Advances in the treatment of refractory nephrotic syndrome in children

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Abstract: Nephrotic syndrome is the most common glomerular disease in children, mostly seen in children aged 3-5 years, and is often treated clinically with hormone therapy, but some children with hormone therapy have poor results, manifesting as frequent relapses, hormone dependence or hormone-resistant refractory nephrotic syndrome, and children with long-term hormone use show adverse effects such as growth inhibition, fat metabolism disorders, and infections, which seriously affect the quality of survival. In recent years, various types of immunosuppressants have been used in children with refractory nephrotic syndrome to maintain disease remission, reduce relapses, and reduce the adverse effects of hormones, providing more therapeutic options for treatment.

Keywords: refractory nephrotic syndrome in children; rituximab; pharmacotherapy

1. Introduction

Nephrotic syndrome (NS) is a glomerular disease with massive proteinuria, hypoproteinemia, edema and hyperlipidemia as the main symptoms [1]. Oral glucocorticoids are the first-line agents in nephrotic syndrome, and approximately 80% of children are glucocorticoid-sensitive, but approximately 80-90% of patients will relapse. In 50% of these patients, frequent relapsing nephrotic syndrome (FRNS) can progress to hormone-dependent nephrotic syndrome (SDNS) [2], and 20% of patients eventually develop Steroid-Resistant Nephrotic Syndrome (SRNS) [3], as shown in Figure 1. Clinically, FSNS, SDNS and SRNS are referred to as Refractory Nephrotic Syndrome (RNS). FSNS and SDNS are called Steroid-sensitive Nephrotic Syndrome (SSNS). In the process of glucocorticoid application, adverse effects such as obesity, growth inhibition, hypertension and hyperglycemia are likely to occur, which seriously affect the quality of life and psychological growth of children, as shown in Figure 2. Therefore, immunosuppressive drugs such as rituximab, cyclophosphamide, tacrolimus and mycophenolate ester are mostly used to treat RNS clinically, which can reduce adverse effects and prevent disease recurrence. In this paper, we describe the research progress of drugs for the treatment of RNS, as shown in Figure 3.
2. Pathogenesis of refractory nephrotic syndrome

1. Podocyte hiatus membrane associated protein gene
2. Podocyte mitochondria-associated protein genes
3. Podocyte GBM-related protein gene

Gene mutation

Podocyte injury

Damage to the glomerular filtration barrier

albuminuria

RNS

Figure 2: Adverse effects of glucocorticoid use

Figure 3: Drugs and properties for the treatment of RNS

Figure 4: Pathogenesis of refractory nephrotic syndrome
The clinical pathogenesis is unclear, but in recent years it is mainly thought to be related to genetic factors and immune mechanisms. Mutations in the foot cell lytic membrane-associated protein gene, foot cell mitochondria-associated protein gene, and foot cell GBM-associated protein gene can lead to foot cell damage, causing damage to the glomerular filtration barrier and proteinuria, leading to the development of RNS [4]. Mutations in the SMARCL1 gene can lead to kidney damage and predispose to the formation of SRNS [5]. Mutations in cyclophosphamide metabolizing enzymes, cytochrome P450 enzymes, glucocorticoid receptor-related genes, and multi-drug resistance protein 2 can lead to RNS, and these genes are determined to determine the optimal treatment regimen and help predict the best starting dose and maintenance regimen for individualized treatment [6]. Immune factors are another factor contributing to RNS, which is thought to be related to T-cell dysfunction, leading to impaired cellular immune function and affecting impaired glomerular filtration [7], as shown in Figure 4.

### 3. Rituximab for RNS

Rituximab for RNS is not yet a definitive regimen, and the following dosing regimens exist: 375 mg/m2, 2 doses at 1-week intervals, with B-cell detection after the second dose, and if B-cells are <5 cells/ul, receive additional injections of RTX for a total of up to four doses [12]; 375 mg/m2 twice at 2-week intervals [13]; 375 mg/m2 (up to 500 mg/m2) four times every six months [14]; 375 mg/m2 once a week for four times, mostly for induction therapy [15]. In clinical practice, the treatment regimens used and their efficacy vary depending on the age of the child, the type of disease, the type of previous immunosuppression, and geographic factors. Topaloglu et al. [16] reported that RTX had better efficacy and long-term prognosis than SRNS in SSNS patients. RTX was given to 21 SSNS patients and 20 SRNS patients, respectively. SSNS patients were on hormones and calcium phosphatase inhibitors before RTX, and the median cumulative number of RTX given was 4. After RTX treatment, 10 patients were given cyclosporine A and 2 patients were given mycophenolate, and during follow-up, 2 patients relapsed, 3 patients were in partial remission, and 16 patients were in complete remission; the cumulative hormone and calcium-regulated phosphatase inhibitors were reduced 1 year after RTX treatment compared with before RTX treatment. Of the SRNS patients, 2 patients were given RTX 2 doses, 4 patients were given 3 doses, and 14 patients were given 4 doses. At the time of maintenance therapy, 14 patients were given every 6-12 months, 5 patients discontinued RTX therapy and developed chronic renal failure, and the median cumulative number of RTX given was 6. During follow-up, 4 patients were in complete remission, 4 patients were in partial remission, and 6 patients had disease that was still active. Chan et al. [17] reported that the risk of relapse was reduced by 5% for each 1-year increase in age at first rituximab administration; children receiving low-dose RTX with maintenance immunosuppression had a lower risk of relapse and improved outcomes that were comparable to those of medium and high doses. Maxted et al. [18] showed that a low-dose regimen of RTX at a single dose of 375 mg/m2 and a high-dose regimen of 750 mg/m2 were equally effective in the treatment of SDNS or SRNS at 6 and 12 months, but that the higher dose regimen was beneficial for initial treatment at 24 months, which was a retrospective and observational study without treatment or blinded randomization, so selection and systematic bias could not be excluded and patients differed in their access to other treatments.

RTX is effective and safe for the treatment of SDNS or SRNS, Gao et al. [19] searched five databases and retrieved a total of 347 articles, which were screened to include six articles, and data analysis was performed by RevMan. 234 children were included in this trial, including 124 in the RTX group, 46 children relapsed and 20 patients discontinued hormone or and calcium phosphatase inhibition and achieved remission; 110 in the control group, 84 children relapsed and one withdrew Hormone and/or calcium phosphatase inhibition, RTX treatment reduced the number of relapses in children and reduced the use of hormone and calcium phosphatase inhibition. However, this study has some limitations, as the follow-up period for its inclusion was short and inconsistent, so larger, long-term, comprehensive studies are needed to assess the efficacy and safety of RTX in SDNS or SRNS. Taşdemir et al. [20] performed a retrospective study analyzing 21 patients with SSNS and 22
patients with SRNS, 11 patients with SSNS relapsed after initial RTX treatment; among patients with SRNS, 41% were in complete remission at year 1 follow-up and 36% at year 2 follow-up; at year 2 follow-up, 8 patients with SSNS and 4 patients with SRNS did not The SSNS group had a significantly lower number of relapses and longer relapse-free survival, significantly improved serum albumin levels, increased albumin and remission rates in the SRNS group, and reduced use of immunosuppressive drugs. Studies [21] have shown that RTX treatment can reduce the use of immunosuppressive drugs, with a 96.5% reduction in steroid use in the last 3 months of follow-up after RTX treatment with steroids only. It has also been shown [14] that RTX can also improve the quality of life of the children.

Current testing for RTX treatment is based on peripheral B-cell counts to assess for relapse [22], and most patients relapse as their B-cells recover. Each additional month of B-cell depletion duration was associated with a 22% reduction in the risk of recurrence [23], and one study [24] showed a correlation between periods of B-cell depletion in the same patient, with 90% of patients having their next B-cell depletion duration 2 months prior to the previous time. The younger the age of RTX initiation, the higher the risk of early B-cell recovery, and to prevent B-cell recovery associated with immediate relapse of the nephrotic syndrome, it is recommended to avoid rapid reduction of hormones [25]. B-cell depletion is associated with a decrease in the number of Th17 and Th2 lymphocytes and an increase in T regulatory cells [20]. RTX in the treatment of other immune diseases, due to repeated infusions, can develop anti-rituximab antibodies (ARA), which may reduce circulating RTX levels and lead to a shorter period of B-cell depletion [22], and studies [27] have shown that low age of onset is an important factor in the formation of ARA, and the risk of ARA formation increases with higher treatment doses, and the efficacy of additional RTX treatment is reduced if ARA is present.

The most common adverse reactions to RTX are infusion reactions, and respiratory reactions are the main type of infusion reactions, mostly seen 3 hours after the infusion reaction, and often present clinically as sore throat, cough, dyspnea, and wheezing [28]. Subun et al. [29] experienced acute infusion reactions such as rash, fever, red eyes, headache, chest discomfort, nausea and vomiting during the study, which resolved on their own after discontinuation of the infusion. In addition, RTX has been associated with several adverse events, including RTX reactivation of hepatitis B virus-induced fatal hepatitis and progressive multifocal white matter encephalopathy, and more serious adverse reactions, including pulmonary fibrosis, fulminant myocarditis, neutrophil deficiency, and hypogammaglobulinemia [30]. Hypogammaglobulinemia is mostly present in children with low IgG levels, and low IgG levels and a previous history of SRNS are high risk factors for hypogammaglobulinemia after RTX treatment [31], and most patients with hypogammaglobulinemia are complicated by infections [32]; therefore, regular testing of immunoglobulins before and after RTX requires replacing immunoglobulins with individualized regimens [33]. Previous studies [34-38] have reported more specific adverse effects, including serum sickness, hypokalemia, hypersensitivity reactions, disseminated intravascular coagulation, and Crohn's disease. Kobayash et al. [39] conducted a retrospective study to monitor the safety of RTX for RNS, adverse drug reactions were observed in 53.7% of patients, infections in 24% of patients, infusion reactions in 31.9% of patients, and no RTX-specific adverse reactions were observed, consistent with the known safety profile of RTX, which can be safely used in patients with RNS.

In the general environment of COVID-19 epidemic, RTX can still be used to treat RNS. Sinha et al. [40] conducted a questionnaire-based study in which 436 children were treated with 701 doses of RTX during the COVID-19 epidemic and 30 children tested positive for SARS-CoV-2, all of whom improved after hospitalization; the study also had some limitations in that the ability to detect asymptomatic may have affected the results and some children were still on other immunosuppressants, making it difficult to assess the specificity of RTX for COVID-19 outcomes.

4. Tacrolimus for RNS

Tacrolimus (TAC) is an immunosuppressant and calcium-regulated neurophosphatase inhibitor; TAC acts by inhibiting IFN-γ and IL-2, a molecule that promotes T-cell development and value-added, and TAC blocks its activation and transcription, thereby slowing the progression of the disease [41]. In combination with low-dose hormone therapy, TAC can be used as a hormone retention agent to reduce the adverse effects of hormones on the child. Chen et al. [42] conducted a study to evaluate the efficacy and safety of TAC combined with low-dose tacrolimus in SRNS, which involved 76 children given low-dose prednisone 0.25-0.5 mg/kg/d and TAC 0.1 mg/kg/d. If the disease remitted after 6 months, TAC was continued at that dose, and if the disease did not remit, the dose of TAC was adjusted the mean follow-up time was 18+6 months, and the total number of remissions at months 1, 3, and 6 was...
72, 72, and 73, respectively. In patients in remission, the glomerular filtration rate of patients did not deteriorate at 1 year, and the incidence of relapse at years 1, 2, and 3 was 39.7% (29/73), 28.9% (11/39) [43]. The common adverse reactions during the follow-up period were bacterial and viral respiratory infections, and no adverse reactions such as acute kidney injury, chronic renal insufficiency, or cancer were found; all patients who reached the initial tacrolimus blood concentration achieved remission, therefore, TAC combined with low-dose prednisone was safe and effective in treating SRNS patients, and relapse was uncommon, but as the initial tacrolimus blood concentrations decreased and gradually increased. Guo et al. [43] reviewed 118 patients with RNS treated with TAC (0.03-0.15 mg/kg-d) and low-dose prednisone (2 mg/kg-d) and diltiazem 1-3 mg/(kg-d) as a preservative depending on tacrolimus trough concentration, with a 6-month follow-up, 95% of patients achieved partial or complete remission, and 79% of children after 1 year Complete remission was achieved with low nephrotoxicity and no adverse effects such as hypertension, hyperlipidemia, or newly detected cancer. Therefore, TAC combined with low-dose hormones can be used to treat RNS.

AC has a narrow therapeutic window, its adverse effects are closely related to nephrotoxicity, and its blood concentration is related to the patient's age and body weight genes. A study [41] showed that at maintenance doses, the age, body weight, and CYP3A5 polymorphism of the children affected the ratio of patient's trough concentration to dose (C0/Dose), with children weighing <20 Kg having a C0/Dose that was 1.5 times lower than that of children weighing >40 Kg. The C0/Dose was 1.5 times higher in children weighing <20 Kg than in children weighing >40 Kg; C0/Dose was 25% and 48% lower in patients aged 1-6 and 1-12 years, respectively, compared with patients aged 12-18 years; CYP3A5 genotyping was not sexually correlated with C0/Dose in younger patients, so genotyping should be performed in children aged 6 years or older. In addition, certain drugs also affect blood concentrations; enzyme inhibitors such as proton pump inhibitors, macrolide antibiotics, and azole antibiotics may increase TAC blood concentrations, and carbamazepine and phenytoin sodium may decrease them [43]. Diltiazem or pentoxifylline capsules are often applied clinically to elevate TAC blood levels.

### 5. Cyclophosphamide for RNS

Cyclophosphamide (CTX) is an immunosuppressant that inhibits the proliferation and differentiation of the body's immune cells and helps restore the glomerular filtration barrier and charge [44]. In developing countries, CTX is still used to reduce the frequency of relapses to achieve long-term remission, and age differences affect the efficiency of its use. Sandhu et al. [45] studied 100 children, 18 with FRNS and 91 with SDNS, given oral CTX2-2.5 mg/(kg-d) for 10-12 weeks, followed by oral prednisone (2 mg/kg-d) or prednisone (1.5 mg/kg-d) given every other day for four weeks after remission, then tapered and discontinued after 2-3 months. At 1 year follow-up, the relapse-free survival rate was 31% and the 2-year relapse-free survival rate was 11%; 61.2% of patients with age at onset <3 years responded; 61% of children with age >5 years responded; therefore, better efficacy can be achieved with CTX in children with age at onset over 3 years and age over 5 years. CTX can also be used in SRNS patients who do not respond to TAC, in a retrospective study by Haddad et al. [46], eight children with SRNS who did not respond to TAC were given intravenous CTX, the first at 500 mg/m2, the second at 750 mg/m2, followed by 1000 mg/m2, four patients went into complete remission, one into partial remission, and three did not respond; no serious infections or hemorrhagic cystitis developed during treatment, and intravenous administration can be used to induce treatment in children who do not respond to TAC.

Certain inflammatory factors can predict the therapeutic effect before the start of CTX treatment, Widiasta et al. [47] conducted a prospective study, which collected sera from 88 children with SRDS before receiving CTX treatment, and after 6 months of CTX treatment, the children were divided into responding and non-responding groups according to the presence or absence of proteinuria. After 6 months, the mean value of TGF-β levels was lower in the responding group TGF-β is a pleiotropic cytokine involved in renal disease progression and CTX was avoided in children with SRNS who had high levels of it.

### 6. Mortyl mescaline for RNS

Mycophenolate Mofetil (MMF) is a pre-ester of the immunosuppressant Mycophenolic acid (MPA), which inhibits cell-mediated immune responses and antibody formation and induces apoptosis in immune cells, and it also acts on non-immune cells, inhibiting thylakoid cell proliferation, thylakoid stromal expansion and podocyte apoptosis, and has a direct urinary protein-lowering effect [48]. MMF
can reduce proteinuria and reduce relapse in children with SDNS, Karunamoorthy et al. \[49\] studied 87 patients with SDNS, 72 patients were sensitive to MMF and the amount of prednisone was reduced from 1.28 mg/kg to 0.35 mg/kg; a total of 31 patients took MMF for at least 2 years and sustained remission; 88% did not experience adverse effects and a few adverse reactions such as diarrhea and urinary tract infections occurred; with continuous application of MMF, 83% of patients got remission and discontinuation led to relapse, but due to the safety of MMF, it can be used as a long-term drug. Xiang et al. \[50\] concluded that MMF has an advantage over tacrolimus or levamisole in reducing the number of relapses and accumulating prednisone doses over 1 year in the treatment of SDNS or FRNS, and that MMF is superior to levamisole but inferior to tacrolimus in terms of relapse-free survival. After RTX treatment, most children develop FSNS or SDNS after B-cell depletion, but the application of MMF after RTX treatment reduces the risk of failure. Iijima K et al. \[30\] performed a study in which 39 children treated with RTX and then given MMF had an 80% reduction in the risk of failure and a 74% and 57% reduction in the recurrence rate and daily dose of hormone use, respectively, but the relapse prevention effect disappeared after MMF was discontinued.

7. Cyclosporine A for RAS

Cyclosporine A (Cs A) is an immunosuppressive agent extracted from Columnaris, a filamentous fungus, which exerts immunosuppressive effects by selectively binding to receptor proteins on the T cell membrane, inhibiting the transcriptional process of interleukins and blocking the mutual aggregation of toxic T cells \[51\]. Li et al. \[52\] included seven studies to evaluate the efficacy and safety of Cs A in SRNS, complete remission was higher in the Cs A group, albumin levels were elevated, serum creatinine and serum cholesterol levels were lower, gingival hyperplasia was higher in the Cs A group than in the control group, and the incidence of infection and hypertension was similar to the control group. Cs A is a safe and effective agent for the treatment of SRNS. Cs A may cause nephrotoxicity when this side effect is more pronounced when it is used for a longer period of time, at higher doses, in the presence of hypertension or with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers \[53\], and blood Cs A levels greater than 200 ng/ml or elevated basal serum creatinine may also be responsible for nephrotoxicity \[54\]. In order to reduce adverse effects and control Cs A blood concentrations, Shen Lihong et al. \[55\] concluded that the older the patient, the higher the creatinine and urea levels, and the lower the hemoglobin, the lower the Cs A dosage should be reduced, which has some limitations with the age of their patients >14 years and the lack of data related to the patients' body mass index. In summary, the advantages and disadvantages associated with each drug are shown in Table 1.

Table 1: Advantages and disadvantages of drugs for RNS

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<tr>
<th>Drugs</th>
<th>Advantages</th>
<th>Disadvantages</th>
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| Rituximab      | 1. Safe and effective  
2. Rapid hormone reduction  
3. Reduce disease recurrence | 1. More expensive  
2. Infusion reactions: sore throat, cough, dyspnea and wheezing  
3. Infection |
| Tacrolimus     | 1. Oral, easy to carry  
2. Low-dose hormone retention  
3. Low nephrotoxicity | 1. Bacterial and viral respiratory tract infections  
2. Vulnerable to other drugs affecting blood levels  
3. Narrower treatment window  
4. High price |
| Cyclophosphamide| 1. Cheap price  
2. Remarkable healing effect | 1. Severe infections and hemorrhagic cystitis  
2. Gastrointestinal symptoms: nausea, vomiting  
3. Hepatic impairment and leukopenia |
| Mortyylimeskalini| 1. Safe for long-term use  
2. Reduced recurrence rate | 1. High price  
2. Hepatic impairment and leukopenia  
3. Susceptible to infection |
| Cyclosporin A  | 1. Safe and effective  
2. Complete remission is higher | 1. Can cause nephrotoxicity  
2. Presence of high blood pressure  
3. Lack of data on patient body mass index |
8. Summary

There are many immunosuppressive drugs for the treatment of RNS, from traditional CTX, Cs A, TAC, MMF to RTX, each of which has its own advantages and disadvantages. TAC has a higher remission rate than CTX and is effective in prolonging relapse-free survival, but its therapeutic window is narrow and its blood levels need to be tested regularly to guide its use. Cs A can safely and effectively treat SDNS, but there are fewer studies on the efficacy of SDNS, FRNS, and its adverse effects such as nephrotoxicity, and it is used as a second-line treatment drug. MMF can safely and effectively bring patients into long-term remission, and its adverse effects are fewer, so it can be used as a long-term drug. RTX has been widely used in RNS in recent years, and its efficacy is remarkable, which can reduce the use of other immunosuppressants and improve the quality of patient survival, but there is no clear treatment plan and it is expensive, so it has not been used on a large scale. Today, treatment experience is still limited in the treatment of RNS, but with the advancement of technology, it is believed that new and better drugs will emerge.

References


