Rituximab in steroid-dependent idiopathic nephrotic syndrome in childhood—follow-up after CD19 recovery

Anne-Laure Sellier-Leclerc1,2, Véronique Baudouin1, Théréesa Kwon1, Marie-Alice Macher1, Valérie Guérin3, Hélène Lapillonne4,5, Georges Deschênes1,6 and Tim Ulinski2,5

1Department of Pediatric Nephrology, Robert Debré Hospital, Assistance Publique - Hopitaux de Paris, Paris, France, 2Department of Pediatric Nephrology, Armand Trousseau Hospital, Assistance Publique - Hopitaux de Paris, Paris, France, 3Laboratory of Immunology, Robert Debré Hospital, Assistance Publique - Hopitaux de Paris, Paris, France, 4Laboratory of Hematology, Armand Trousseau Hospital, Assistance Publique - Hopitaux de Paris, Paris, France, 5University Pierre et Marie Curie, Paris, France and 6University Denis Diderot, Paris, France

Correspondence and offprint requests to: Anne-Laure Sellier-Leclerc; E-mail: anne-laure.sellier-leclerc@trs.aphp.fr

Abstract
Rituximab (RTX) is a new treatment strategy in high-degree steroid-dependent idiopathic nephrotic syndrome (SDNS) in childhood. Thirty patients (nine girls) with SDNS with steroid side effects and previously treated with immunosuppressive drugs, mostly calcineurin inhibitors, were treated with RTX and included in this non-controlled single-centre study. Patient age at first RTX infusion was 12.9 ± 0.7 years. Our aim was to evaluate disease outcome after a minimum CD19 depletion period of 15 months obtained by repeated RTX infusion. Minimum follow-up after initial CD19 depletion was 24 months. During the RTX treatment period, seven patients had nephrotic syndrome relapses, six among them at the time of an intermittent CD19 recovery and one patient relapsed under CD19 depletion. The risk for these patients to relapse after the RTX treatment period was higher than in those without intermittent relapses. After definitive CD19 recovery over a follow-up of 17.4 ± 1.9 months, 19 patients (63%) did not relapse and 11 (37%) relapsed 4.3 ± 1 months after definitive CD19 recovery. Among these 11 patients, 6 already had intermittent relapses during the RTX treatment period. Steroid and immunosuppressive treatment could be discontinued in all patients during CD19 depletion and was re-introduced in two after CD19 recovery. Fourteen patients had mostly benign and transitory side effects, which did not require RTX discontinuation. In conclusion, RTX treatment with a 15-month CD19 depletion period induced long-term remission after definitive CD 19 recovery in almost two-thirds the of patients without oral immunosuppressive drugs.

Keywords: B cell depletion; idiopathic nephrotic syndrome; immunosuppressive treatment; paediatric; rituximab; side effects

Introduction
Rituximab (RTX) has been proposed as a new treatment strategy to reduce high-degree steroid dependency in childhood idiopathic nephrotic syndrome [1–9]. RTX is used in paediatric idiopathic nephrotic syndrome since 2006, but long-term observational studies are still lacking. Oral immunosuppressive drugs, classically used in this setting, are cyclophosphamide (CP), mycophenolate mofetil (MMF) and calcineurin inhibitors, such as cyclosporine A (CyA) or tacrolimus. Further, steroid dependency is generally replaced by dependency on calcineurin inhibitors and/or MMF, exposing the patient to the risk of relapses in case of non-compliance. Long-term use of calcineurin inhibitors is often limited by nephrotoxicity, while gonadotoxicity has to be considered for patients treated with CP.

Despite the absence of pathophysiological evidence, CD19 depletion was shown to be correlated with remission in the absence of other immunosuppressive drugs [2, 9, 10].
In previous reports and in our own clinical practice, nephrotic syndrome relapse occurred frequently a few months after CD19 recovery. It could therefore be hypothesized that RTX has beneficial effects only as long as CD19 depletion persists. Therefore, we proposed a prolonged B-cell depletion period of 15 months.

We studied (i) long-term outcome after a prolonged CD19 depletion, (ii) side effects after a medium term follow-up and (iii) possible predictability of high relapse risk after CD19 recovery.

We analysed clinical and biological parameters in 30 patients with steroid-dependent idiopathic nephrotic syndrome (SDNS) treated with repeated RTX infusions in order to maintain CD19 depletion for a minimum of 15 months and a follow-up of at least 2 years from the first rituximab infusion.

**Patients and methods**

We report the data of 30 patients with a follow-up of at least 24 months after the first RTX infusion. High-degree steroid dependency was defined as at least one relapse under >0.5 mg/kg/day (or >1.0 mg/kg every other day) of oral prednisone with steroid-induced side effects. All patients were dependent on MMF or calcineurin inhibitors during disease course.

Initial RTX infusion was performed in remission with either negative proteinuria or non-nephrotic range proteinuria (<1 g/L) without significant decrease of serum albumin levels (>30 g/L). Initial RTX course was performed in one to four RTX infusions of 375 mg/m². CD19 depletion was controlled by flow cytometry assay 1 week after RTX infusion and then monthly in a prospective manner. In the case of reappearance of circulating CD19-positive cells (>10/mm³), a new RTX infusion (375 mg/m²) was performed over a period of 15 months in all patients except one who had an RTX side effect and therefore a reduced CD19 depletion period of 11 months. Serum albumin levels, proteinuria and urinary creatinine were checked monthly in order to confirm persistent remission of the nephrotic syndrome (NS).

Pre-medication with dexchlorpheniramine (2.5–5 mg) and methylprednisolone (0.5 mg/kg) was given prior to each RTX treatment. Cotrimoxazole (20 mg/kg; three times a week) was systematically given to all patients during the period of B-cell depletion for pneumocystosis prophylaxis.

Oral immunosuppressive drugs were tapered down or discontinued for all patients: MMF was discontinued immediately after CD19 depletion was obtained. Calcineurin inhibitors and prednisone were tapered off over 3 months following CD19 depletion. In the case of relapse during the RTX treatment period, prednisone was re-started at 60 mg/m²/day until remission and rapidly tapered off over 2 months after RTX re-infusion.

No systematic RTX re-treatment was performed after 15 months and CD19 recovery was tolerated in all patients. Patients with a CD19 recovery were seen every 3 months and checked for persistent remission of NS. A new RTX treatment course was proposed only in the case of NS relapse.

Side effects of oral immunosuppressive drugs and RTX were documented in the patients’ file.

All patient data were collected from the electronic patient file and collected on a separate electronic data sheet.

The study was approved by the local ethical committee and all parents gave their written informed consent to this treatment modality. Eighteen of the reported patients in the present long-term follow-up study had been included in a previous report from our center [9].

**Statistical analysis**

Analysis was performed with Sigmapstat (version 3.5), and graphs were created with Sigmaplot (version 8). Data were summarized as mean ± SEM if normally distributed and as median and range if not normally distributed. Normality was tested using Kolmogorov–Smirnov test. Patient groups were compared using t-test or Mann–Whitney test or analysis of variance. Chi-square was used to analyse a 2 × 2 contingency table. P-values <0.05 were considered statistically significant.

**Definitions**

Remission of nephrotic syndrome was defined as absent or trace proteinuria on dipstick analysis on three consecutive days. Relapse was defined as nephrotic-range proteinuria with serum albumin <30 g/L. Intermittent relapses were defined as nephrotic-range proteinuria during the RTX treatment period between two RTX infusions. RTX treatment period was defined as a period of 15 months in which we aimed to maintain CD19 depletion with repeated RTX infusions. CD19 depletion was defined as CD19 peripheral blood count <10/mm³ and CD19 recovery as CD19 peripheral blood count ≥10/mm³. Intermittent CD19 recovery was defined as CD19 increase >10/mm³ between two RTX infusions and definitive CD19 recovery was defined as CD19 increase after the 15-month treatment period.

**Results**

**Patient characteristics**

Thirty patients (nine girls) were included. Fifteen patients were Caucasians, seven Africans, six Northern Africans and two Asians. Age at onset of NS was 2.60 (0.7–12.6) years; disease duration before first RTX infusion was 9.5 ± 0.83 (0.3–17.5) years. Oral immunosuppressive treatment before RTX was prednisone (30 patients), calcineurin inhibitor (28 patients), mycophenolate mofetil (27 patients) and levamisole (9 patients). Fourteen patients received oral cyclophosphamide (cumulative dose was 150 mg/kg over 3 months).

Immunosuppressive treatment at first RTX infusion was co-administration of prednisone and calcineurin inhibitor and MMF (20 patients), co-administration of calcineurin inhibitor and prednisone (5 patients) and co-administration of MMF and prednisone (5 patients). During RTX treatment, all oral immunosuppressive drugs were discontinued. Renal function was normal in 24 patients. Five patients had mild-to-moderate renal failure [glomerular filtration rate (GFR) 40–80 mL/min/1.73 m²] and one patient had severe renal failure (GFR 30 mL/min/1.73 m²). All patients with renal failure were on oral CyA. All patients with renal failure had kidney biopsies performed and revealed minimal change nephrotic syndrome (MCNS) with low-degree fibrosis (5–10%) in four patients. The patient with severe renal failure had experienced sudden increase of serum creatinine level, probably explained mainly by a severe relapse of his nephrotic syndrome, concomitant vasomotor effect of cyclosporine treatment and low-degree chronic renal function impairment due to long-term cyclosporine exposure and interstitial fibrosis in 10% of the analysed biopsy specimen. During and after RTX treatment, renal function recovered concomitantly with CyA withdrawal and was normal in all patients at last follow-up. Details of patient characteristics are summarized in (Table 1).

Follow-up after first RTX infusion was 38.0 ± 1.3 (25.5–51.7) months and follow-up after CD19 recovery was 16.2 (3.1–31.7) months.

**RTX infusions**

Initial RTX treatment consisted of one single RTX infusion in 2 patients, two infusions in 10 patients, three in 3 patients and four injections in 15 patients. After initial CD19 depletion, all patients had RTX re-treatment to sustain B-cell depletion for a minimal of 15 months. Nine patients had one RTX injection, 13 had two and 8 had three.
**Table 1.** Patient characteristics

<table>
<thead>
<tr>
<th>#</th>
<th>Age at NS onset (years)</th>
<th>Age at RTX onset (years)</th>
<th>Treatment at first RTX infusion</th>
<th>Duration of CNI treatment (years)</th>
<th>Treatment at first RTX infusion</th>
<th>RTX courses</th>
<th>Initial B-cell depletion (months)</th>
<th>Total B-cell depletion (months)</th>
<th>Relapses during RTX treatment</th>
<th>Relapses after RTX treatment with full CD19 recovery</th>
<th>Treatment at last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9</td>
<td>12.7</td>
<td>Pred, FK</td>
<td>9.5</td>
<td>4+1</td>
<td>9.6</td>
<td>19.9</td>
<td>19.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.7</td>
<td>13.7</td>
<td>Pred, CyA, MMF</td>
<td>9.0</td>
<td>4+1</td>
<td>12.2</td>
<td>32.6</td>
<td>32.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>10.8</td>
<td>Pred, CyA, MMF</td>
<td>6.0</td>
<td>4+1</td>
<td>8.3</td>
<td>18.9</td>
<td>18.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
<td>13.0</td>
<td>Pred, FK, MMF</td>
<td>11.0</td>
<td>4+1</td>
<td>8.0</td>
<td>15.6</td>
<td>15.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>9.5</td>
<td>Pred, CyA, MMF</td>
<td>5.5</td>
<td>4+1</td>
<td>3.0</td>
<td>14.5</td>
<td>14.5</td>
<td>2</td>
<td>1</td>
<td>Pred, MMF, CyA, RTX</td>
</tr>
<tr>
<td>6</td>
<td>11.8</td>
<td>13.2</td>
<td>Pred, CyA, MMF</td>
<td>1.2</td>
<td>3+1</td>
<td>19.7</td>
<td>30.7</td>
<td>30.7</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>12.6</td>
<td>Pred, CyA, MMF</td>
<td>9.0</td>
<td>4+1</td>
<td>8.1</td>
<td>15.0</td>
<td>15.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>7.0</td>
<td>Pred, FK, MMF</td>
<td>4.0</td>
<td>4+1</td>
<td>6.0</td>
<td>20.2</td>
<td>20.2</td>
<td>1</td>
<td>1</td>
<td>Pred, MMF</td>
</tr>
<tr>
<td>9</td>
<td>2.8</td>
<td>16.7</td>
<td>Pred, CyA, MMF</td>
<td>14.0</td>
<td>4+1</td>
<td>15.3</td>
<td>21.4</td>
<td>21.4</td>
<td>1</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>8.6</td>
<td>Pred, FK, MMF</td>
<td>2.6</td>
<td>4+1</td>
<td>8.7</td>
<td>18.8</td>
<td>18.8</td>
<td>0</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>13.5</td>
<td>Pred, CyA, MMF</td>
<td>6.8</td>
<td>4+1</td>
<td>10.1</td>
<td>31.6</td>
<td>31.6</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.7</td>
<td>12.6</td>
<td>Pred, CyA</td>
<td>10.0</td>
<td>4+1</td>
<td>4.6</td>
<td>23.2</td>
<td>23.2</td>
<td>1</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>13</td>
<td>5.4</td>
<td>5.7</td>
<td>Pred, CyA</td>
<td>0.2</td>
<td>4+1</td>
<td>5.5</td>
<td>15.1</td>
<td>15.1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2.3</td>
<td>19.7</td>
<td>Pred, MMF</td>
<td>14.0</td>
<td>4+1</td>
<td>8.5</td>
<td>17.8</td>
<td>17.8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4.2</td>
<td>14.8</td>
<td>Pred, CyA, MMF</td>
<td>9.5</td>
<td>3+1</td>
<td>8.7</td>
<td>22.6</td>
<td>22.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.1</td>
<td>13.7</td>
<td>Pred, CyA, MMF</td>
<td>12.5</td>
<td>3+1</td>
<td>7.9</td>
<td>21.0</td>
<td>21.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>3.5</td>
<td>17.7</td>
<td>Pred, CyA, MMF</td>
<td>13.5</td>
<td>4+1</td>
<td>6.7</td>
<td>21.5</td>
<td>21.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2.9</td>
<td>12.4</td>
<td>Pred, MMF</td>
<td>0</td>
<td>4+1</td>
<td>6.4</td>
<td>19.9</td>
<td>19.9</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>2.4</td>
<td>10.5</td>
<td>Pred, MMF, MMF</td>
<td>5.5</td>
<td>1+1</td>
<td>5.1</td>
<td>11.0</td>
<td>11.0</td>
<td>1</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>20</td>
<td>1.8</td>
<td>18.1</td>
<td>Pred, MMF</td>
<td>0</td>
<td>2+1</td>
<td>15.8</td>
<td>25.7</td>
<td>25.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.2</td>
<td>3.7</td>
<td>Pred, CyA, MMF</td>
<td>0.1</td>
<td>2+1</td>
<td>9.9</td>
<td>17.7</td>
<td>17.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>3.4</td>
<td>15.3</td>
<td>Pred, CyA, MMF</td>
<td>8.5</td>
<td>2+1</td>
<td>5.9</td>
<td>22.3</td>
<td>22.3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0.7</td>
<td>12.8</td>
<td>Pred, FK, MMF</td>
<td>12.0</td>
<td>2+1</td>
<td>7.4</td>
<td>20.7</td>
<td>20.7</td>
<td>0</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>24</td>
<td>12.6</td>
<td>15.3</td>
<td>Pred, CyA</td>
<td>2.5</td>
<td>2+1</td>
<td>6.3</td>
<td>17.3</td>
<td>17.3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2.3</td>
<td>19.7</td>
<td>Pred, CyA, MMF</td>
<td>15.8</td>
<td>2+1</td>
<td>5.7</td>
<td>16.8</td>
<td>16.8</td>
<td>0</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>26</td>
<td>4.4</td>
<td>17.4</td>
<td>Pred, CyA, MMF</td>
<td>6.6</td>
<td>1+1</td>
<td>7.6</td>
<td>17.6</td>
<td>17.6</td>
<td>0</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>27</td>
<td>5.7</td>
<td>14.4</td>
<td>Pred, FK, MMF</td>
<td>7.4</td>
<td>2+1</td>
<td>6.2</td>
<td>21.6</td>
<td>21.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>2.0</td>
<td>12.9</td>
<td>Pred, MMF</td>
<td>10.6</td>
<td>2+1</td>
<td>8.7</td>
<td>18.8</td>
<td>18.8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>2.2</td>
<td>12.1</td>
<td>Pred, CyA, MMF</td>
<td>2.4</td>
<td>2+1</td>
<td>5.5</td>
<td>17.3</td>
<td>17.3</td>
<td>0</td>
<td>1</td>
<td>RTX</td>
</tr>
<tr>
<td>30</td>
<td>2.5</td>
<td>8.4</td>
<td>Pred, CyA, MMF</td>
<td>5.8</td>
<td>2+1</td>
<td>7.8</td>
<td>22.3</td>
<td>22.3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*B-cell depletion*

Mean CD19 depletion period was 20.5 ± 0.92 (11.0–32.6) months. One patient (No. 5, Table 1) did not respond to the second RTX re-treatment and did not achieve CD19 depletion any more. His CD19 depletion period was 14.5 months. RTX antibodies were not detected in this patient. One patient (No. 19, Table 1) developed a neutropenia after the first RTX re-treatment. Therefore, no further RTX infusions were performed in this patient. His CD19 depletion period was 11 months. Duration of B-cell depletion in patients with three or four initial RTX infusions (8.2; 3.0–19.7 months) and those with one or two RTX infusions (6.9; 5.1–15.8 months) was not significantly different (P = 0.26).

**Nephrotic syndrome relapses during RTX treatment**

Seven patients had nephrotic syndrome relapses during the RTX treatment period: six at the time of intermittent CD19 recovery and one patient (No. 9) under complete CD19 depletion. Two patients (No. 10 and 23) had transient...
non-nephrotic proteinuria with spontaneous decrease of proteinuria 1 week later.

Among the six patients who relapsed with intermittent CD19 recovery, four had one, one patient had two and one had three nephrotic syndrome relapses. Relapses in these patients occurred at the time of CD19 recovery detection. In these six patients, a new remission could be obtained by short-term steroid therapy followed by a new RTX infusion. At the time of relapse, CD19 count ranged from 10 to 360/μL.

**Nephrotic syndrome outcome after RTX treatment period and CD19 recovery**

After RTX treatment period and despite CD19 recovery, 19 patients did not relapse (Table 1, Figure 1). Mean follow-up period for these patients was 17.4 ± 1.9 (4.7–33.3) months after CD19 recovery.

Eleven patients relapsed with nephrotic syndrome after the RTX treatment period. All these patients had definitive CD19 recovery at the time of relapse. Time period between CD19 recovery and relapse was 4.3 ± 1.0 (0.1–10.9) months. Among these 11 patients, 6 already had intermittent relapses during the RTX treatment period (Table 1). These 11 patients were re-started on high-dose prednisone 60 mg/m²/day and oral CyA, if appropriate. Once a remission was obtained, RTX was proposed to these patients. Ten accepted RTX re-treatment and one refused (No. 8, Table 1). In nine patients, a new CD19 depletion was obtained, whereas no CD19 depletion could be obtained in one patient (No. 5, Table 1) for whom no further RTX injection has been performed. He requires CyA, MMF and steroids to sustain remission (Figures 2 and 4).

**Prediction of relapse risk after CD19 recovery**

Patients who experienced nephrotic syndrome relapses during the RTX treatment at the time of intermittent CD19 recovery had a higher probability for relapse after RTX treatment. Among the 11 patients with relapses after RTX treatment, 6 (55%) had one or several intermittent relapses during the RTX treatment, whereas among the 19 patients without relapses after the RTX treatment, only 1 (5%) had an intermittent relapse (P = 0.009). Therefore, six of seven patients (86%) with relapses after RTX also had intermittent relapses during the RTX treatment (Figure 3).

**Analysis of patient age and outcome after RTX treatment**

In order to analyse the impact of patient age on outcome after RTX treatment and CD19 recovery, we separated patients into three groups: <10 years (Group A), 10–13 years (Group B), >13 years (Group C). In Group A, three of six patients (50%) relapsed after CD19 recovery. In
Group B, 4 of 9 relapsed and in Group C, 4 of 15 (27%) relapsed. These differences did not reach statistical significance, as patient number in each sub-group was small.

**Oral immunosuppressive and steroid treatment changes after RTX treatment**

In all patients treated with RTX, oral immunosuppressive and steroid treatment could be discontinued during the CD19 depletion period. However, seven patients had intermittent steroid treatment during relapses before RTX re-treatment could be performed (Figure 4). At the end of the follow-up, immunosuppressive treatment was re-introduced in two patients, MMF and prednisone in one and co-administration of MMF, CyA and prednisone in another patient. In nine other patients, rituximab treatment has been re-introduced (Tables 1 and 2).

**RTX side effects**

Fourteen patients (47%) had mostly benign and transitory side effects, which did not require RTX discontinuation. Some patients had more than one side effect. Side effects were separated in those that appeared during or immediately after RTX infusion and those that appeared after a longer time interval from the RTX infusion.

Six patients had a mild cytokinic shock with general dizziness, tachypnea and tachycardia, all after the first RTX infusion. Two patients had severe neutropenia, one patient had a peripheral vein thrombosis, two had a transient hepatic cytolysis (SGOT and SGPT activities were five times above the upper normal range) and one a transient thrombopenia (120 000/mm³). Neutropenia, hepatic cytolysis and thrombopenia were detected after RTX re-treatment. Four patients had infectious manifestations: one case of gastroenteritis, one case of fever due to human herpes virus 6 (HHV6) and two cases of high fever with unidentified microbial agent.

Five patients had long-term side effects such as benign lymphadenopathy in two patients and erythrocytaemia in three patients.

**Discussion**

This is a single-centre study of a large number of paediatric patients treated with RTX for high-degree steroid-dependent nephrotic syndrome. The particular difference to other reported series with paediatric patients [2, 10, 11] is a homogenous RTX infusion protocol with RTX injection in complete remission and a systematic RTX re-infusion in order to maintain CD19 depletion for at least 15 months. Further, the mean follow-up period after the onset of RTX treatment in our study was 35 months (with a minimum of 24 months), which is considerably higher than in the other reported studies [2, 9–11].

Two-thirds of the patients did not relapse with definitive CD19 recovery after the 15-month CD19 depletion period. Mean follow-up period after CD19 recovery was 14.4 months, which is longer than in previous studies [9–11], suggesting that RTX treatment might be able to sustain remission even after the CD19 depletion period. One important limitation of the present study is the absence of a control group. Therefore, results need to be interpreted with caution and require confirmation by a controlled study in the future.

A recent report from Gulati et al. [10] demonstrated a sustained remission in ~70% of patients with SDNS after a single course of two RTX injections with a mean follow-up of 16.8 months after RTX injection. Prytula et al. [11] observed 82% of patients with SDNS having a good initial response but after a mean follow-up of 4.5 months, and only 36% of patients were still in remission. There was no systematic CD19 counts follow-up in these two series and for the second report, no information on the status of nephrotic syndrome (in relapse or in remission) at the time of RTX infusion was given.

In our study, 19/30 patients (63%) had a sustained remission at the last follow-up. Comparison of the study by Gulati et al. [10] and our study showed similar remission rates at the end of the follow-up period. However, the follow-up period after the first RTX treatment is approximately two times longer in our study. Further investigation and long-term follow-up is needed to evaluate the impact of the longer B-cell depletion compared to short-term treatment with a single RTX course. The study by Prytula et al. [11] showed a lower proportion of patients (36%) with SDNS in remission after a follow-up of 4.5 months post-RTX treatment. Despite the absence of pathophysiological or clinical evidence about when to inject RTX, performance of RTX infusion in patients during nephrotic proteinuria may be less efficient and might be a possible explanation for these results. From our own experience (G.D., T.U., unpublished results), bad outcome with early relapses or no response to RTX has been observed in a considerable number of patients with RTX infusion performed during relapses. Therefore, we chose to perform RTX infusions only in patients in remission.

We have shown that mean time interval from B-cell recovery until nephrotic syndrome relapse was 4.5 months, with only two patients relapsing after >6 months of definitive B-cell recovery. The hypothesis that relapse frequency decreases after 6 months of B-cell recovery has to be evaluated in larger long-term follow-up studies.

Several patients in our cohort had relapses during the RTX treatment period due to reappearance of B cells and rapid relapse before a new RTX infusion could be performed. For practical reasons, it is often impossible to perform RTX infusion quickly enough to avoid a relapse in these patients. Therefore, clinicians might want to discuss the possibility to re-infuse RTX before the re-appearance of B cells. To date, there are no data available to discuss this potential strategy.

Patients with relapses during the RTX treatment period were at a higher risk (86%) to relapse after RTX treatment under CD19 recovery. Such patients might need a different follow-up than those without intermittent relapses. Clinicians have to discuss the possibility of re-introduction of oral immunosuppressive drugs or a prolonged CD19 depletion period >15 months. However, among patients without intermittent relapses, only 24% of patients relapsed.
At the end of the RTX treatment and definitive CD19 recovery.

Among patients who relapsed after the RTX treatment period, two different strategies can be discussed: (i) RTX re-treatment in order to prolong the CD19 depletion or (ii) introduction of oral immunosuppressive drugs. Similar to what has been observed in patients on oral immunosuppressive drugs with dependency on MMF or CyA [12–15], there can be RTX dependency. However, the initial RTX treatment period might have an impact on disease activity in the future and therefore, there might be a probability that oral immunosuppressive drugs after RTX treatment are less (nephro)toxic than those before [2, 9, 10]. Even for patients for whom a re-introduction of calcineurin inhibitors is necessary, the RTX treatment period can be considered as a (at least temporary) calcineurin inhibitor-sparing effect.

The relapse frequency of nephrotic syndrome is thought to decrease with patient age [16]. Therefore, we analysed if patient age plays a role for the risk of relapse after RTX treatment. Unfortunately, patient number in each sub-group was very small and therefore, no definitive conclusion can be made, even though patients <10 years tended to have a higher relapse risk after CD19 recovery. Even if the toxicity profile of RTX seems to be rather favorable in the short term, yet, no data are available about potential long-term side effects.

Side effects occurred in about half of the patients but were mostly benign without necessity to stop RTX treatment. The duration of the CD19 depletion does not seem to impact on the incidence of infectious complications if compared to other case series [2, 11]. Progressive multi-focal leukoencephalopathy [17] has never been reported in patients with idiopathic nephrotic syndrome; however, fatal lung fibrosis [18] and severe colitis [19] appeared exceptionally in this setting.

**Fig. 4.** Clinical course of patients with a relapse after the CD19 depletion period.

**Table 2.** Number of patients under different immunosuppressive treatments before, during and after the rituximab treatment period of repeated rituximab infusions in order to maintain a CD19 depletion of 15 months.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BEFORE RTX</th>
<th>DURING RTX</th>
<th>AT LAST FOLLOW-UP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>30</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>MMF</td>
<td>25</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Anti-calcineurin</td>
<td>25</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RTX</td>
<td>0</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
</table>

*Nine patients received RTX re-treatment after the 15-month depletion protocol due to nephrotic syndrome relapse. RTX, rituximab; MMF, mycophenolate mofetil.*
In conclusion, RTX treatment with a 15-month CD19 depletion period induces long-term remission after definitive CD19 recovery in two-thirds of patients mostly with previous dependency on calcineurin inhibitors in this uncontrolled study. No data are available to analyse long-term side effects after prolonged B-cell depletion in such patients, but no severe side effects have occurred in the medium term. However, benefit-risk ratio has to be carefully analysed in comparison to other oral immunosuppressive drugs. To date, such a strategy should probably be reserved to patients dependent on calcineurin inhibitors or with side effects of immunosuppressive or steroid treatment.

Conflict of interest statement. None declared.

References


Received for publication: 2.3.11; Accepted in revised form: 16.6.11