Efficacy and safety of rituximab in children with difficult-to-treat nephrotic syndrome

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ABSTRACT

Background. Rituximab has emerged as an important medication for patients with steroid-dependent or steroid-resistant nephrotic syndrome.

Patients. We report the efficacy and safety of therapy with intravenous rituximab, administered once weekly for 2–4 doses, in 193 patients (mean age 10.9, range 2.2–18.7 years) with difficult-to-treat steroid dependence (n = 101), calcineurin inhibitor (CNI)-dependent steroid resistance (n = 34) and...
INTRODUCTION

The management of patients with steroid-dependent and steroid-resistant nephrotic syndrome is challenging. Therapy for the former is comprised of levamisole, cyclophosphamide, mycophenolate mofetil (MMF) and calcineurin inhibitors (CNI) [1–3]. Despite therapies, a proportion of patients with steroid dependence continue to relapse and/or show medication-related toxicities. The treatment of patients with steroid-resistant nephrotic syndrome is as difficult. While CNIs induce remission in the majority, a significant proportion has steroid-sensitive relapses [4]. Prolonged therapy with a CNI is associated with multiple adverse effects, including glucose intolerance and risks of nephrotoxicity, necessitating consideration of alternative medications [5, 6]. Finally, the management of patients with steroid resistance who are refractory to therapy with CNIs is empirical with limited evidence base [7].

During the last few years, rituximab has been used for treatment of patients with steroid- and/or CNI-dependent or resistant nephrotic syndrome with variable results [8]. Most reports on the efficacy of rituximab in patients with steroid-dependent and/or resistant nephrotic syndrome are based on case series [8–16], with limited data from controlled studies [17–20]. It is difficult to determine the role of rituximab because of lack of clarity on disease categories, variable regimens for steroid tapering and use of concomitant medications. We previously reported the effectiveness and safety of rituximab in 55 patients with steroid-dependent and resistant nephrotic syndrome [9, 10], and its efficacy compared with tacrolimus [21]. We present the cumulative 8 year, follow-up experience at a single center on use of rituximab in children with difficult-to-treat nephrotic syndrome. Results are presented separately for patients administered rituximab for steroid-sensitive and steroid-resistant illness; the latter were further categorized based on whether rituximab was given during steroid- or CNI-induced remission or for refractory proteinuria.

MATERIALS AND METHODS

Records of patients, 1–18 years old, with steroid-dependent or steroid-resistant nephrotic syndrome treated with rituximab between January 2006 and October 2013 and followed for at least 6 months were reviewed. Standard definitions were used for nephrotic syndrome, remission, relapse and steroid resistance [1, 2]. Steroid-dependent nephrotic syndrome was defined as occurrence of two consecutive relapses while receiving prednisolone on alternate days or within 15 days of its discontinuation. Therapy for these patients comprised of prolonged treatment with tapering doses of alternate-day prednisolone; those requiring prednisolone >0.5 mg/kg on alternate days or showing steroid toxicity received alternative medications, including levamisole (2 mg/kg on alternate days), cyclophosphamide (2 mg/kg/day for 12 weeks), mycophenolate mofetil (600–750 mg/m²/day), cyclosporine (4–5 mg/kg/day) or tacrolimus (0.1–0.15 mg/kg/day) [1, 2]. Satisfactory response was defined as remission for ≥6 months after a course of cyclophosphamide, or sustained remission or infrequent relapses during therapy with other agents. Steroid toxicity was defined as body mass index (BMI) >2 standard deviation scores (SDS), height below −2 SDS [22], cataract, glaucoma or hyperglycemia. Patients with steroid dependence or toxicity despite therapy with ≥2 alternative agents were considered for treatment with rituximab.

Steroid resistance was absence of remission despite therapy with prednisolone at 2 mg/kg/day for 4 weeks [7]. Patients with initial or late resistance received therapy with cyclosporine or tacrolimus. Patients with complete remission (urine protein-to-creatinine ratio, Up/Uc<0.2) or partial remission (Up/Uc 0.2–2, serum albumin >2.5 g/dL) during therapy were termed CNI-dependent steroid-resistant nephrotic syndrome. Rituximab was administered to these patients during or following CNI therapy if steroid-sensitive relapses were associated with (i) steroid dependence or steroid toxicity and (ii) prolonged (≥3-year) CNI therapy, nephrotoxicity (striped fibrosis, nodular arteriolar hyalinosis), seizures or diabetes. Patients with steroid resistance and primary or delayed non-response to 6 months’ therapy with CNI (Up/Uc >2, dipstick 3–4+, serum albumin ≤2.5 g/dL) were termed steroid- and
CNI-resistant nephrotic syndrome. Rituximab was not given to patients with (i) estimated GFR (eGFR) <60 mL/min/1.73 m² [23], (ii) congenital or secondary nephrotic syndrome, (iii) positivity for hepatitis B surface antigen or antibodies to hepatitis C or HIV or (iv) serious infections or tuberculosis.

**Therapy with rituximab**

IV rituximab was given at a dose of 375 mg/m² weekly for 2–4 doses, aiming to achieve CD19 depletion <1% of total lymphocyte count (Supplementary data). In patients with steroid dependence and CNI-dependent steroid resistance, rituximab was administered during remission, following which prednisolone was tapered by 0.25 mg/kg every 2–4 weeks and discontinued at 3–5 months. Other immunosuppressive agents including CNI were either discontinued immediately or tapered over 2–3 months. The decision to use MMF to prevent post-rituximab relapses followed discussion with parents about its potential utility, and associated adverse effects and costs. Relapses were treated with prednisolone at 2 mg/kg/day until remission, followed by 1.5 mg/kg on alternate days for 4 weeks. Patients with late steroid resistance or ≥2 relapses within 6 months were considered treatment failure. Following therapy with rituximab, patients with steroid- and CNI-resistant nephrotic syndrome received tapering doses of prednisolone at 0.3–0.5 mg/kg on alternate days, calcium and vitamin D3 supplements and enalapril for 6–18 months. Treatment failure was defined as non-response at 3 months following rituximab. Last effective follow-up was time from therapy with rituximab to the use of alternative agents for treatment failure, or last available follow-up.

Patients were asked to monitor urine albumin daily at home and were screened for infections at clinic visits. Blood counts and levels of creatinine, glucose, albumin, cholesterol and transaminases were estimated every 3 months. Height and BMI SDS were estimated using reference charts [22]. Information on frequency of relapses and cumulative prednisolone dosage was compiled at 6 and 12 months and last effective follow-up. Among patients with CNI-resistant nephrotic syndrome, the proportion in remission or showing chronic kidney disease (CKD) stage 4–5 was recorded. B lymphocytes (CD19+) were estimated by flow cytometry at 2-month intervals in a subset of patients with steroid dependence.

**Statistics**

Results were analyzed using STATA version 12.0 (StataCorp, Texas, USA). Continuous variables were expressed as mean ± standard deviation (interquartile range, IQR), except for time to event which was reported as median (IQR). Variables were compared using paired or unpaired t-test and χ² test; P < 0.05 was considered significant. Survival analysis was used to determine time to relapse, need for alternative medications and progression to CKD stage 4–5. Cox and logistic regression were used to determine hazard ratio (HR) and odds ratio (OR) for risk factors.

**RESULTS**

Of 193 patients administered rituximab and followed for at least 6 months, 101 had steroid-dependent nephrotic syndrome, 34 showed steroid-sensitive relapses during CNI therapy for steroid resistance and 58 had steroid- and CNI-resistant nephrotic syndrome (Table 1).

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Steroid dependence (n = 101)</th>
<th>CNI-dependent steroid resistance (n = 34)</th>
<th>Steroid- and CNI-resistance (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys (%)</strong></td>
<td>69 (68.3)</td>
<td>19 (55.9)</td>
</tr>
<tr>
<td><strong>Age at onset, months</strong></td>
<td>38.6 ± 26.4 (21–49)</td>
<td>35.9 ± 37.2 (16–32)</td>
</tr>
<tr>
<td><strong>Age at steroid dependence, months</strong></td>
<td>49.2 ± 29.7 (30–59)</td>
<td>64.1 ± 38.6 (36–77)</td>
</tr>
<tr>
<td><strong>Age at steroid resistance, months</strong></td>
<td>–</td>
<td>46.5 ± 41.6 (18–45)</td>
</tr>
<tr>
<td><strong>Age at therapy with rituximab, months</strong></td>
<td>145.9 ± 47.6 (115–177)</td>
<td>116.3 ± 57.0 (80–143)</td>
</tr>
<tr>
<td><strong>Initial resistance/late resistance</strong></td>
<td>–</td>
<td>22 (64.7)/12 (35.3)</td>
</tr>
<tr>
<td><strong>Minimal change disease/FSGS</strong></td>
<td>51 (83.6)/10 (16.4)*</td>
<td>22 (64.7)/12 (35.3)</td>
</tr>
<tr>
<td><strong>eGFR at therapy, mL/min/1.73 m²</strong></td>
<td>104.6 ± 30.3 (84–123.1)</td>
<td>96.7 ± 30.9 (77.5–108.7)</td>
</tr>
<tr>
<td><strong>Previous therapies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>82 (81.2)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>Cyclophosphamide, oral/intravenous</td>
<td>91 (90.1)/15 (14.9)</td>
<td>16 (47.1)/7 (21.9)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>69 (68.3)</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>Cyclosporine/tacrolimus/both</td>
<td>18 (17.8)/26 (25.7)/8 (7.8)</td>
<td>12 (35.3)/13 (38.2)/9 (26.5)</td>
</tr>
<tr>
<td>Use of ≥2 steroid sparing agents</td>
<td>93 (92.1)</td>
<td>24 (70.6)</td>
</tr>
<tr>
<td><strong>Medication-related toxicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index &gt;2 SDS</td>
<td>34 (33.7)</td>
<td>10 (29.4)</td>
</tr>
<tr>
<td>Height SDS &lt;–2 SDS</td>
<td>50 (49.5)</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>15 (14.9)</td>
<td>16 (47.0)</td>
</tr>
<tr>
<td>Cataract/glaucoma</td>
<td>31 (30.7)/3 (3.0)</td>
<td>7 (20.6)/0</td>
</tr>
<tr>
<td>Calciuminur inhibitor nephrotoxicity</td>
<td>11 (10.9)</td>
<td>6 (17.6)</td>
</tr>
<tr>
<td>Hyperglycemia or diabetes</td>
<td>10 (9.9)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Psychosis/seizures</td>
<td>1 (1.0)</td>
<td>2 (5.9)</td>
</tr>
</tbody>
</table>

Values represent n (percentage) or mean ± standard deviation (interquartile range).

eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; SDS, standard deviation score.

*Not performed in 40 patients.
Steroid-dependent nephrotic syndrome

Rituximab was administered to 101 patients (68.3% boys), 8.9 ± 3.9 (IQR 6.3–11.4) years from disease onset; 75 had received therapy with ≥3 steroid sparing agents and 85 showed one or more features of steroid toxicity (Table 1). Eighty-two patients received two doses of rituximab, 13 received three doses and 3 patients each received 1 and 4 doses. Patients were followed for a mean duration of 30.6 ± 19.1 (15–43) months; 85 were followed for at least 1 year. Therapy with rituximab resulted in 81.8% decline in frequency of relapses and reduction in corticosteroid requirement by 70.6% at 12 months follow-up (both P < 0.0001; Table 2). Prednisolone was discontinued in 90 patients at 4.8 ± 2.0 months after rituximab; 19 of 20 patients stopped concomitant therapy with CNI. Remission was sustained for a median (IQR) 16 (9–27) months after therapy. Sustained remission or infrequent relapses were seen in 99 (98.0%) and 73 (85.9%) patients at 6 and 12 months, respectively; 53 required additional therapy for treatment failure (frequent relapses in 43 and late resistance in 2) or single relapse with steroid toxicity (n = 8) (Table 3). At last effective follow-up

Table 2. Efficacy of rituximab in steroid-dependent nephrotic syndrome and calcineurin inhibitor (CNI)-dependent steroid resistance

<table>
<thead>
<tr>
<th>Steroid-dependent nephrotic syndrome</th>
<th>6 months prior</th>
<th>6 months after</th>
<th>Mean difference [95% CI] P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapses during 6 months</td>
<td>2.1 ± 1.0 (1–3)</td>
<td>0.09 ± 0.3 (0–0)</td>
<td>2.0 [1.8, 2.2] &lt;0.0001</td>
</tr>
<tr>
<td>Cumulative prednisolone, mg/kg</td>
<td>84.1 ± 46.7 (44.6–115)</td>
<td>28.6 ± 17.9 (15.3–37.3)</td>
<td>55.5 [47.5, 63.6] &lt;0.0001</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>97.7 ± 25.3 (80.4–112.4)</td>
<td>105.1 ± 27.7 (85.1–118.9)</td>
<td>7.4 [2.4, 12.4] 0.004</td>
</tr>
<tr>
<td>n = 101</td>
<td>12 months prior</td>
<td>12 months after</td>
<td></td>
</tr>
<tr>
<td>Relapses during 12 months</td>
<td>3.3 ± 1.6 (2–4)</td>
<td>0.6 ± 0.8 (0–1)</td>
<td>2.7 [2.3, 3] &lt;0.0001</td>
</tr>
<tr>
<td>Cumulative prednisolone, mg/kg</td>
<td>148.1 ± 82.3 (90.7–200)</td>
<td>43.6 ± 36.6 (17.4–57.1)</td>
<td>104.5 [87.4, 121.6] &lt;0.0001</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>100.4 ± 32.6 (78.6–115.1)</td>
<td>106.9 ± 32.7 (82.6–131.3)</td>
<td>6.5 [0.7, 13.7] 0.078</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CNI-dependent steroid resistance</th>
<th>6 months prior</th>
<th>6 months after</th>
<th>Mean difference [95% CI] P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapses during 6 months</td>
<td>2.0 ± 1.2 (1–3)</td>
<td>0.2 ± 0.5 (0–0)</td>
<td>1.8 [1.3, 2.2] &lt;0.0001</td>
</tr>
<tr>
<td>Cumulative prednisolone, mg/kg</td>
<td>93.8 ± 51.6 (50.6–124.1)</td>
<td>37.4 ± 20.9 (21.6–53.2)</td>
<td>56.4 [39.0, 73.9] &lt;0.0001</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>88.4 ± 244.8 (77.5–102.5)</td>
<td>99.8 ± 31.7 (79.8–118.9)</td>
<td>11.3 [−1.2, 23.9] 0.074</td>
</tr>
<tr>
<td>n = 34</td>
<td>12 months prior</td>
<td>12 months after</td>
<td></td>
</tr>
<tr>
<td>Relapses during 12 months</td>
<td>3.1 ± 1.9 (2–4)</td>
<td>0.9 ± 0.8 (0–2)</td>
<td>2.2 [1.5, 3.0] &lt;0.0001</td>
</tr>
<tr>
<td>Cumulative prednisolone, mg/kg</td>
<td>180.8 ± 93.2 (95.0–239.4)</td>
<td>67.1 ± 43.2 (31.3–118.9)</td>
<td>113.6 [82.7, 144.6] &lt;0.0001</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>95.3 ± 35.1 (70.4–122.5)</td>
<td>103.0 ± 28.7 (70.4–118.7)</td>
<td>7.7 [−5.0, 20.4] 0.23</td>
</tr>
<tr>
<td>n = 30</td>
<td>12 months prior</td>
<td>12 months after</td>
<td></td>
</tr>
<tr>
<td>Relapses during 12 months</td>
<td>3.1 ± 1.9 (2–4)</td>
<td>0.9 ± 0.8 (0–2)</td>
<td>2.2 [1.5, 3.0] &lt;0.0001</td>
</tr>
<tr>
<td>Cumulative prednisolone, mg/kg</td>
<td>180.8 ± 93.2 (95.0–239.4)</td>
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</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>95.3 ± 35.1 (70.4–122.5)</td>
<td>103.0 ± 28.7 (70.4–118.7)</td>
<td>7.7 [−5.0, 20.4] 0.23</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation (interquartile range); or mean difference [95% confidence interval, CI]. eGFR, estimated glomerular filtration rate.

Includes data at last effective follow-up in 2 and 15 children who were switched to alternative therapy prior to 6 and 12 months, respectively.

Includes data at last effective follow-up in 2 and 10 children who were switched to alternative therapy prior to 6 and 12 months, respectively.

Table 3. Outcomes in steroid-dependent nephrotic syndrome and calcineurin inhibitor (CNI)-dependent steroid resistance

<table>
<thead>
<tr>
<th>Steroid-dependent nephrotic syndrome</th>
<th>6 months (n = 101)</th>
<th>12 months (n = 85)</th>
<th>At last effective follow-up (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained remission</td>
<td>93 (92.1)</td>
<td>49 (57.6)</td>
<td>38 (37.6)</td>
</tr>
<tr>
<td>Infrequent relapses</td>
<td>6 (5.9)</td>
<td>24 (28.2)</td>
<td>18 (17.8)</td>
</tr>
<tr>
<td>Frequent relapses</td>
<td>2 (2.0)</td>
<td>7 (8.2)</td>
<td>35 (34.7)</td>
</tr>
<tr>
<td>Steroid dependence</td>
<td>0</td>
<td>4 (4.7)</td>
<td>8 (7.9)</td>
</tr>
<tr>
<td>Late steroid resistance</td>
<td>0</td>
<td>1 (1.2)</td>
<td>2 (2.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CNI-dependent steroid resistance</th>
<th>6 months (n = 34)</th>
<th>12 months (n = 30)</th>
<th>At last effective follow-up (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained remission</td>
<td>27 (79.4)</td>
<td>10 (33.3)</td>
<td>9 (26.3)</td>
</tr>
<tr>
<td>Infrequent relapses</td>
<td>5 (14.7)</td>
<td>10 (33.3)</td>
<td>9 (26.3)</td>
</tr>
<tr>
<td>Frequent relapses</td>
<td>1 (2.9)</td>
<td>5 (16.7)</td>
<td>7 (20.6)</td>
</tr>
<tr>
<td>Steroid dependence</td>
<td>0</td>
<td>2 (6.7)</td>
<td>4 (11.8)</td>
</tr>
</tbody>
</table>

Continued
of 22 (14–39) months, relapse rate (1.0 ± 1.0 relapses/year) was lower than before rituximab (mean difference 2.4; 95% CI 2.0, 2.8; P < 0.0001).

In order to prevent relapses, 16 patients received MMF pre-emptively, beginning 3.8 ± 1.6 months after rituximab. Pre-emptive therapy did not delay the time to first relapse (Figure 1a; log rank P = 0.39) or need for alternative therapy (log rank 0.13) despite some reduction in the frequency of relapses during 12 months (mean difference 0.36 relapses, 95% CI −0.06, 0.78; P = 0.094). Patients given rituximab without prior CNI use (n = 49) had a briefer duration of illness compared with 52 patients treated following CNI (mean difference 1.9 years; 95% CI 0.4, 3.4; P = 0.014). There was no difference in median time to first relapse (18 versus 10 months, respectively; log rank P = 0.11) and frequency of relapses during 12 months (mean difference 0.007 relapses; 95% CI −0.36, 0.37; P = 0.97).

On Cox regression, we found no relationship between baseline features, age at onset of nephrotic syndrome or at therapy, prior relapse rates, response to MMF or CNI, or use of pre-emptive MMF and hazards for first relapse or need for alternative medications (Supplementary data, Table S1). Multivariable analysis showed that satisfactory response to cyclophosphamide independently reduced the hazards for relapse (HR 0.56; 95% CI 0.31, 0.99; P = 0.045) and need for alternative medications (HR 0.47; 95% CI 0.26, 0.86; P = 0.014).

CNI-dependent steroid resistance

Thirty-four patients were treated with rituximab, 6.7 ± 3.8 (3.9–7.9) years after the diagnosis of initial (64.7%) or late (35.3%) steroid resistance. All patients had responded to therapy with CNI that they received for 3.6 ± 1.6 (2.7–4.8) years (Table 1). Indications for administration of rituximab were steroid-sensitive relapses associated with steroid toxicity (n = 30) and/or CNI therapy that was prolonged (n = 24), or associated with frequent relapses (n = 16) or toxicity (n = 8).

Following rituximab therapy, prednisolone was discontinued in 25 (73.5%) patients at 5.4 ± 2.2 months; 18 of 19 patients discontinued concomitant therapy with CNI. Treatment with rituximab was associated with decrease in frequency of relapses by 71.0% and 60.1% reduction in need for corticosteroids (Table 2). Twenty-five patients relapsed at a median 10 (6.5–15) months, earlier than patients with steroid dependence (log rank P < 0.0001). Time to first relapse was longer in patients with late compared with initial resistance (Figure 1b; log rank P = 0.011). At last effective follow-up, 18 (12–47) months from rituximab, nine patients each were in sustained remission or had infrequent relapses (Table 3). Eighteen (52.9%) patients received an alternative agent due to frequent relapses (n = 11), recurrence of resistance (n = 5) or single relapse with steroid toxicity (n = 2). The relapse rate at last effective follow-up was 1.4 ± 1.3 relapses/year, lower than before rituximab (mean difference 1.7 relapses; 95% CI 0.85, 2.5; P < 0.0001). Patients with initial resistance relapsed more frequently during 12 months than those with late resistance (mean difference 0.76; 95% CI 0.21, 1.30; P = 0.0084) and required earlier institution of alternative therapy (log rank P = 0.047). Pre-emptive MMF therapy (n = 14), beginning 3.5 ± 1.2 months after rituximab, was associated with longer
time to relapse (Figure 1c; P = 0.024) and trend for fewer relapses at 12 months (mean difference 0.5 relapses; 95% CI −0.10, 1.10; P = 0.10) and reduced need for alternative therapy (log rank P = 0.087). Patients with minimal change disease and focal segmental glomerulosclerosis (FSGS) had similar frequency of relapses (P = 0.75), comparable time to first relapse (log rank P = 0.57) and need for alternative medications (P = 0.15).

Cox regression confirmed that initial resistance was associated with risk of first relapse (HR 3.04; 95% CI 1.22, 7.58; P = 0.017), and pre-emptive MMF therapy was protective (HR 0.36; 95% CI 0.14, 0.93; P = 0.034) (Supplementary data, Table S2). On multivariable analysis, initial resistance was an independent risk factor for relapse (HR 2.66; 95% CI 1.04, 6.82; P = 0.042) and predicted the need for alternative medications (HR 2.65; 95% CI 0.86, 8.22; P = 0.091).

### Steroid- and CNI-resistant nephrotic syndrome

Of 58 patients with steroid- and CNI-resistant nephrotic syndrome treated with rituximab 2.5 ± 4.4 (1.5–8.4) years after onset of disease, 41 had FSGS and 17 had minimal change disease (Table 1). Indications for therapy with rituximab were primary (n = 34) or delayed (n = 24) non-response to CNI; 28.6% had also failed treatment with cyclophosphamide. Of 14 patients screened, 3 showed the heterozygous variation p.R229Q including one with a compound heterozygous mutation (p.A297V; exon 8:c890C>T) in NPHS2 gene; one had heterozygous WT1 mutation (IVS9+5G>A in exon 9). Patients received four (n = 39), three (n = 10) or two (n = 9) doses of rituximab. Seven (12.1%) patients achieved complete remission and 10 (17.2%) had partial remission at 2.0 ± 1.4 (IQR 1–2) months after rituximab therapy, which lasted for a median duration of 9 (7–14) months. No response to rituximab was seen in 41 (70.7%) patients. Remission was seen in 7 (21.9%) patients with initial resistance and 10 (38.5%) patients with late resistance (P = 0.17). A higher proportion of patients with minimal change disease compared with FSGS showed remission (9/17 versus 8/41; P = 0.011). Of the patients screened for genetic mutations, non-response was noted in all 4 patients with mutations in NPHS2 or WT1 genes and in 9 of 10 patients without mutations.

Table 4 shows outcomes on follow-up. Nine patients with rituximab-induced remission received treatment for steroid sensitive frequent relapses with cyclophosphamide (n = 2), MMF (n = 2), tacrolimus (n = 2) and redose of rituximab (n = 3). Recurrence of resistance was seen in seven patients at median of 15 (12–17) months, and was managed with rituximab (n = 5) or tacrolimus (n = 2). Compared with baseline, eGFR at last effective follow-up was higher among patients with rituximab-induced remission (difference 16.0 mL/min/1.73 m²; 95% CI −2.4, 34.4; P = 0.084) and declined in those with non-response (difference −17.6 mL/min/1.73 m²; 95% CI −32.1, 31; P = 0.019). Twenty (48.8%) of 41 non-responders progressed to CKD stages 4–5, compared with one and two patients with complete and partial remission, respectively (log rank P = 0.016; Figure 2). Response to rituximab was associated with prolonged duration of nephrotic syndrome (P = 0.048) and prior response to immunosuppression (P = 0.031); FSGS (P = 0.014) and male sex (P = 0.059) predicted non-response (Table 5). On multivariable regression, FSGS was the only significant risk factor for relapse (HR 3.04; 95% CI 1.22, 7.58; P = 0.017), and pre-emptive MMF therapy was protective (HR 0.36; 95% CI 0.14, 0.93; P = 0.034) (Supplementary data, Table S2). On multivariable analysis, initial resistance was an independent risk factor for relapse (HR 2.66; 95% CI 1.04, 6.82; P = 0.042) and predicted the need for alternative medications (HR 2.65; 95% CI 0.86, 8.22; P = 0.091).

### Table 4: Outcomes in patients with steroid- and CNI-resistant nephrotic syndrome, in relation to response to rituximab

<table>
<thead>
<tr>
<th>Status</th>
<th>At 12 months</th>
<th>Last effective follow-up</th>
<th>Last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Responders</td>
<td>Non-response</td>
<td>Responders</td>
</tr>
<tr>
<td>Remission (complete/partial)</td>
<td>(n = 17)</td>
<td>(n = 41)</td>
<td>(n = 17)</td>
</tr>
<tr>
<td>Infrequent relapses</td>
<td>12 (6/6)</td>
<td>6 (2/4)</td>
<td>1 (1/0)</td>
</tr>
<tr>
<td>Frequent relapses, dependence</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Steroid resistance, non-response</td>
<td>3</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>CKD 4–5 (transplant)</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

With or without alternative therapy.

Table 5 contains additional information about the patients and their outcomes.
rituximab (Table 2). eGFR was preserved 6 and 12 months following therapy with rituximab, 19.1 ± 9.5 months after the treatment (Figure 4). Therapy with rituximab was associated with increase in height SDS (difference 0.25; 95% CI 0.01, 19.1). Therapy with rituximab was associated with B-cell recovery at 4 months (OR 0.94; 95% CI 0.01, 5.34), 6 months (OR 2.44; 95% CI 0.55, 10.83), and 12 months (OR 0.5; 95% CI 0.17, 5.34). B-cell (CD19) depletion

B-cell depletion was documented in 96.5% of 85 patients with steroid dependence, 95.8% of 24 with CNI-dependent steroid resistance and 96.8% of 31 with steroid and CNI resistance. In patients with steroid dependence, B-cell recovery (CD19+ cells >5% lymphocytes) was seen in 2/57 (3.5%) patients at 2 months, 9/53 (17.0%) at 4 months, 25/40 (62.5%) at 6 months, 19/23 (82.6%) at 8 months, and 100% of 13 and 15 patients tested at 10 and 12 months, respectively. The occurrence of relapse within 12 months of rituximab therapy was not associated with B-cell recovery at 4 months, 5 patients tested at 10 and 12 months, respectively. The occurrence of remission within 12 months of rituximab therapy was not associated with B-cell recovery at 4 months, 5 patients tested at 10 and 12 months, respectively. The occurrence of relapse within 12 months of rituximab therapy was not associated with B-cell recovery at 4 months, 5 patients tested at 10 and 12 months, respectively.

Other outcomes

Among patients with steroid dependence and CNI-dependent steroid-resistant nephrotic syndrome (n = 135), there was decline in height SDS (mean difference −0.17; 95% CI −0.10, −0.23; P < 0.0001) and increase in BMI SDS (difference 0.26; 95% CI 0.40, 0.12; P = 0.0005) in the year preceding therapy with rituximab (Figure 3). Therapy with rituximab was associated with increase in height SDS (difference 0.25; 95% CI −0.02, 0.51; P = 0.069) and decline in BMI SDS (difference −0.27; 95% CI −0.03, −0.51; P = 0.029) at 12 months. Similarly, SDS for systolic and diastolic blood pressure declined significantly during the year following therapy with rituximab (Figure 4). eGFR was preserved 6 and 12 months following therapy with rituximab (Table 2).

Retreatment

Thirty-one patients with steroid dependence and 11 with CNI-dependent steroid resistance who received 1–3 doses of rituximab, 19.1 ± 9.5 months after the first course were followed for ≥6 months. Twenty-three (54.8%) patients relapsed at median 14 (9–30) months. There was no difference in the time to relapse among patients with steroid dependence and CNI-dependent steroid resistance (15 versus 12 months, respectively; log rank P = 0.28), and those given or not given pre-emptive MMF (15 versus 10 months, respectively; log rank P = 0.41). Of nine patients with steroid- and CNI-resistant nephrotic syndrome retreated for steroid-sensitive relapses, seven relapsed at 4–15 months. Redosing for recurrent steroid resistance was associated with remission in three of five patients.

Adverse effects

Infusion-related reactions included urticaria during three infusions, and fever, chills, throat pain and hypertension in one each. The patients responded to cessation of the infusion, therapy with diphenhydramine and slower rate of administration. Three patients each had transient synovitis and delayed drug eruptions at 2–7 days. Other events were transient leukopenia, hematuria, tachypnea, peritonitis, varicella and malaria in one patient each.

DISCUSSION

This case series summarizes an 8-year single-center experience with use of rituximab in a large group of patients with steroid-dependent and steroid-resistant nephrotic syndrome. Since a significant proportion of the latter respond to therapy with CNI, but subsequently show CNI dependence or steroid-sensitive relapses, data on these patients are presented separately from those receiving rituximab for steroid and CNI resistance. Using uniform policies regarding treatment, rituximab was effective and safe in maintaining remission, reducing the frequency of relapses and ensuring steroid sparing in difficult-to-treat steroid-dependent nephrotic syndrome. While patients with CNI-dependent steroid resistance also showed reduction in steroid-sensitive relapses, the duration of remission was briefer compared with the former group. Although less than one-third

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds of remission [95% CI]</th>
<th>P-value</th>
<th>Hazards of progression to CKD 4–5 [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>0.33 [0.10, 1.05]</td>
<td>0.059</td>
<td>1.64 [0.62, 4.35]</td>
<td>0.31</td>
</tr>
<tr>
<td>Age at onset, years</td>
<td>0.96 [0.80, 1.14]</td>
<td>0.62</td>
<td>0.99 [0.86, 1.16]</td>
<td>0.99</td>
</tr>
<tr>
<td>Age at steroid resistance, years</td>
<td>1.06 [0.93, 1.21]</td>
<td>0.37</td>
<td>0.96 [0.85, 1.09]</td>
<td>0.52</td>
</tr>
<tr>
<td>Age at therapy with rituximab, years</td>
<td>1.096 [0.96, 1.25]</td>
<td>0.16</td>
<td>1.01 [0.90, 1.12]</td>
<td>0.90</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis</td>
<td>0.22 [0.06, 0.73]</td>
<td>0.014</td>
<td>12.09 [1.61, 90.85]</td>
<td>0.015</td>
</tr>
<tr>
<td>Initial resistance</td>
<td>0.45 [0.14, 1.42]</td>
<td>0.17</td>
<td>1.54 [0.62, 3.87]</td>
<td>0.35</td>
</tr>
<tr>
<td>Duration of disease, years</td>
<td>1.15 [1.00, 1.31]</td>
<td>0.048</td>
<td>0.99 [0.88, 1.10]</td>
<td>0.83</td>
</tr>
<tr>
<td>Estimated GFR at therapy, mL/min/1.73 m²</td>
<td>1.00 [0.98, 1.01]</td>
<td>0.68</td>
<td>0.98 [0.96, 0.99]</td>
<td>0.012</td>
</tr>
<tr>
<td>Prior response to calcineurin inhibitor or IV cyclophosphamide</td>
<td>3.54 [1.08, 11.57]</td>
<td>0.031</td>
<td>0.59 [0.23, 1.50]</td>
<td>0.27</td>
</tr>
<tr>
<td>Duration of CNI therapy, months</td>
<td>1.03 [0.99, 1.06]</td>
<td>0.11</td>
<td>0.99 [0.97, 1.02]</td>
<td>0.55</td>
</tr>
<tr>
<td>Nephrotoxicity with CNI</td>
<td>0.88 [0.20, 3.83]</td>
<td>0.87</td>
<td>2.37 [0.85, 6.60]</td>
<td>0.099</td>
</tr>
<tr>
<td>Tubulointerstitial changes</td>
<td>0.95 [0.28, 3.16]</td>
<td>0.93</td>
<td>1.80 [0.56, 5.76]</td>
<td>0.32</td>
</tr>
<tr>
<td>More than two doses of rituximab</td>
<td>0.80 [0.18, 3.65]</td>
<td>0.77</td>
<td>1.72 [0.40, 7.51]</td>
<td>0.47</td>
</tr>
<tr>
<td>Response to rituximab</td>
<td>–</td>
<td>–</td>
<td>0.14 [0.03, 0.62]</td>
<td>0.009</td>
</tr>
</tbody>
</table>

CI, confidence interval; CNI, calcineurin inhibitor; GFR, glomerular filtration rate.
of patients with steroid and CNI resistance showed remission, those that responded showed satisfactory relapse free interval and preserved renal function. Therapy with rituximab was safe with minimal risk of adverse effects or infections, and allowed reversal of steroid toxicities.

Multiple case series have reported benefits of therapy with rituximab in patients with steroid-dependent nephrotic syndrome who fail treatment with immunosuppressive agents [10–17]. Our results show that therapy with rituximab was associated with 81.8% reduction in relapse rates, comparable to 62–95% in previous reports [10, 14, 16]. Treatment with one or more doses of rituximab is reported to result in remission lasting 3–12 months, with 25–83% patients showing sustained remission during follow-up [10–16]. The median time to relapse in our patients, administered two doses of rituximab was 16 months. Since it is suggested that rituximab administration during nephrotic-range proteinuria may result in its reduced efficacy [16, 24], we preferred to administer the agent during remission.

A systematic review on 14 studies that included 86 adult patients concluded that rituximab was effective in reducing relapses and had a steroid-sparing effect in patients with steroid-dependent nephrotic syndrome [25]. Clinical practice guidelines suggest that treatment with rituximab be considered in patients with steroid-dependent nephrotic syndrome who fail to respond satisfactorily to conventional agents, including CNI [1, 7, 8]. Having demonstrated that two doses of rituximab were as effective as 12 months’ treatment with tacrolimus [21], we offer rituximab as an alternative to CNI in patients failing therapy with levamisole, alkylating agents and MMF.

**FIGURE 3**: Standard deviation scores (SDS) for height (interrupted line) and body mass index (continuous line) at 6 and 12 months before and after administration of rituximab and at effective last follow-up in 135 patients with steroid-dependent nephrotic syndrome and CNI-dependent steroid resistance. Significant differences in mean SDS between time points are indicated by horizontal lines, with P-values indicated as follows: *P < 0.05, **P < 0.01 and ***P < 0.001. The panel below the figure indicates mean differences in SDS between values at therapy with rituximab and those at 1 year before and after therapy.
Results from the present study suggest that response to therapy was similar in patients receiving rituximab with or without prior use of CNI. In conformity with recent results [19, 26], we found that use of rituximab enables steroid sparing and improved SDS scores for height and BMI. Despite multiple reports on efficacy of rituximab in patients with steroid dependence, predictors of response are not identified. The reason why post-cyclophosphamide remission for 6 months or longer predicted a reduced risk for post-rituximab relapses in the present patients is unclear. Reports suggest that young age at onset [19, 27] and early B-cell recovery [27] were associated with increased risk of relapse. Based on the assumption that B-cell repletion might be a predictor for relapses, some experts propose redosing with rituximab at frequent intervals to ensure sustained remission [27–29]. While B-cell recovery, in the present study, occurred in most patients by 6 months, this was not temporally associated with relapses; similar findings have been reported by others [12]. Since frequent redosing might also lead to development of antichimeric antibodies and risk of adverse effects or non-response, our practice of redosing patients if they have recurrence of steroid dependence appears rational [30].

A number of reports suggest that pre-emptive therapy with MMF begun shortly after rituximab enables sustained remission in patients with steroid dependence [11, 12, 16, 31, 32]. Our decision to administer pre-emptive MMF was not based on severity of disease, as evidenced by similar baseline features and prior frequency of relapses in patients not receiving such therapy (data not shown). However, the benefits of this strategy in our patients were equivocal. Although not confirmed on multivariable analyses, pre-emptive use of MMF was
associated with a trend to sustained remission in patients with CNI dependence (Figure 1c).

CNIs are effective in the majority of patients with steroid-resistant nephrotic syndrome [33, 34]. Later, a significant proportion shows a relapsing illness and CNI dependence, requiring use of other agents [8, 35]. The present study shows that therapy with rituximab resulted in a 71% reduction in relapse rates, with withdrawal of CNI in 95% and corticosteroids in 72% patients. Patients with initial resistance showed a 3-fold higher risk of relapse and treatment failure than those with late resistance, a finding that has not been reported earlier. Compared with patients with steroid dependence, the median duration of sustained remission in these patients was briefer at 10 months. While data from patients with CNI-dependent steroid-resistant nephrotic syndrome are often reported together with steroid dependence [12, 16, 20], our findings suggest that response to rituximab is not similar in the two groups.

Despite initial interest, the efficacy of rituximab in inducing remission in patients with steroid- and CNI-resistant nephrotic syndrome is limited [8, 9]. Treatment with rituximab is believed to result in complete remission in 0–27.3% and partial remission in 21.2–37.5% patients at 4–8 weeks [8]. A randomized controlled trial, on 31 children with steroid- and CNI-resistant nephrotic syndrome, failed to show benefits of additional rituximab therapy [18]. Experience from the present study confirms the limited efficacy, with complete and partial remission in 12.1 and 17.2% patients, respectively. In absence of genetic screening in all patients with steroid resistance, we could not ascertain the effect of mutations in podocyte genes on determining response to rituximab. Similar to findings from a review, response to rituximab was better in patients with prior response to CNI and unsatisfactory in those with FSGS [8].

The present report confirms the variable efficacy of rituximab in patients with difficult-to-treat nephrotic syndrome. Remission was longer in patients with steroid-sensitive disease compared with those with CNI-dependent steroid-resistant nephrotic syndrome. The outcomes were less satisfactory in those with steroid- and CNI-resistant nephrotic syndrome. Prospective studies are required to examine the comparative efficacy of rituximab and CNI in order to define the sequence of therapy for patients with difficult-to-treat nephrotic syndrome. Controlled trials should also examine the utility of preemptive MMF in sustaining rituximab-induced remission, keeping in view the relative costs and adverse effects of additional immunosuppression.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no financial or non-financial relationships for any of the authors with any pharmaceutical company, any other industry or source of funding, that requires disclosure. Further, the results presented in this paper have not been published previously in whole, except in abstract format.

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