing to its chimeric nature, the immunogenicity of rituximab was reported in several diseases, such as rheumatoid arthritis and systemic lupus erythematosus.[S4–S6] Recent findings have reported that the development of anti-rituximab antibodies may affect the efficacy of rituximab in children with SDNS[3] and in adults with idiopathic membranous nephropathy.[4]

However, the impact of anti-rituximab antibodies on the safety/efficacy of further infusions in SDNS still has to be fully elucidated.[4,5,S7,S8] We aimed to evaluate the development of anti-rituximab antibodies in a cohort of patients with SDNS treated with rituximab as part of a large randomized controlled trial[6] and to establish the association between these antibodies and safety/efficacy of further rituximab administrations and B-cell depletion and reconstitution.

## RESULTS

### Population Characteristics

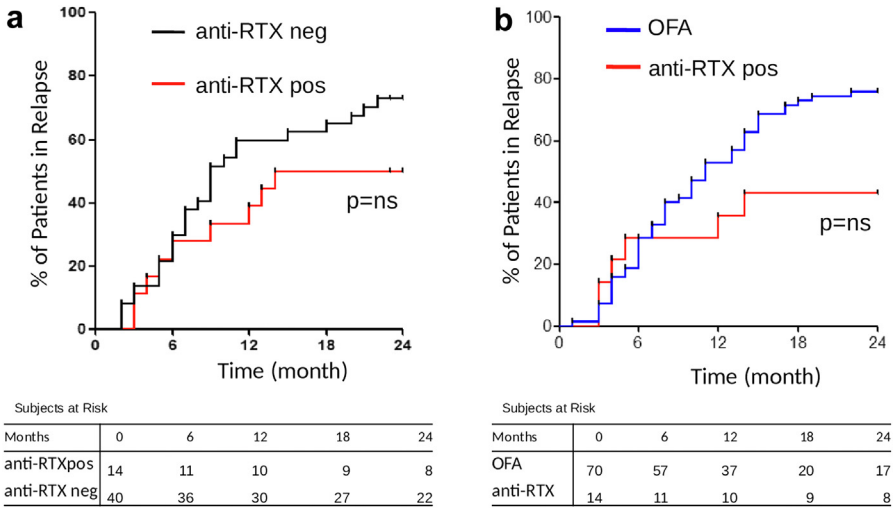
A total of 140 patients with SDNS were included in the study, and 64 of 140 had previously received at least 1 infusion of rituximab (average of previous infusions: 1.5 ± 0.7) in a median of 36 (13–54) months before enrollment. The closest infusion was 13 months before enrollment. Furthermore, 54 of 70 patients (77%) enrolled in the rituximab arm consented to participate to this ancillary study (Supplementary Figure S1).

### Detection of Anti-Rituximab Antibodies Before (T0) and 6 Months (T6) After Rituximab Treatment

Anti-rituximab antibodies were detected in none of the 64 patients receiving ≥1 infusions before enrollment. At 6 months, anti-rituximab antibodies were detected in 14 of 54 patients (26%) who received rituximab (Supplementary Figure S2A). Rituximab infusions before enrollment did not affect the development of anti-rituximab antibodies at T6 (Supplementary Figure S2B). There were no differences in the baseline characteristics (Supplementary Table S1).

### Relapse and Anti-Rituximab Antibodies

Of 54 patients treated with rituximab, 35 (65%) relapsed during the 24 months of follow-up. Incidence of anti-rituximab antibodies at 6 months




Subjects at Risk

| Months | 0 | 6 | 12 | 18 | 24 |
|---|---|---|---|---|---|
| anti-RTXpos | 14 | 11 | 10 | 9 | 8 |
| anti-RTX neg | 40 | 36 | 30 | 27 | 22 |

Subjects at Risk

| Months | 0 | 6 | 12 | 18 | 24 |
|---|---|---|---|---|---|
| OFA | 70 | 57 | 37 | 20 | 17 |
| anti-RTX | 14 | 11 | 10 | 9 | 8 |

**Figure 1.** Presence of circulating anti-rituximab antibodies does not affect response to rituximab. (a) Time to relapse was not statistically different between patients with and without development of anti-rituximab antibodies at T6. (b) Time to relapse was not statistically different between patients with anti-rituximab antibodies at T6 and subjects randomized to ofatumumab in the original trial. neg, negative; ns, not significant; OFA, ofatumumab; pos, positive; RTX, rituximab.

postrandomization was not statistically different between patients who relapsed versus who did not (Supplementary Figure S2C). Time to relapse is reported in Figure 1a. We also compared time to relapse in patients developing anti-rituximab antibodies with subjects randomized to ofatumumab in the original trial (Figure 1b).

The relapse rate was not statistically different between patients with (7/14, 50%) and without (28/40, 70%) anti-rituximab antibodies (Supplementary Figure S3A). According to the protocol study, the 35 relapsing received a further infusion of rituximab at each relapse: 23 of 35 experienced 2 or more relapses (2 or more rituximab infusions after enrollment) and 12 of 35 (34%) had only 1 relapse after enrollment (only 1 rituximab infusion after enrollment). In the 35 relapsing patients who received further rituximab doses, presence of anti-rituximab antibodies at 6 months of follow-up did not correlate with incidence of further relapses (Supplementary Figure S3B).

### Impact of Anti-Rituximab Antibodies on Circulating Total and Memory B Cells Post-Rituximab Treatment

We measured changes in B-cell subsets in 33 patients (10 and 23 from the anti-rituximab antibodies positive and negative subjects, respectively). Patients with and without anti-rituximab antibodies had similar level of total B-cell count before rituximab therapy. At months 6 and 12 after rituximab, both groups had similar B-cell reconstitution (Figure 2a). Similar results were reported for memory B cells (Figure 2b).

Of note, time to relapse and B-cell reconstitution were not statistically different even considering only subjects with serum levels of anti-rituximab antibodies above the manufacturer threshold of 5 ng/ml (not found).

### Safety

All the 54 participants remained free from severe complications during the entire duration of the study. Moreover, none of the subjects reported infusion-related reactions.

## DISCUSSION

In this study, we investigated the development of anti-rituximab antibodies and their association with clinical outcome in subjects with SDNS. In accordance with previous reports,[3,7] we found that 26% of patients developed anti-rituximab antibodies.

Bertrand *et al.*[8] recently described development of anti-rituximab antibodies in 7 of 24 patients receiving single infusion (375 mg/m$^2$) in children with frequent-relapsing or SDNS. Moreover, in a retrospective study, Boyer-Suavet *et al.*[4] described 44 patients affected by membranous nephropathy and treated with two 1 g infusions of rituximab at 2-week interval, which developed anti-rituximab antibodies in 23% of cases at 6 months of follow-up.

In the 24 months of follow-up, development of anti-rituximab antibodies in SDNS was not associated with increased risk of disease recurrence after rituximab treatment. Therefore, in contrast to previous studies in other diseases such as systemic lupus erythematosus or membranous nephropathy, we did not find unfavorable outcomes after additional rituximab doses.[4,5] The lower rituximab doses in patients with SDNS compared with those used to treat individuals with systemic

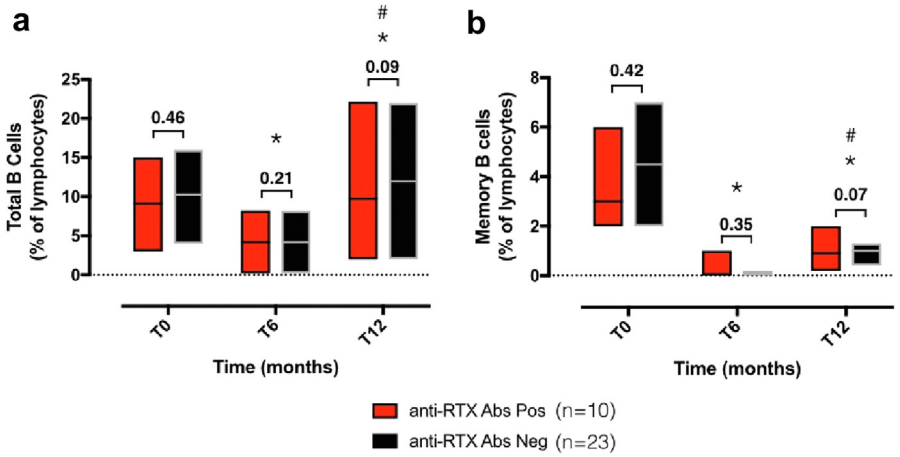



**Figure 2.** Reconstitution of total and memory B cells is not affected by the development of anti-rituximab antibodies. (a) Total B-cell counts at month 6 and at month 12 had similar reconstitution in patients with ($n$ = 10) and without ($n$ = 23) anti-rituximab antibodies at T6, with nonsignificant statistical differences. (b) Memory B-cell counts at month 6 and at month 12 had similar reconstitution in patients with and without anti-rituximab antibodies at T6, with nonsignificant statistical differences. * refers to T0; # refers to T6. Abs, antibodies; neg, negative; pos, positive; RTX, rituximab.

lupus erythematosus or membranous nephropathy may partially explain the inconsistency with previous studies using higher rituximab doses.[4,5,8] Therefore, the development of anti-rituximab antibodies may have a dose-dependent mechanism. However, the optimum dosing schedule for rituximab has not been established for NS.[9] Some previous studies reported that a single dose of 375 mg/m$^2$ would have comparable outcomes to higher doses in reducing the frequency of relapse and time to B-cell reconstitution.[S9,S10] Therefore, our findings suggest that single low dose may also limit the development of anti-rituximab antibodies.

Moreover, in contrast to previous findings,[4,8,S11] we found low serum titers of circulating anti-rituximab antibodies, which may in part explain the lack of correlation between development of anti-rituximab antibodies and clinical outcome.

Importantly, incidence of relapse in patients developing anti-rituximab antibodies and in subjects receiving ofatumumab was comparable.

Depletion levels and the recovery of circulating total and memory B cells after rituximab were not affected by the presence of anti-rituximab antibodies. Previous retrospective studies presented contrasting results. Bertrand *et al*.[8] revealed that anti-rituximab antibodies were associated with incomplete $CD19^{+}CD20^{-}$ B-cell depletion and low serum rituximab levels in SDNS. However, the development of anti-rituximab antibodies was not associated with shorter B-cell depletion. In contrast, in children with SDNS receiving single dose of rituximab (375 mg/m$^2$), the median time of B-cell recovery was significantly shorter in 9 of 13 developing anti-rituximab antibodies.[3] Difference in rituximab doses and anti-rituximab antibody levels may explain inconsistencies in the impact of anti-rituximab antibodies across studies.

We also reported that, at randomization, anti-rituximab antibodies were negative in all patients who previously received at least 1 infusion of rituximab. This suggests that the persistence of serum anti-rituximab antibodies is temporary, although serial measurement is required to substantiate this hypothesis.

Rituximab is generally well tolerated, with adverse events mostly limited to infusion reactions.[S12] Previous studies have reported a possible correlation between the incidence of infusion-related reactions and anti-rituximab antibodies,[4,5,S13] but data are inconsistent.[S5] In this study, we did not report any adverse event nor infusion-related reactions in subjects presenting anti-rituximab antibodies.

The prospective design represents a major strength of this work. In addition, considering SDNS is a rare condition, we present data on a large cohort of subject. We also recognize some limitations: not all patients assigned to the rituximab arm consented to participate to the ancillary study, we performed only a single detection of anti-rituximab antibodies at 6 months of follow-up, and B-cell analysis was performed on 33 of 54 subjects, but there was no bias in the selection of patients because all were asked to participate.

In conclusion, we found that approximately one-fourth of patients with SDNS develop anti-rituximab antibodies after a single rituximab infusion. The presence of these antibodies does not affect the safety/efficacy profile of rituximab in this population nor circulating B-cell kinetics.

## DISCLOSURE

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## ACKNOWLEDGMENTS

This work was supported by the Italian Ministry of Health—Ministero della Salute, Ricerca Corrente 2022.

### Data Availability Statement

The data sets generated for this study are available on request to the corresponding author.

### Ethics Statement

Study approval was obtained from the local independent ethics committee (ComitatoEticoRegione Liguria) and the Italian Drug Agency (Agenzia Italiana del Farmaco, AIFA). We registered the study at https://clinicaltrials.gov (study number: NCT02394119) and at https://eudract.ema.europa.eu (Eudract study number 2015-000624-28).

## AUTHOR CONTRIBUTIONS

AA and GMG contributed to the conception and design of the work. AA wrote the letter. PC largely revised the letter. PR and GMR designed the original trial. MB performed laboratory and statistical analysis. MC and MV performed laboratory analysis on B cells. AA, GC, FL, ELP, MC, MV, PC, and GMG revised it critically and contributed to the interpretation of data. All the authors provide approval for publication of the content.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Supplementary Methods**.

**Supplementary References.**

**Figure S1.** Study flowchart.

**Figure S2.** Development of anti-rituximab antibodies is not different in patients with relapse versus patients without relapse.

**Figure S3.** Presence of circulating anti-rituximab antibodies does not affect response to further infusions of rituximab.

**Table S1.** Baseline characteristics.

## REFERENCES

1. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. *Lancet*. 2018;392:61–74. https://doi.org/10.1016/S0140-6736(18)30536-1
2. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. *Kidney Int*. 2021;100(4S):S1–S276. https://doi.org/10.1016/j.kint.2021.05.021
3. Fujinaga S, Nishino T, Endo S, Umeda C, Watanabe Y, Nakagawa M. Unfavorable impact of anti-rituximab antibodies on clinical outcomes in children with complicated steroid-dependent nephrotic syndrome. *Pediatr Nephrol*. 2020;35:2003–2008. https://doi.org/10.1007/s00467-020-04629-w
4. Boyer-Suavet S, Andreani M, Lateb M, et al. Neutralizing anti-rituximab antibodies and relapse in membranous nephropathy treated with rituximab. *Front Immunol*. 2020;10:3069. https://doi.org/10.3389/fimmu.2019.03069
5. Wincup C, Menon M, Smith E, et al. Presence of anti-rituximab antibodies predicts infusion-related reactions in patients with systemic lupus erythematosus. *Ann Rheum Dis*. 2019;78:1140–1142. https://doi.org/10.1136/annrheumdis-2019-215200
6. Ravani P, Colucci M, Bruschi M, et al. Human or chimeric monoclonal anti-CD20 antibodies for children with nephrotic syndrome: a superiority randomized trial. *J Am Soc Nephrol*. 2021;32:2652–2663. https://doi.org/10.1681/ASN.2021040561
7. 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. https://doi.org/10.1136/ard.2007.083162
8. Bertrand Q, Mignot S, Kwon T, et al. Anti-rituximab antibodies in pediatric steroid-dependent nephrotic syndrome. *Pediatr Nephrol*. 2022;37:357–365. https://doi.org/10.1007/s00467-021-05069-w
9. Chan EY, Yu ELM, Angeletti A, et al. Long-term efficacy and safety of repeated rituximab to maintain remission in idiopathic childhood nephrotic syndrome: an international study. *J Am Soc Nephrol*. 2022;33:1193–1207. https://doi.org/10.1681/ASN.2021111472