Short-Term Effects of Rituximab in Children with Steroid- and Calcineurin-Dependent Nephrotic Syndrome: A Randomized Controlled Trial

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Summary

Background and objectives Prednisone and calcineurin inhibitors are the mainstay therapy of idiopathic nephrotic syndrome (INS) in children. However, drug dependence and toxicity associated with protracted use are common. Case series suggest that the anti-CD20 monoclonal antibody rituximab (RTX) may maintain disease remission.

Design, setting, participants, & measurements This open-label randomized controlled trial was powered to show that a strategy based on RTX and lower doses of prednisone and calcineurin inhibitors was noninferior to standard doses of these agents in maintaining 3-month proteinuria as low as baseline or up to 1 g/d greater (noninferiority margin). Participants were stratified by the presence of toxicity to prednisone/calcineurin inhibitors and centrally assigned to add RTX (Mabthera, 375 mg/m^2 intravenously) to lower doses of standard agents or to continue with current therapy alone. The risk of relapse was a secondary outcome.

Results Fifty-four children (mean age 11 ± 4 years) with INS dependent on prednisone and calcineurin inhibitors for >12 months were randomized. Three-month proteinuria was 70% lower in the RTX arm (95% confidence interval 35% to 86%) as compared with standard therapy arm (intention-to-treat); relapse rates were 18.5% (intervention) and 48.1% (standard arm) ($P = 0.029$). Probabilities of being drug-free at 3 months were 62.9% and 3.7%, respectively ($P < 0.001$); 50% of RTX cases were in stable remission without drugs after 9 months.

Conclusions Rituximab and lower doses of prednisone and calcineurin inhibitors are noninferior to standard therapy in maintaining short-term remission in children with INS dependent on both drugs and allow their temporary withdrawal.

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Introduction

Idiopathic nephrotic syndrome (INS) is characterized by severe proteinuria, hypoalbuminemia, dyslipidemia, and thrombogenic tendency. INS affects 2 to 2.7 new children per 100,000 children per year, in Western countries, with a prevalence of 16 cases per 100,000 children (1). Mechanisms underlying the disorder include different genetic and pathologic variants (2–4), with polymorphic podocyte injury as a unifying feature (5–7). All described phenotypes are considered part of a pathology continuum from minimal lesions (minimal change disease) to podocyte depletion and glomerulosclerosis (focal and segmental glomerular sclerosis) (7).

Prednisone is the cornerstone of therapy for INS, inducing remission within 4 weeks in approximately 90% of cases. However, the risk of clinical relapse can be as high as 85% at 5 years (8), requiring reiteration of prednisone courses, often with the additional use of calcineurin inhibitors, that is, cyclosporin A or tacrolimus (9,10). Long-term treatment with prednisone and calcineurin inhibitors increases the risk of complications such as neurotoxicity, renal failure, malignancy, growth retardation, hypertension, and diabetes. On the other hand, accelerated progression of the underlying kidney disease is also associated with morbidity and mortality, in addition to a high rate of disease recurrence in renal grafts (11). Thus, balancing the risks and benefits of a protracted course of immunosuppression in patients with relapsing INS presents a significant challenge for pediatric nephrologists.

Rituximab (RTX) is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen on the surface of B lymphocytes that inhibits B cell proliferation and differentiation. Rituximab was first introduced in clinical prac-

tice for the treatment of non-Hodgkin's lymphoma and then extended to autoimmune diseases (12), such as rheumatoid arthritis (13), lupus erythematosus (14,15), vasculitic disorders (16), and membranous nephropathy (12,17,18). Recent uncontrolled studies suggest that RTX may maintain remission of INS as effectively as standard therapy based on prednisone and calcineurin inhibitors (19–22). This randomized controlled trial tested this hypothesis comparing a new strategy based on RTX and reduced/suspended doses of prednisone and calcineurin inhibitors *versus* the standard approach in children with INS dependent on both these agents.

Materials and Methods

Design Overview

Eligible participants entered a 1-month run-in period during which proteinuria was monitored, compliance assessed, calcineurin inhibitors dose maintained constant, and prednisone dose tentatively reduced to the minimum amount of the previous 6 months. During run-in, instructions on urine collection and dipstick readings were carefully reviewed. After run-in, children were centrally allocated to continue standard therapy alone or add RTX independent of the current level of proteinuria. Assignments followed permuted block randomization lists (stratified by center and signs of toxicity) with blocks of variable size. Clinical investigators and study nurses enrolling patients were not blinded to group assignment. However, study staff responsible for facilitating follow-up data measurements were blinded. An independent data safety monitoring board reviewed safety data when half of the participants had been enrolled and at study end. The protocol and consent documents were approved by the ethics committees at each participating center.

Setting and Participants

Participants in this study had to be 16 years old or younger with an estimated creatinine clearance >60 ml/min per 1.73 m^2. They had to have a history of INS responsive to, and dependent on, both prednisone and calcineurin inhibitors for at least 1 year with a remission of at least 6 months (daily proteinuria <4 mg/m^2 per hour). INS was diagnosed in the presence of nephrotic range proteinuria (>40 mg/m^2 per hour or >1 g/d per m^2, or protein to creatinine ratio [P/C] >4 in a single urine specimen) or between 5 and 40 mg/m^2 per hour associated with hypoalbuminemia or dyslipidemia. Genetic testing and renal biopsy were not required as childhood INS is a clinical diagnosis based on the response to prednisone and calcineurin inhibitors. Criteria for exclusion were as follows: infantile onset (<1 year); previous history of macro-hematuria, hepatitis B, hepatitis C, or HIV infection; positivity of any marker of autoimmunity (ANA, nDNA, ANCA); and low C3 levels. Patients requiring diuretics, albumin, or anticoagulant therapy were also temporarily excluded.

Steroid dependence was defined as responsiveness to full doses of prednisone (2 mg/kg) and calcineurin inhibitors (5 mg/kg) with two consecutive relapses occurring during prednisone tapering or within 2 weeks of prednisone withdrawal. Steroid dependence could have been present since the beginning of prednisone therapy or developed after an initial resistance to prednisone monotherapy. In the first case, the addition of a calcineurin inhibitor was required after at least 6 months of prednisone monotherapy to maintain remission. In the other case, calcineurin inhibitors modified the response to therapy. Angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs) were used at the discretion of the investigators but were kept constant during the study. Criteria for toxicity due to steroids and/or calcineurin inhibitors are summarized in Table 1.

Overall, between 2007 and 2008, we screened 150 INS patients, most of whom were excluded because of sensitivity to, or dependency on, low-dose prednisone monotherapy. Children with INS resistant to prednisone and calcineurin inhibitors, or dependent on high prednisone doses (0.7 mg/kg per day), were also excluded (Figure 1). Children dependent on steroids and calcineurin inhibitors who showed signs of toxicity were initially enrolled separately (EudraCT 2007-007796-16) from those without toxicity (EudraCT 2008-004486-26) and subsequently treated as separate strata of the same trial.

Intervention Strategy and Standard Therapy

The intervention strategy was based on RTX and reduced doses of prednisone and calcineurin inhibitors. Ta-

Table 1. Criteria for toxicity to standard medications

Steroids	
(1)	Growth retard (height >2 SD below normality according to Tanner standard [35] without other causes of short height)
(2)	Cataract diagnosed by an ophthalmologist
(3)	Osteoporosis (Z-score >2 SD below age-matched normal bone mass plus one spontaneous bone fracture at dual-energy X-ray absorptiometry scan)
Calcineurin inhibitors	
(1)	Arterial hypertension (values >95° based on three consecutive measures) lasting for >3 months and not linked to the acute phase of the disease
(2)	Posterior reversible encephalopathy syndrome (NMR criteria [36])
(3)	Presence of pathological signs of cyclosporine nephropathy (tubulointerstitial fibrosis, epithelia vacuolization, calcineurin inhibitors crystals)
(4)	Deterioration of renal function in the last 6 months (20% increase of serum creatinine levels)

NMR, nuclear magnetic resonance.

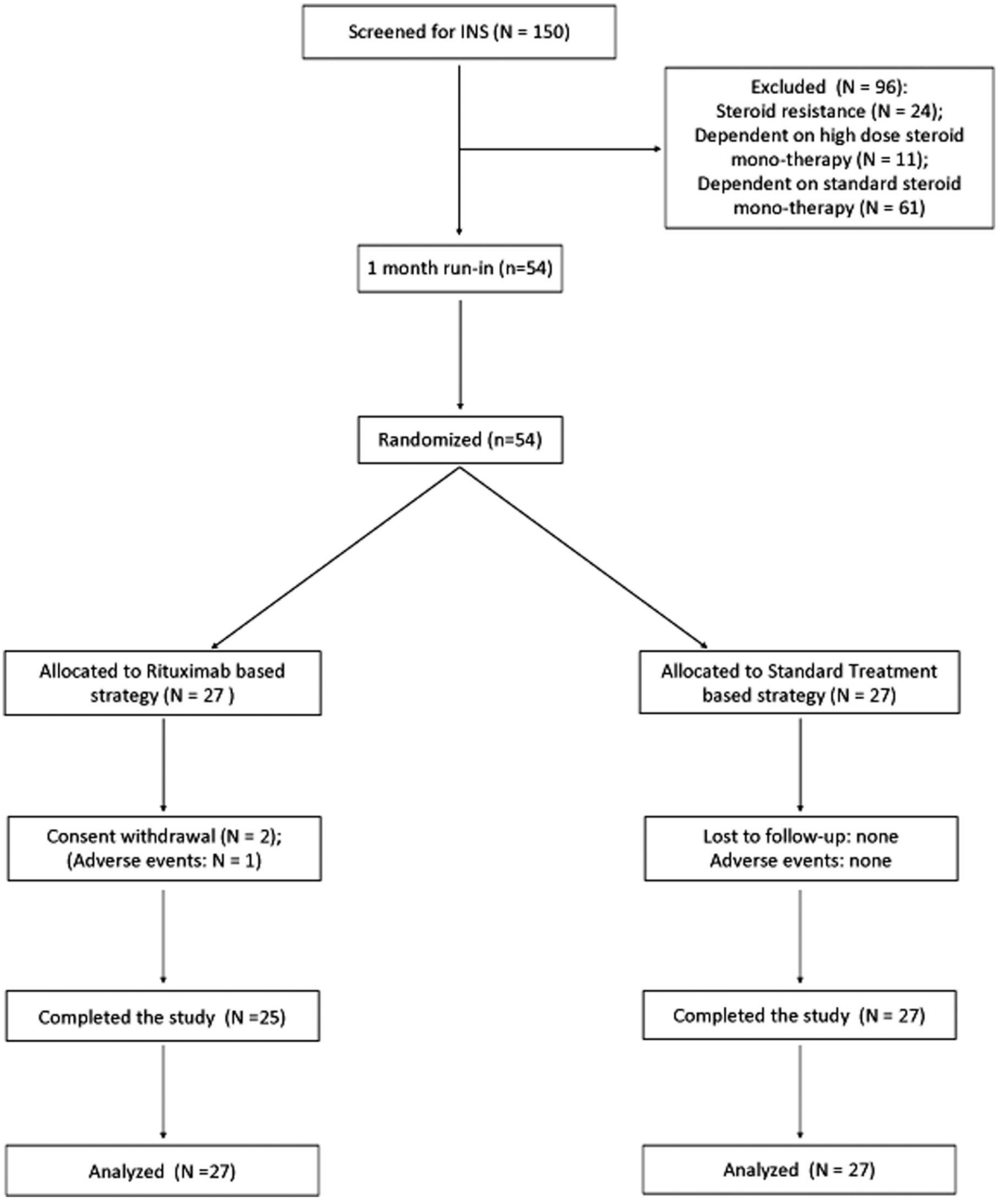

Figure 1. | **Number of patients who were screened for the study, who underwent randomization, and who completed the study**.

pering was done following the scheme below that allows drug withdrawal within 45 days. Rituximab (Mabthera; 375 mg/m^2) was given intravenously once (at randomization in the absence of clinical signs of toxicity secondary to steroids and/or cyclosporine; see Table 1) or twice (at randomization and after 2 weeks in the presence of toxicity). The medication was diluted in normal saline (1 mg/ml) and administered at increasing speeds (0.5 to 1.5 ml/min) over approximately 6 hours. The infusion was preceded by 2.5 to 5 mg of intravenous chlorfenamine maleate, 2 mg/kg methyl prednisolone in normal saline, and 8 mg/kg of oral paracetamol. Starting at 30 days, prednisone was tapered off by 0.3 mg/kg per week if proteinuria was <1 g/d. After 2 weeks, calcineurin inhibitors were also decreased by 50% and withdrawn after 2 additional weeks.

The standard strategy was based on prednisone and calcineurin inhibitors alone. Doses of these agents could be tapered off as in the intervention strategy if proteinuria was <1 g/d. In both groups relapses had to be treated with prednisone (maximum dose of 2 mg/kg per day).

Outcomes and Follow-up

Children were seen by the nephrologist responsible for the study at the beginning and at the end of the study, and as many times as necessary in case of complications or relapses. Study coordinators maintained ongoing contact with the children and the families to monitor clinical status and report potential adverse events. BP was measured at regular intervals (2 times per week) by the family pediatrician who contacted the referent nephrologist for data reporting and therapy update.

Urine protein excretion was measured weekly on 24-hour urine collection, or more often in case of dipstick positivity. Determination of proteinuria at baseline and after 3 months was performed at a central laboratory. Dipstick for proteinuria determination was evaluated daily. In case of dipstick positivity, the presence of proteinuria was confirmed with 24-hour urine collection. Kidney function, plasma proteins, and cholesterol were obtained monthly. Counts of white blood cells and lymphocyte populations were monitored monthly in the RTX group. The primary efficacy measure was the percentage change in daily proteinuria at 3 months in children receiving RTX *versus* those on standard care.

The risk of relapse at 3 months was a secondary outcome. Relapse was diagnosed in the presence of proteinuria >40 mg/h per m^2 (P/C >4; full relapse) or of proteinuria of 20 to 40 mg/h per m^2 (P/C 2 to 4; partial relapse) and hypoalbuminemia or dyslipidemia.

Statistical Analyses

We estimated that 54 participants were necessary to demonstrate with a power of 90% that RTX was noninferior to standard care in maintaining proteinuria at 3 months as low as baseline levels or lower, or up to 1 g/d greater (noninferiority margin). We assumed a log-normal distribution of the response, with a coefficient of variation of 1.5 on the original scale (mean 0.5 and SD 0.75 g/d), and a geometric mean ratio of 3 as noninferiority margin. The estimation accounted for a 5% risk of withdrawals and multiple comparisons (effect of RTX by presence/absence of toxicity) (23). Analysis of the primary outcome was conducted according to the intention-to-treat principle. Three-month log-transformed proteinuria was modeled using an analysis of covariance (ANCOVA) model with treatment, toxicity, and their interaction term as factors and log-transformed baseline proteinuria as covariate. Missing values at 3 months in those who did not complete the study were replaced, carrying forward their last available value. The risk of relapse at 3 months was studied using logistic regression. Missing data at 3 months were treated as "event present" in the RTX arm and "event absent" in the control group (assuming the worst case scenario). Kaplan-Meier analysis was used to describe 1-year relapse-free survival of children assigned to RTX. Comparisons by treatment group were conducted using two-sided tests with a significance level of 0.05. Analyses were performed with STATA 11.1 (StataCorp, College Station, Texas) and R 2.11.0 (http://www.R-project.org).

Results

Patient Characteristics

Fifty-four patients were randomized. Baseline characteristics are summarized in Table 2. Children were on average

Table 2. Baseline characteristics

Cases	All (n = 54)	Control (n = 27)	Intervention (n = 27)
Age (year)	10.8 ± 4.2	11.3 ± 4.3	10.2 ± 4.0
Body weight (kg)	42.6 ± 17.5	45.5 ± 19.3	39.6 ± 15.2
Men	43 (79.6)	19 (70.4)	24 (88.9)
Disease duration (years)	6.7 ± 3.9	7.8 ± 4.0	5.7 ± 3.5
Renal histology			
Unavailable	18 (33.3)	11 (40.7)	7 (25.9)
FSGS	17 (31.5)	10 (37.1)	7 (25.9)
MCD	19 (35.2)	6 (22.2)	13 (48.1)
Steroid toxicity	19 (35.1)	9 (33.3)	10 (37.0)
CNI toxicity	5 (9.2)	3 (11.1)	2 (7.4)
CTX therapy	31 (57.4)	13 (48.1)	18 (66.7)
Prednisone (mg/kg per day)	0.58 ± 0.44	0.60 ± 0.47	0.57 ± 0.42
CsA/FK506[a]	38 (70)/16 (30)	19 (70)/8 (30)	19 (70)/8 (30)
ARB/ACEI	25 (46.3)	15 (55.5)	10 (37.1)
Urinary protein (g/d)	1.6 ± 2.8	1.6 ± 2.1	1.6 ± 3.4
Serum albumin (g/dl)	3.4 ± 0.9	3.2 ± 0.8	3.6 ± 0.9
Serum cholesterol (mg/dl)	276 ± 161	283 ± 116	268 ± 200
Serum creatinine (mg/dl)	0.55 ± 0.2	0.55 ± 0.2	0.55 ± 0.3
White blood cells (μl^{-1})	10,534 ± 4,150	10,703 ± 4,121	10,365 ± 4,250
IgG (g/dl)	533 ± 267	488 ± 265	575 ± 268

Mean ± SD reported for quantitative variables (unit) and absolute (%) for qualitative variables. FSGS, focal and segmental glomerular sclerosis; MCD, minimal change disease; CNI, calcineurin inhibitors (cyclosporine or tacrolimus) used at full dose (necessary to maintain trough levels of 50 to 100 ng/ml for cyclosporine [CsA] and 5 to 10 ng/ml for tacrolimus [FK506]); CTX therapy, previous use of cytotoxic agents (Endoxan, Leukeran).

[a]In two patients, CsA was stopped during the run-in for severe toxicity.

11 years old and tended to be male. Disease duration was on average 6.7 years. Signs of toxicity were present in 24 children. In these patients, calcineurin inhibitor treatment had been carried out for longer periods than in those without toxicity (6.25 ± 3.3 *versus* 5.0 ± 3.5 years, $P = 0.184$) and both prednisone doses and levels of proteinuria tended to be higher (Figure 2). All children but two were still on full maintenance doses of calcineurin inhibitors at randomization, defined as the minimum oral amount necessary to achieve trough levels of 50 to 100 and 5 to 10 ng/ml, respectively, for cyclosporine and tacrolimus (for details, see Table 2). Average proteinuria was similar by treatment arm at screening and baseline (although overall greater than anticipated), as were average prednisone doses. There were no differences between groups in terms of additional therapies (ARBs and ACEIs), levels of kidney function, serum albumin, and cholesterol levels. ARBs and ACEIs use was limited in patients with hypotension and calcineurin inhibitors toxicity because of their known additive effect (24). Two patients without toxicity assigned to RTX did not complete the study for consent withdrawal.

Effectiveness

Table 3 summarizes the results of regression analysis. Rituximab use allowed the maintenance of remission despite progressive reduction and withdrawal of prednisone. Overall 3-month proteinuria was 70% lower in children who received RTX as compared with controls (95% confidence interval [CI] 35% to 86% lower). Prednisone was withdrawn in all but one child in the RTX arm. Control patients without toxicity had a modest increment of proteinuria during the prednisone reduction at 4 weeks that required resuming previous prednisone dose (Figure 2). In children with toxicity, 3-month proteinuria was not significantly different by treatment group (average change −35.66%, 95% CI −78.62% to 93.66%, *i.e.*, below the noninferiority margin of 3 times). However, the use of RTX made it possible to withdraw prednisone in all but four children with toxicity, two of whom had, however, a 50% and 60% reduction of the initial dose. Overall, the proportion of children in whom calcineurin inhibitors withdrawal was possible without change in proteinuria was also higher in the RTX group (56% *versus* 4%, $P < 0.001$). *Vice*

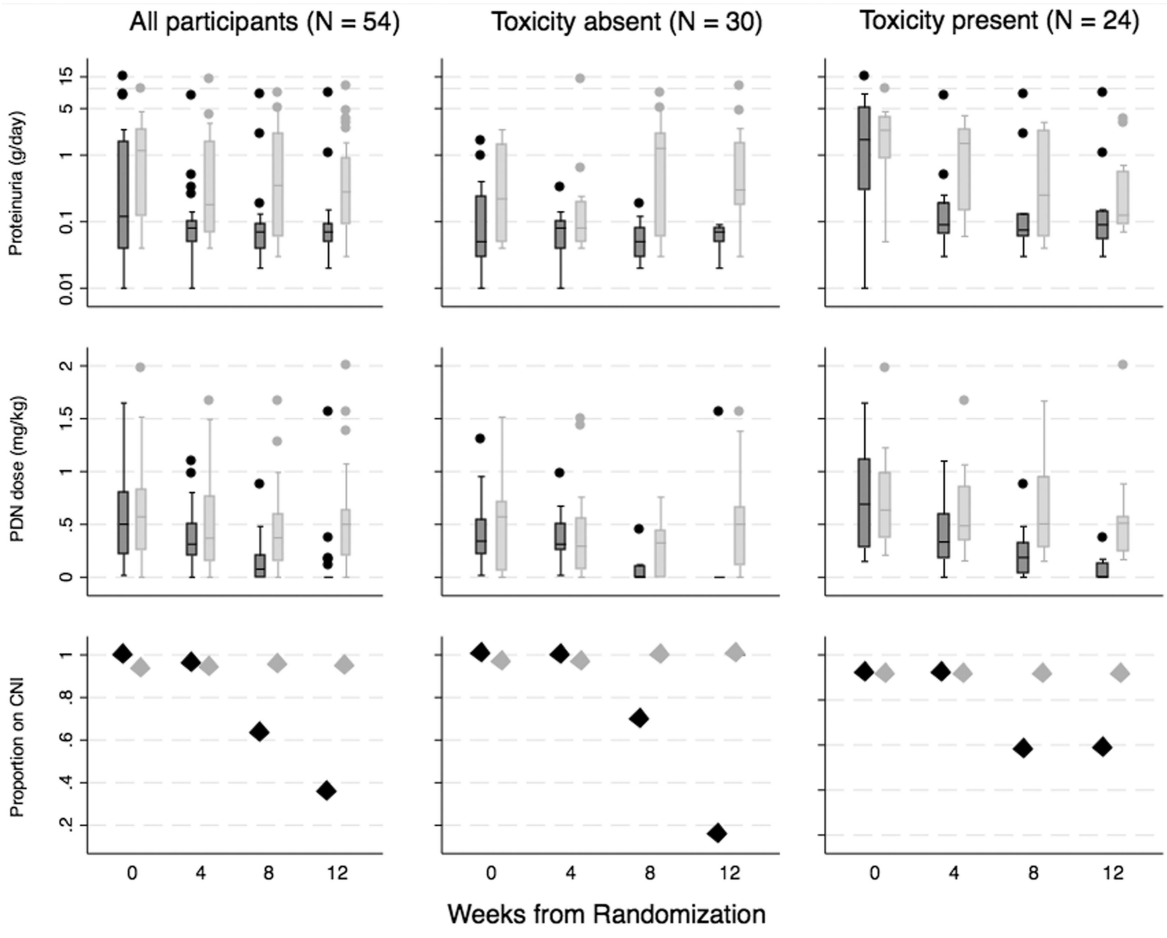

Figure 2. | The plots summarize the distribution of proteinuria (g/d [log-scale]), prednisone [PDN] in mg/kg per day, and proportion of children on full dose of calcineurin inhibitors [CNI, *i.e.*, cyclosporine or tacrolimus] over time in weeks from randomization (time zero). Dark gray bars refer to patients assigned to RTX-based strategy; light gray bars refer to standard therapy strategy. The plot on the left (all patients) refers to model 1 in Table 3; the middle and right plots refer to model 2 in Table 3. The line across the box plots (proteinuria and prednisone plots) is the median, the box hinges are the 25th and 75th percentiles, and the outliers are represented as dots lying beyond 1.5 times the interquartile range.

Table 3. Proteinuria at study end (ANCOVA model of log-proteinuria)

	Group Mean (95% CI)	% Reduction (95% CI)	*P*
Model 1 (R2 0.23)			
control	0.36 (0.21 to 0.62)	−69.80 (−86.04 to −34.68)	0.003
intervention	0.11 (0.06 to 0.20)		
Model 2 (R2 0.29)			
control (no toxicity)	0.55 (0.26 to 1.18)	−83.59 (−94.24 to −53.18%)	0.001
intervention (no toxicity)	0.09 (0.03 to 0.22)		
control (toxicity)	0.22 (0.10 to 0.48)	−35.66 (−78.62 to 93.66%)	0.425
intervention (toxicity)	0.14 (0.06 to 0.30)		

Geometric means (95% confidence intervals [CIs]) of daily proteinuria at study end and percentage change due to intervention (exponentiated differences of log-values) without considering toxicity (model 1) and by absence and presence of drug toxicity (model 2). Both models include baseline proteinuria, intervention group, and prednisone dose. The models failed to reject the null hypothesis of inferiority of rituximab (RTX) as compared to standard therapy. In model 2 (which includes also the interaction between toxicity and intervention) the average proteinuria values at 3 months are similar to (children with toxicity [$P = 0.425$] but upper 95% CI <400%, the noninferiority margin of 3 times) or lower than baseline values (children without toxicity [$P = 0.001$]). Of note, the use of prednisone and calcineurin inhibitors was reduced only in the RTX arm of both strata (Figure 2). ANCOVA, analysis of covariance.

versa, remission was maintained at the cost of unchanged doses of prednisone and calcineurin inhibitors in the standard therapy group. Children with toxicity required overall higher doses of all medications. No other factor (including histology) affected the outcome or modified the effect of RTX.

Secondary Outcome and Follow-up Data

The risk of disease relapse at 3 months was 48.1% in the control group ($n = 13$) *versus* 18.5% ($n = 5$) in the intervention group (odds ratio 4.08, 95% CI 1.19 to 13.9; $P = 0.029$). The probabilities of being prednisone-free and prednisone and calcineurin inhibitors–free at 3 months were 77.8% ($n = 21$) and 62.9% ($n = 17$) in the intervention group and 7.4% ($n = 2$) and 3.7% ($n = 1$) in the control group ($P < 0.001$ for both comparisons), respectively. One-year relapse-free survival after RTX therapy is described in Figure 3. At 6 and 12 months, respectively, 50% and 25% of the children assigned to RTX were still in remission without prednisone or calcineurin inhibitors. Sixteen patients in the control group were switched to RTX after 3 months because of family request and ethical committee approval. Eleven children continued with standard doses of prednisone and calcineurin inhibitors for continuous relapsing episodes (not shown).

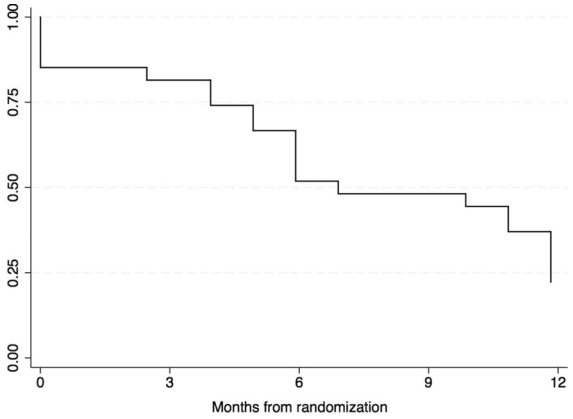

Figure 3. | Twelve-month relapse-free survival from randomization in children assigned to RTX therapy ($n = 27$). Tapering of steroids and calcineurin inhibitors started 30 days after RTX infusion and was completed in 45 days.

Adverse Events

One patient developed bronchospasm and hypotension at the second RTX infusion. Treatment was discontinued with spontaneous recovery. Two other cases required RTX infusion in intensive care for initial bronchospasm, which improved after slowing the infusion rate. Two patients presented fever with migrating skin rash and acute arthritis at the hip joint after 2 and 6 days from RTX infusion. Resolution was rapidly and completely achieved (24 to 48 hours) with nonsteroidal anti-inflammatory medications. CD20 counts were reduced to <1% at the first month in all RTX-treated patients. After 3 months CD20 count was still undetectable in all but three cases (one with toxicity and two without toxicity).

Discussion

The present trial shows that in children with INS responsive to and dependent on prednisone and calcineurin inhibitors, RTX therapy is noninferior to standard therapy in maintaining disease remission with reduced use of standard agents. In our study, disease remission was maintained for 3 months with one single dose of RTX in children without toxicity and with two doses administered 2 weeks apart in children with toxicity despite prednisone and calcineurin inhibitors reduction or withdrawal. This is the first randomized study showing the potential utility of RTX in achieving the dual goal of maintaining disease remission and reducing/withdrawing standard agents in children with INS sensitive to a combination of prednisone and calcineurin inhibitors. In these children, calcineurin inhibitors are often added to prednisone because of development of steroid toxicity or steroid resistance after initial steroid dependence, or because of steroid resistance "ab initio". Overall, they differ from patients

who manifest a clear resistance to any drug and belong to potentially different disease cohorts, including genetically mediated forms of INS (4,25).

Although responsiveness to therapies makes INS less dramatic in these children, the need for long-term use of prednisone and calcineurin inhibitors exposes them to serious side effects (26). In several cases, the clinical burden of drug toxicity is so heavy that periods of symptomatic therapy of the nephrotic syndrome become acceptable trade-offs. Toxicity to either or both prednisone and calcineurin inhibitors is, in fact, relatively common in children with INS who respond to these agents (44% in this study). This opens the question of how pediatric nephrologists can use these medications and whether new agents may change the course of the disease (19–22). The more urgent need for alternative solutions to the management of children with toxicity was the reason we administered two doses of RTX in this stratum.

A noninferiority study design was chosen to address the question of whether RTX allowed maintenance of remission in dependent INS while reducing/withdrawing prednisone and calcineurin inhibitors therapy. We hypothesized that RTX and lower/suspended doses of standard therapies were as effective as a combination of standard doses of prednisone and calcineurin inhibitors in maintaining 3-month remission. Although attempts to lower the doses of prednisone and calcineurin inhibitors were possible in the standard strategy arm, only in children who received RTX was disease remission maintained with lower doses of (or without) standard agents. The presence of drug toxicity to prednisone and calcineurin inhibitors and the pathology score did not substantially modify the effect of RTX. Most importantly, prednisone and calcineurin inhibitors withdrawal was rapidly possible in most children who received RTX with a median relapse-free survival longer than 1 year. Importantly, at 6 and 12 months, respectively, as many as 50% and 25% of the children assigned to RTX were still in remission without prednisone or calcineurin inhibitors. On clinical grounds this is a clear benefit that, in case of significant toxicity, is extremely required. Individual and disease-related factors may affect the duration of the response to RTX. Whether additional RTX doses can prolong remission remains to be tested in clinical trials once the safety profile of RTX is confirmed by long-term follow-up data.

The rationale for use of RTX in INS is, unfortunately, based on limited biologic knowledge. Interference of RTX with T helper cells and T regulatory and circulating plasma factors has been proposed (27–29). Accumulating evidence suggests that B cells play a role in regulating the immune response in both physiologic and pathologic conditions (30). B cell depletion therapy with RTX may be effective not only in autoantibody-associated but also in T cell–mediated autoimmune diseases (31). Data on the positive effects of RTX in INS support this role of B cells including effects on podocytes. However, given the absence of infiltrating cells in the glomeruli of patients with INS and the apparent lack of CD20 on podocytes, how the interaction between RTX therapy and T and B lymphocyte function translates into glomerular effects is currently unknown. It is possible that RTX modifies the oxygen radical–mediated innate response to infectious triggers (32), a highly regulated process in which CD20 cells play a key role.

Our study has limitations. In terms of study design, lack of blinding raises the issue of measurement bias and differential cointerventions. However, proteinuria is an objective outcome, staff responsible for patient follow-up were kept blind, the use of medications interfering with the renin-angiotensin system was maintained constant during the study, and prednisone and calcineurin inhibitors dose adjustment was defined per protocol.

Although our study suggests that RTX is effective in achieving the dual goal of maintaining disease remission and reducing the use of toxic agents, several questions remain unaddressed. First, we do not know the extent to which one or two doses of RTX affect the long-term outcomes of the disease including relapse rates, need for additional prednisone and calcineurin inhibitors courses, impairment of kidney function, and management costs (*e.g.*, 2 RTX doses cost approximately the same as a 1-year treatment with cyclosporine or as 6 months therapy with tacrolimus).

Second, studies with adequate follow-up are necessary to assess the long-term safety of RTX. Potential complications have been described, such as lung fibrosis (33) and progressive multifocal leukoencephalopathy (34). Virus monitoring (*e.g.*, JC virus—the etiologic agent of progressive multifocal leukoencephalopathy) in urine and blood cells and data on B cell deprivation are essential requirements for a long-term follow-up program. Finally, kinetic studies are also necessary as proteinuria may affect the metabolism of RTX and its urinary elimination.

In conclusion, our study suggests that RTX is noninferior to prednisone and calcineurin inhibitors in maintaining short-term remission of INS dependent on both these agents. One-year relapse-free survival probabilities of 50% can be achieved with one or two doses of RTX and reduced use of calcineurin inhibitors and prednisone. Longer follow-up studies are necessary to confirm efficacy and safety of RTX in children with INS dependent on both prednisone and calcineurin inhibitors.

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Disclosures

None.

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