Rituximab shows no effect on remission in patients with refractory nephrotic syndrome
A MOOSE-compliant meta-analysis

Supei Yin, MM, Ting He, MM, Yi Li, MM, Jingshuang Wang, MM, Wei Zeng, MM, Sha Tang, MM, Jinghong Zhao, PhD∗

Abstract
To assess the efficacy of rituximab in treatment of refractory nephrotic syndrome (NS) compared with other agents.

Studies were searched from Web of Science, PubMed, and CNKI up to April 2016. The standardized mean difference or relative risk or odds ratio and 95% confidence intervals were used to assess the efficacy of rituximab treatment compared with other agents in refractory NS.

Totally, 8 studies were included. The present study showed that there was a significant higher relapse-free survival rate in rituximab group than that in the other agents group. Compared with other agents, rituximab did not significantly improve the complete and overall remission rate, serum albumin levels. Rituximab also did not decrease the serum creatinine, urinary protein, and serum cholesterol levels. However, compared with other agents, the adult patients had a higher serum cholesterol levels after treatment with rituximab.

Rituximab promised to be a new agent in the treatment of refractory NS; it also could be used as an alternative to conventional immunosuppressive drugs-dependent or drugs-resistant. However, more high-quality, large sample, and multicenter randomized controlled trials are needed to further confirm the efficacy of rituximab in treatment of refractory NS.

Abbreviations: CI = confidence intervals, NS = nephrotic syndrome, OR = odds ratio, RCT = randomized controlled trial, RR = relative risk, SDNS = steroid-dependent nephrotic syndrome, SRNS = steroid-resistant nephrotic syndrome.

Keywords: meta-analysis, refractory nephrotic syndrome, rituximab

1. Introduction
Nephrotic syndrome (NS) is defined by the presence of heavy proteinuria (more than 3.5 g/24 h), hypoaluminemia (less than 30 g/L), hyperlipidemia, and peripheral edema.1 It is a common renal disorder in adults and children,2,3 for example, it affects 15 to 20 per 100,000 children, often with a frequently remitting and relapsing course.3,4 Normally, NS patients are usually treated with glucocorticoid, most cases are steroid-sensitive; however, especially about 20% children cases experience a complicated course with steroid resistance and have a poor renal survival.5,6 Moreover, most of the steroid-sensitive NS cases would relapse and develop into steroid-dependent NS (SDNS), even steroid-resistant NS (SRNS).1,5 Cyclophosphamide, calcineurininhibitor, mycophenolate mofetil, and alkylating agents, or various combinations of these drugs are the most commonly used steroid-sparing protocols in SRNS and SDNS;4 however, most of these drugs not only present serious adverse, but also so many cases cannot achieve completely remission and will develop end-stage renal failure.6 Because of the complicated patient states, ineffective treatments, and high relapse rate, NS seriously influences the health of humans and takes a huge challenge on the patient’s family and society; novel drugs should be taken consideration to the treatment of NS.

In the past decade, some studies have acquired some success in the treatment SRNS and SDNS with rituximab.7,8 However, these studies did not set control groups, the statistical efficiency is low. Subsequently, several studies assessed the efficacy and safety of rituximab in treatment of SRNS and SDNS by cohort–case-control study, and randomized controlled trial (RCT)9–16; however, these studies showed contradictory remission rate, different changes of biochemical indicators. Although a previous study has assessed the efficacy and safety of rituximab in treating refractory NS by a meta-analysis,17 this study only based on the childhood, and only 5 studies were included; hence, with the accumulating evidences, we conducted an update meta-analysis based on all refractory NS patients to assess the use of rituximab in treating refractory NS.

2. Materials and methods
2.1. Study selection
A study selection was performed with Web of Science, PubMed, and CNKI to search studies that reported the efficacy and safety...
of rituximab in treatment of refractory NS published up to April 2016 with the following search terms: “rituximab” or “CD20” in combination with “refractory nephrotic syndrome” or “nephrotic syndrome” or “steroid-dependent nephrotic syndrome” or “steroid-resistant nephrotic syndrome” with no restrictions. The references of the potential studies were reviewed.

2.2. Inclusion criteria
If the study met the following conditions, it should be included: the patients should meet the diagnostic criteria of NS and were not cured with common drugs; the patients should be treated with rituximab, the controls should be treated with other immunotherapy; the study should be published as full-length articles in English or Chinese; and available data that could be extracted from the article or obtained by calculation.

2.3. Data collection
We conducted the data extraction with a standardized form. The following information was collected from the included studies: the first author’s last name, publication year, study population, size of sample size, outcome indicators, and follow-up. Because the data included in this study were retrieved from the literature, ethical approval from ethics committees was not needed.

2.4. Bias and quality assessments
For the nonrandomized study, the authors completed the quality assessment based on the primary criteria for nonrandomized and observational studies of the Newcastle–Ottawa Quality Assessment scale for meta-analyses. For the RCT, the risk of bias was assessed using the Cochrane Collaboration’s “Risk of bias” tool.

Two independent authors performed all the above procedures, any disagreements were resolved by discussion.

3. Results
3.1. Study selection process and study characteristics
Initially, 705 articles were searched from the above databases according to the study selection strategy, after reviewing titles, abstracts, and full-text, most of them were excluded. At last, 8 articles (154 patients with rituximab treatment and 189 controls with other drug treatment) were included. Eight were from Web of Science or PubMed, and none were from CNKI. The study selection process was presented in Fig. 1.

As shown in Table 1, 4 of them were conducted in the Italian, 1 in Indians, 1 in French, 1 in Japanese, and 1 in England. Three were matched-cohort study, 3 were RCT, and 2 were case–control study. Two studies were based on the adults, and 6 were based on the children. Most of them had a longer follow-up period and higher quality. However, the sample size of each study was small.

3.2. Complete and overall remission events
In the included studies, 6 studies reported the complete and overall remission events. 2 of them were based on the adults, and 4 were children. The results showed that

---

Figure 1. The study selection process.
there was no difference in complete and overall remission events between rituximab and control groups, and these results were not changed by the ages of patients. The RR (95% CI) for the complete and overall remission events was 1.41 (0.95, 2.10) and 1.25 (0.93, 1.68), respectively. There was no evidence of significant heterogeneity among these studies (P > 0.05, I^2 < 50%). The results of complete and overall remission events are presented in Figs. 2 and 3.

### 3.3. Serum albumin

Figure 4 shows 4 studies (2 based on adults[9,10] and other 2 based on children[12,13]) assessed the difference in serum albumin levels between 2 groups after treatment.[9,10,12,13] After treatment, no difference in serum albumin levels between rituximab and control groups, SMD (95% CI) was 0.06 (−0.32, 0.44), P > 0.05. NO matter in adults or children patients, the difference was also not found, the SMD (95% CI) were −0.12 (−0.65, 0.42) and 0.23 (−0.30, 0.77), respectively.

### 3.4. Serum creatinine

Two studies based on the adult patients and 2 based on the children reported serum creatinine levels at the end of treatment, respectively.[9,10,12,13] None of them showed a difference between 2 groups. The pooled analysis showed that serum creatinine levels in rituximab group were not different from those in control group, the SMD and 95% CI was −0.01 (−0.47, 0.44), P > 0.05. The results are presented in Fig. 5.

### 3.5. Urinary protein

Figure 6 indicates 4 studies reported the urinary protein levels after treatment,[9–12] only 1 study that was based on the children showed a lower urinary protein level after treatment with rituximab[11]; however, the combined analysis did not show a significant difference in urinary protein levels between the 2 groups, the SMD and 95% CI were −0.80 (−2.30, 0.71), P > 0.05. Meanwhile, there was a strong evidence of heterogeneity among these studies (P > 0.05, I^2 < 50%).

---

### Table 1

| Reference          | Population     | Study design case/control | Age of subjects case/control | Sample size (n) case/control | Follow-up time (mo) | Quality assessment |
|--------------------|----------------|---------------------------|------------------------------|-------------------------------|---------------------|--------------------|
| Cravedi et al[9]   | Italian        | Matched-cohort, single-center, and controlled study | 57.00±13.0/[55.00±15.0] | 12/24                         | 24                  | 8                  |
| Cravedi et al[10]  | Italian        | Matched-cohort, single-center, and controlled study | 48.68±13.9/[50.18±12.3] | 11/11                         | 12                  | 7                  |
| Ravani et al[11]   | Italian        | Single-center and parallel RCT | 10.2±4.0/[11.3±4.3]       | 27/27                         | 12                  | Low bias           |
| Magnasco et al[12] | Italian        | Multicenter RCT           | 8.5±4.4/[7.3±3.7]         | 16/15                         | 12                  | Low bias           |
| Sinha et al[13]    | Indians        | Retrospective case-control study | 12.2±2.3/[12.3±3.0] | 10/13                         | 12                  | 7                  |
| Delbe-Bertin et al[14] | French       | Single-center prospective case-control study | 7.9±5.0/[5.7±3.7] | 12/16                         | 18                  | 7                  |
| Iijima et al[15]   | Japanese       | Multicenter double-blind and RCT | 11.5±5.0/[13.6±6.9] | 24/24                         | 12                  | Low bias           |
| Webb et al[16]     | England        | Matched-cohort single-center and controlled study | N/A/N/A | 42/59                         | >24                 | 8                  |

RCT = randomized controlled trial.
3.6. Serum cholesterol

As shown in Fig. 7, 2 studies were based on the adult patients and only 1 was based on the children reported serum cholesterol levels at the end of treatment, respectively.[9,10,13] The pooled analysis showed that there was a significant difference between 2 groups among the adults; however, no difference was found among all patients, the SMD and 95% CI were 0.59 (0.04, 1.14), \( P < 0.05 \), and 0.43 (−0.03, 0.89), \( P > 0.05 \), respectively.

3.7. Relapse-free survival

In total, 4 studies were based on the children reported the relapse-free survival rate.[11–13] The pooled analysis suggested that there was a significant difference in relapse-free survival rate between the 2 groups, the pooled OR (95% CI) was 0.42 (0.29, 0.62), \( P < 0.05 \). The result is presented in Fig. 8.

3.8. Adverse effect

Almost all included studies reported adverse effects during the treatment in 2 groups. The common adverse effects included nausea, vomiting, sweating, hypotension, bronchospasm, and skin rash. However, after reducing the drug infusion rate or providing temporary treatment interruption, these adverse events were rapidly and completely resolved.
Figure 5. Forest plot shows the efficacy of rituximab versus other drugs on serum creatinine levels.

Figure 6. Forest plot shows the efficacy of rituximab versus other drugs on urinary protein levels.

Figure 7. Forest plot shows the efficacy of rituximab versus other drugs on serum cholesterol levels.
3.9. Publication bias

The funnel plot showed that all the included studies were symmetrically distributed in the triangle area; this means that the results of present study were less affected by publication bias. The funnel plot of the studies is presented in Fig. 9.

4. Discussion

In our study, 8 studies, including 154 patients with rituximab treatment and 189 controls with other drug treatment, were identified. In addition, our study showed that patients with rituximab treatment might have a higher relapse-free survival rate; however, we did not observe significant differences in complete and overall remission rate, serum albumin, serum creatinine, urinary protein, and serum cholesterol levels between the 2 groups.

It is known that the patients with NS often suffer from the presence of heavy proteinuria (>3.5 g/24h), hypoalbuminemia (<30g/L), hyperlipidemia and peripheral edema, the changes or remission of proteinuria, hypoalbuminemia, hyperlipidemia, and serum creatinine are often used to evaluate the efficacy of clinical treatment. Therefore, we assessed the efficacy of rituximab versus other drugs on patients with refractory NS trough these indicators.

The proteinuria and hypoalbuminemia are the indispensable diagnostic criteria of NS; therefore, decreasing proteinuria levels and increasing serum albumin are the main objectives of treatment. In the included studies, only 1 showed that rituximab treatment could reduce proteinuria levels; this study was similar to another study which reported that about 90% of patients with refractory NS achieved complete or partial remission of proteinuria after receiving rituximab treatment. However, pooled analysis of our study did not show a good treatment efficacy of rituximab on proteinuria and hypoalbuminemia. Consequently, the difference in complete and overall remission rate was not remarkable.

In the present study, we also assessed the changes of serum creatinine levels after treatment. None of the included studies showed a significant difference in serum creatinine levels after different treatments between the 2 groups; the pooled result was coincident with the single included study.

Although we did not find a significant difference in serum cholesterol levels between the 2 groups, the adults achieved higher levels of serum cholesterol after treatment with rituximab; this result implied that patients, especially the adults, should be monitored for serum cholesterol levels when treated with rituximab, even when lipid regulating drugs were administered.

Although we did not find any significant differences between the 2 groups, these results should be discussed further, because the following reasons: a small number of included study and sample size in each analysis, which unquestionably influence the statistical result; the patients in each included study had different pathological types of NS, different dosages of rituximab, and different treatment responses to rituximab.

The present study suggested that rituximab treatment significantly improved relapse-free survival rate. This result was consistent with previous studies. The mechanism of this treatment efficacy is unclear, but as is well known that, NS is an autoimmune disease, rituximab is a monoclonal anti-CD20 antibody. CD20 expression is localized on B cells from prolymphocytes to lymphoplasmacyte. It is also weakly expressed on about 20% of plasma cells responsible for immunoglobulin (Ig)G and IgA secretion. Rituximab (RTX) binding to the CD20 antigen leads to rapid destruction of the CD20-expressing cell. B-cell depletion after RTX injection is complete and lasts for several weeks to several months. These may be a reasonable explanation of the treatment efficacy of rituximab on improving relapse-free survival rate.

Although a previous meta-analysis had assessed the efficacy of rituximab versus other drugs on patients with refractory NS, it was found that rituximab significantly improved relapse-free survival, complete remission rate, and reduced the occurrence of proteinuria; only 5 studies based on the children were included, the sample size was small; moreover, an increased complete remission rate (P=0.09) was based on the P < 0.1, the
statistical efficiency was lower. In the present study, we included more published studies, a larger sample size, and we conducted subgroup-analysis to assess the efficacy of rituximab versus other drugs on the adults and children patients with refractory NS; it provided more strong evidence.

However, several limitations in the present study should be considered. First, we identified all published studies; however, only 3 RCTs were included; recall bias and selection bias of case-control and cohort studies were not excluded.[26] Second, most of included studies were based on the European populations, whether these findings were supported by other populations was needed to be further assessed. Third, although we included more studies and patients than the previous patients, the sample size was also small. Fourth, we did not find evidence of publication bias, but we were not able to completely rule out such bias because of the limited number of studies. Fifth, although publication bias, but we were not able to completely rule out such findings were supported by other populations was needed to be further assessed. Third, although we included more studies and patients than the previous patients, the sample size was also small. Fourth, we did not find evidence of publication bias, but we were not able to completely rule out such bias because of the limited number of studies. Fifth, although publication bias, but we were not able to completely rule out such bias because of the limited number of studies. Sixth, the included studies were not only based on the children, but also on the adults; however, there were only 2 studies based on the adults,[3,5] and the number of subgroups was fewer; hence, the results of our study should be weighed and considered. These limitations should be taken into consideration in the future studies.

5. Conclusion
Our study suggested that rituximab might be a new agent in the treatment of refractory NS, it also could be used as an alternative to conventional immunosuppressive drugs-dependent or drugs-resistant. However, more high-quality, large sample, multicenter, double-blind, randomized, and placebo-controlled trials are needed to further confirm the efficacy of rituximab on the patients with refractory NS in the future.

References
[1] Crew RJ, Radhakrishnan J, Appel G. Complications of the nephrotic syndrome and their treatment. Clin Nephrol 2004;62:245–59.
[2] Sahay M, Vali PS, Ismail K, et al. An unusual case of nephrotic syndrome. Indian J Nephrol 2016;26:55–6.
[3] Gipson DS, Massengill SF, Yao L, et al. Management of childhood onset nephrotic syndrome. Pediatrics 2009;124:747–57.
[4] Basu B, Mahapatra TK, Mondal N. Mycophenolate mofetil following rituximab in children with steroid-resistant nephrotic syndrome. Pediatr Drugs 2013;1366:132–9.
[5] Lombel RM, Gipson DS, Hodgson EM, et al. Treatment of steroid-sensitive nephrotic syndrome: new guidelines from KDIGO. Pediatr Nephrol 2013;28:415–26.
[6] Sinha A, Bagga A. Rituximab therapy in nephrotic syndrome: implications for patients’ management. Nat Rev Nephrol 2013;9:134–69.
[7] Benz K, Dotsch J, Rascher W, et al. Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. Pediatr Nephrol 2004;19:794–7.
[8] Gilbert RD, Hulse E, Rigden S. Rituximab therapy for steroid dependent minimal change nephrotic syndrome. Pediatr Nephrol 2006;21:1698–700.
[9] Cravedi P, Ruggenenti P, Sghirlanzoni MC, et al. Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranoproliferative glomerulonephritis. Clin J Am Soc Nephrol 2007;2:932–7.
[10] Cravedi P, Sghirlanzoni MC, Marasà M, et al. Efficacy and safety of rituximab second-line therapy for membranoproliferative nephropathy: a prospective, matched-cohort study. Am J Nephrol 2011;33:461–8.
[11] Ravani P, Magnusso A, Edelfonti A, et al. Short-term effects of rituximab in children with steroid- and calcineurin-dependent nephrotic syndrome: a randomized controlled trial. Clin J Am Soc Nephrol 2011;6:1308–15.
[12] Magnusso A, Ravani P, Edelfonti A, et al. Rituximab in children with resistant idiopathic nephrotic syndrome. J Am Soc Nephrol 2012;2:1117–24.
[13] Sinha A, Bagga A, Gulati A, et al. Short-term efficacy of rituximab versus tacrolimus in steroid-dependent nephrotic syndrome. Pediatr Nephrol 2012;27:235–41.
[14] Delbe-Bertin L, Aoun B, Tudorache E, et al. Does rituximab induce hypogammaglobulinemia in patients with pediatric idiopathic nephrotic syndrome? Pediatr Nephrol 2013;28:447–51.
[15] Iijima K, Sako M, Nouro K, et al. Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2014;384:1273–81.
[16] Webb H, Jaureguiberry G, Dušek S, et al. Cyclophosphamide and rituximab in frequently relapsing/steroid-dependent nephrotic syndrome. Pediatr Nephrol 2016;31:589–94.
[17] Zhao Z, Liao G, Li Y, et al. The efficacy and safety of rituximab in treating childhood refractory nephrotic syndrome: a meta-analysis. Sci Rep 2015;5:8219.
[18] Zeng X, Zhang Y, Kwong JS, et al. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. J Evid Based Med 2015;8:2–10.
[19] Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928.
[20] Bowden J, Tienney JP, Copas AJ, et al. Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. BMC Med Res Methodol 2011;11:81.
[21] Kong WY, Swaminathan R, Irish A. Our experience with rituximab therapy for adult-onset primary glomerulonephritis and review of literature. Int Urol Nephrol 2013;45:795–802.
[22] Guigonis V, Dallocchio A, Baudouin V, et al. Rituximab treatment for severe steroid- or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. Pediatr Nephrol 2008;23:1269–79.
[23] Gulati A, Sinha A, Jordan SC, et al. Efficacy and safety of treatment with rituximab for difficult steroid-resistant and dependent nephrotic syndrome: multicentric report. Clin J Am Soc Nephrol 2010;5:2207–12.
[24] Fujisaka S, Hirano D, Nishizaki N, et al. Single infusion of rituximab for persistent steroid-dependent minimal-change nephrotic syndrome after long-term cyclosporine. Pediatr Nephrol 2010;25:339–44.
[25] Seller-Leclerc AL, Baudouin V, Kwon T, et al. Rituximab in steroid-dependent idiopathic nephrotic syndrome in childhood—follow-up after CD19 recovery. Nephrol Dial Transplant 2012;27:1083–9.
[26] Chen C, Huang Y, Yi S, et al. Association of vitamin E intake with reduced risk of kidney cancer: a meta-analysis of observational studies. Med Sci Monit 2015;21:3420–6.