Efficacy and safety of rituximab for systemic lupus erythematosus treatment: a meta-analysis

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Abstract:
Background: Given the inconsistency of previous studies and the newly emerging evidence, we decided to conduct a meta-analysis.
Methods: The meta-analysis included 2 randomized controlled trials and 13 observational studies involving a total of 742 patients. Qualified studies were properly searched from databases. Data were analyzed by the RevMan 5.3 software. Results were demonstrated as WMD, SMD and RR with 95% CIs, I² and P value.
Results: We observed a remarkable increase of complement C3 in the rituximab group than placebo group (WMDfixed=7.67mg/dL, 95%CIs=-0.16~15.50, I²=0%, P=0.05). A significant increase of complement C4 was observed in the rituximab group than placebo group (WMDfixed=3.14mg/dL, 95%CIs=1.06~5.22, I²=0%, P=0.003). Notably decreased peripheral CD19+B cells in rituximab group than placebo group (WMDfixed=-117.93n/μl, 95%CIs=-172.94~62.91, I²=0%, P<0.0001) in RCTs. Patients with severe or refractory SLE got more satisfactory efficacy results after receiving rituximab in observational studies, such as British Isles Lupus Assessment Group index score, SLE Disease Activity Index score, complement C3/C4, anti-dsDNA antibodies, peripheral CD19+B cells and so on. Safety profiles were no difference between rituximab and placebo groups.
Conclusion: Although the efficacy of rituximab is highly controversial for SLE, our study shows that rituximab presents a satisfying efficacy and safety for SLE.
Keywords: Efficacy; safety; rituximab; systemic lupus erythematosus; meta-analysis.
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Introduction
Systemic lupus erythematosus (SLE) is an auto-immune disease that involves widely differing tissues and organs with diverse clinical symptoms. The incidence of SLE in women is estimated to be approximately 10 times higher than that in men. However, the pathogenesis of SLE is still unclear; the production of autoantibodies and deposition of immune complexes in multiple organs leads to various abnormalities, including rash, arthritis, serositis, cytopenia, nephritis, and psychosis. Conventional therapies for SLE include nonsteroidal anti-inflammatory drugs, corticosteroids, hydroxychloroquine (HCQ) and immunosuppressive agents. Among these therapies, corticosteroids and immuno-
suppressive agents are primarily associated with mortality and morbidity. More effective treatments should be developed for SLE. B cells are widely thought to play a crucial role in the pathogenesis of SLE. B cells act as antigen-presenting cells and present autoantigens to T cells; subsequently, T cells activate and produce cytokines. T cell cytokines stimulate and induce B cells to secrete autoantibodies. Autoantigen-specific B cells interact with T cells and produce autoantibodies that are present only in non-healthy individuals. The evidence suggests that depletion of B cells has a favorable effect on SLE. Rituximab is a chimeric monoclonal antibody that targets the CD20 marker. Findings of previous studies have suggested that rituximab has a beneficial effect and satisfactory tolerance profile for serious refractory SLE. However, two randomized placebo-controlled double-blinded trials showed no clinically significant differences between rituximab and a placebo. These previous studies are controversial. Borba found unsatisfactory variations between rituximab and a placebo. These previous studies are controversial. Given the inconsistency of previous studies and newly emerging evidence, we decided to conduct a meta-analysis. The purpose of our study is to determine other parameters to investigate the efficacy and safety of rituximab for SLE patients that may be used for reference by clinicians.

**Methods**
We conducted a meta-analysis to estimate the efficacy and safety of rituximab treatment for SLE and followed the Cochrane Handbook.

**Inclusion and exclusion criteria**
The inclusion criteria were as follows: (1) The SLE diagnosis satisfied the standards specified by the American College of Rheumatology. (2) The trials included rituximab as an intervention treatment for SLE. (3) Placebo group as control group in RCTs. Baseline group when patients did not receive rituximab as control group in observational studies. (4) The study included efficacy and safety results, and the parameters of efficacy were the BILAG score, SLEDAI score, complement C3/C4 levels, anti-dsDNA antibodies, peripheral CD19B cells, serum creatinine, 24-h urinary protein and Up/Ucr. The safety results included the incidence of SAEs, deaths, infections, gastrointestinal disorders, infusion-related SAEs and infusion-related AEs. (5) Both RCT and observational studies that met the above conditions can be included in this study. Trials without clinical outcomes and articles that were merely obtained as abstracts were excluded from the meta-analysis. No language restrictions were implemented.

**Search strategy and data extraction**
The PubMed, Cochrane Library, EMBASE, Clinicaltrials and CNKI and Chinese database of WanFang databases were searched for relevant articles, most of which were published in English. The search was conducted using the following strategy, according to recognized methodologies. Descriptors in the PubMed database included the Medical Subject Headings terms “Lupus Erythematosus, Systemic” and “Rituximab” combined with free terms. The process showed the results of electronic searches with Boolean operators such as “AND” and “OR”. Two reviewers (SSW and JJZ) independently performed electronic searches on several databases. Initial screening was performed by title and abstract. Then, two reviewers read the full-text article during the final screening. In the case of discrepancies between the two reviewers, the results were discussed with a third reviewer. Reviewers assessed the included studies according to the Cochrane Collaborations tool; the evaluation bias risk is reported in the Cochrane Handbook. Two reviewers independently extracted data, and other reviewers verified and ensured that data had been exactly recorded. When data could not be obtained from the full-text article, we contacted the authors by e-mail to obtain raw data.

**Quality assessment**
The quality of included RCTs was estimated by the Jadad scale, which ranges from 0 to 5. Low-quality RCTs frequently receive a score of 2 or less, and high-quality research receives a score of at least 3. According to the Cochrane Collaboration approach, the risk of bias is reported as low, moderate, or high; reporting of bias leads to an uncertain potential risk of bias. The quality of the included observational studies was estimated by the Newcastle-Ottawa Scale (NOS). The NOS score for studies ranges from 5 to 9. Scores ≥6 are defined as high-quality research. Thirteen observational studies were defined as high-quality, and the average score was 7.5, as shown in Table 4.

**Data analysis**
The extracted data are expressed as the means±SD at
baseline and at the endpoint. The results were reported as weighted mean differences (WMDs), standard mean differences (SMDs) and relative risks (RRs) with 95% CIs, I² values and P values. The I² value indicated the heterogeneity among included studies; I² values of over 25%, 50%, and 75% are commonly defined as low, medium and high heterogeneity, respectively. When I²≥50%, heterogeneity is significant, the random effect model is applied. In this case, the inverse variance statistical method was utilized to calculate the WMD or SMD with 95% CI. The RR and 95% CI were calculated with the Mantel-Haenszel statistical method. A value of I²≤25% was regarded, as low heterogeneity, and the fixed-effects model was utilized. To ensure the homogeneity of the included studies, when I²≥75%, a study with obvious heterogeneity would be removed to determine whether it was the source of heterogeneity. All tests were two-tailed, and a value of P≤0.05 was regarded as a significant difference. The statistical analysis was performed using RevMan version 5.3.

Results

Review profiles and included studies

We retrieved 4139 articles in the following electronic databases: PubMed 631, Cochrane Library 12, EMBASE 3465, Clinicaltrials 4, China National Knowledge Infrastructure (CNKI) 9 and Chinese database of WanFang 18. After duplicates were removed (n=650), 3451 articles were deemed unsuitable according to their title or abstract because animal experiments were conducted or the studies were case reports, meeting abstracts or reviews. The remaining 38 articles were assessed independently after a full-text reading by two reviewers (SSW and JJZ). At the end of the screening, 2 RCTs and 13 observational studies were included based on the established inclusion criteria. A flowchart of the literature search and screening procedure is shown in Fig. 1.

Records identified through database searching (n=4139)
  Pubmed (n= 631)
  Embase (n= 3465)
  Cochrane (n= 12)
  Clinicaltrials (n= 4)
  CNKI (n= 9)
  Wanfang (n=18)

Records after duplicates removed (n=650)

Exclude:
  Review articles;
  Animal experiments;
  Case reports;
  (n= 3451)

Full texts assessed for eligibility (n=38)

Full texts excluded with reasons (n=23)
  no evaluation parameters

Studies included in quantitative synthesis (n=15)

Fig.1 Flowchart of study selection

Inherent differences exist between RCTs and observational studies; therefore, they were analyzed separately. RCTs exhibited high quality and low risk of bias. Observational studies with greater numbers of patients were included, but the potential risk for selection bias and residual confounding were increased. The baseline features of the 2 included RCTs are summarized in Table 1. The baseline characteristics of the 13 observational studies are shown in Table 2.
Table 1 Baseline characters of patients in 2 RCTs

| Study        | Jadad Score | Enrolled patients | Endpoint (week) | Age (year) mean±SD | Female sex(%) | SLEduration (year) mean±SD | Treatment | BLA4 score mean±SD | Anti-dsDNA (nL) mean±SD | C3 (mg/dL) mean±SD | C4 (mg/dL) mean±SD | CD19+B cells (nL/μL) mean±SD |
|--------------|-------------|------------------|----------------|-------------------|---------------|-----------------------------|-----------|---------------------|------------------------|-------------------|-------------------|-----------------------------|
| LUNAR        |             |                  |                |                   |               |                             |           |                     |                        |                   |                   |                             |
| rituximab    | 3           | 72               | 52             | 31.0±9.8          | 67.3          | 32.4±48.0 (months)          | Rituximab | 15.3±6.4            | 455.2±795.7            | 73.6±29.4         | 14.7±8.3          |                             |
| placebo      |             | 72               | 29.4±9.3       | 95.1              |               | 28.8±51.6 (months)          | Rituximab | 15.3±6.2            | 350.6±634.0            | 74.1±27.0         | 13.8±9.4          |                             |
| EXPLORER     |             |                  | 88             | 40.5±12.8         | 90.2          | 8.5±7.6                     | Rituximab | 14.0±5.1            | 282.3±799.0            | 99.0±32.5         | 15.6±8.1          |                             |
| rituximab    | 3           | 169              | 40.2±11.4      | 90.9              |               | 8.5±7.2                     | Placebo   | 14.5±5.6            | 209.2±535.2            | 96.3±31.5         | 15.5±8.6          |                             |
| placebo      |             | 88               | 40.5±12.8      | 90.2              |               | 8.5±7.6                     | Placebo   | 14.5±5.6            | 209.2±535.2            | 96.3±31.5         | 15.5±8.6          |                             |

* The number of enrolled patients

Table 2 Safety of rituximab (1,000 mg) at week 52

| Outcome                                      | Rituximab | Placebo | RR(95%CI) | I² | P   |
|----------------------------------------------|-----------|---------|-----------|----|-----|
| Severe adverse events                        | 88        | 61      | 0.94[0.72,1.23] |   | 0.67|
| Deaths                                       | 6         | 1       | 2.86[0.51,16.15] |   | 0.23|
| Infections                                   | 30        | 29      | 0.73[0.46,1.16] | 28 | 0.18|
| Gastrointestinal disorders                   | 11        | 13      | 0.55[0.25,1.22] |   | 0.14|
| Any infusion-related severe adverse events   | 17        | 17      | 0.55[0.29,1.03] |   | 0.06|
| 1st infusion infusion-related adverse events | 62        | 44      | 0.91[0.65,1.27] |   | 0.58|
| 2nd infusion infusion-related adverse events | 35        | 22      | 0.87[0.59,1.61] |   | 0.91|
| 3rd infusion infusion-related adverse events | 31        | 12      | 1.52[0.81,2.88] |   | 0.19|
| 4th infusion infusion-related adverse events | 31        | 6       | 2.95[1.26,6.90] |   | 0.01|

Sensitivity analysis
Sensitivity analysis is often used to evaluate the reliability of results. Ignoring the data of individual studies did not change the overall outcomes, which showed that outcomes were quite stable. Sensitivity analysis of the pooled data from the 13 observational studies was assessed. A significant change was not found in the outcomes, revealing that results of our observational studies are reliable.

Net changes of efficacy parameters in RCTs
A total of 241 patients received rituximab and 160 patients received a placebo in the two RCTs9,10, with 52 weeks as the end point. No heterogeneity was found between the 2 RCTs, and the fixed-effects model was applied. Relative to the placebo group, we observed a remarkable net increase of serum complement C3 in the rituximab group (WMDfixed=7.67 mg/dL, 95% CI=-0.16-15.50, I²=0%, P=0.05), as shown in Fig. 2A.
Compared to the placebo group, a significant increase in serum complement C4 was observed in the rituximab group (WMDfixed=3.14 mg/dL, 95% CI=1.06-5.22, I²=0%, P=0.003), as shown in Fig. 2B. A notable decrease in peripheral CD19⁺ B cells was observed in the rituximab group (WMDfixed=-117.93 n/μL, 95% CI=-172.94--62.91, I²=0%, P<0.0001), as illustrated in Fig. 2C. Changes in serum anti-dsDNA antibodies were not significantly different between the rituximab and placebo groups (WMDfixed=-123.16 I U/mL, 95% CI=-264.55-18.23, I²=0%, P=0.09), as depicted in Fig. 2D. Changes in the BILAG score did not differ between the rituximab and placebo groups (WMDfixed=0.28, 95% CI=1.00-1.56, I²=0%, P=0.67), as shown in Fig. 2E. Clinical responses were assessed as the combination of complete and partial responses. The clinical responses were not significantly different between the rituximab and placebo groups (RRfixed=1.14, 95% CI=0.88-1.48, I²=0%, P=0.31), as shown in Fig. 3.
Safety of rituximab in RCTs
The safety outcomes of rituximab are summarized in Table 3. The occurrence and severity of adverse events (AEs) were classified according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 3.0). We considered the following AEs: SAEs, death, infections, gastrointestinal disorders, infusion-related SAEs and infusion-related AEs over 52 weeks. The above-mentioned safety parameters were dichotomous variables, and no heterogeneity was found between the two RCTs (I²=0%). The statistical analysis revealed no significant differences between the rituximab and placebo group, except for the occurrence ratio of the 4th rituximab infusion, where infusion-related AEs were significantly increased in the rituximab group (RRfixed=2.95, 95% CI=1.26-6.90, I²=0%, P=0.01), as illustrated in Fig. 4J.

### Table 3 Baseline characteristics of 13 observational studies

| No | Study | Eczematous patients characters | n | T. | Female (%) | Rituximab dose | Other treatments | SLEDAI score mean±SD | BLA-scores mean±SD | Anti-dDNA (IU/mL) mean±SD | C1 (mg/dL) mean±SD | C4 (mg/dL) mean±SD | CD19 B cells (% of) mean±SD | Lactate dehydrogenase (IU/mL) mean±SD | 24-hour proteinuria (mg/d) mean±SD | Serum proteinuria ratio (mg/mg) mean±SD |
|----|-------|-------------------------------|---|----|------------|----------------|------------------|---------------------|-----------------|------------------------|----------------|----------------|----------------------|---------------------|------------------|---------------------|
| 1  | Leandro et al. (24) | Patients failed conventional immunosuppressive therapy | 24 | 24 | 91.7 | 6 patients 2 infusions of 500mg, 10 patients 2 infusions of 1000mg given every 2 weeks apart | Inductions IV cyclophosphamide and prednisolone | Not mentioned | 13 (6±5) | 250±231.7 | 65±0.0 | Not mentioned | Not mentioned | Not mentioned | 417±185.0 |
| 2  | Abdih et al. (20) | Patients with severe SLE and lupus nephritis: age: 10 years | 18 | 24 | 88.9 | The initial dose was 100 mg/m² subcutaneous dose every 375 mg/day | Low-dose corticosteroids and HCQ, maintenance doses of MMF or CYC 4 mg/m² start 12.5-50 mg for 2, 375 mg for 1 for 18, 14, 15 and 22 | Not mentioned | Not mentioned | 159.0±402.1 | 1.4±0.4 | 12.8±2.1 | 241±225.0 | 1.2±0.4 | mg/dl | Not mentioned | Not mentioned |
| 3  | Tamimio et al. (21) | Refractory SLE failed to corticosteroids and immunosuppressive therapy | 8 | 46 | 87.5 | 1000 mg for 3, 2300 mg for 1, 375 mg for 2 for 18, 14, 15 and 22 | Prednisolone: 12.5-50 mg, cyclophosphamide: 75-175 mg and low-dose corticosteroids IV | 8.2±3.4 | Not mentioned | 685.0±540.5 | 55.8±21.0 | Not mentioned | Not mentioned | 118±21.2 | 4.6±2.2 | Not mentioned |
| 4  | Li et al. (22) | Patients with class III or intravascular (IV) lupus nephritis | 19 | 24 | 89.5 | Infusions of 1000mg | IV methylprednisolone 250mg, prednisolone reduced from 30 to 5 mg/day, IV infusions CVCC 750mg for 18 | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned | 96±45.5 | 324±240.2 |
| 5  | Popper et al. (23) | Patients with class IIIV lupus nephritis | 18 | 48 | 83.3 | Two infusions 1g on days 1 and 15 | IV methylprednisolone 500mg, maintenance with MMF 1 g/day | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned |
| 6  | Obar et al. (24) | Active SLE with severe manifestations | 18 | 46 | 80.0 | IV infusions of Ig | Deferasirox dose: 1g on days: 1 and 15 | 12.8±5.9 | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned | Not mentioned |

SLE: Systemic lupus erythematosus; SLEDAI: Systemic lupus erythematosus disease activity index; BLA: British Isles Lupus Ass. score; Group index; CVC: cyclophosphamide; HEC: hydroxychloroquine; MMF: mycophenolate mofetil; AZA: azathioprine; n: number enrolled; t: follow-up end point (weeks).
Fig. 4 Safety of rituximab (1,000 mg) in two RCTs.
Evaluation of the efficacy of rituximab in observational studies

Observational studies data were grouped in this meta-analysis. Thirteen observational studies involving 341 patients (254 females) were included. Summarized baseline characteristics of the included studies are shown in Table 3. Depending on whether patients received rituximab, patients were assigned to the baseline group and the “after rituximab” group. The baseline group was considered the control group, and the “after rituximab” group was regarded as the intervention group.

In a total of 6 studies, 153 patients showed a net change in the SLE Disease Activity Index (SLE-DAI) score. We adopted the random-effects model and observed that relative to baseline, rituximab users resulted in a significantly decreased in the “after rituximab” group (WMDrandom=−12.31, 95% CI=−14.09−10.52, I²=57%, P<0.00001, Fig. 5A). Additionally, moderate heterogeneity was found among studies (I²=57%).

Table 5 The two most important patient outcomes are listed in the summary of findings table

Rituximab Versus Placebo
Patient or population: patients with Systemic lupus erythematosus
Settings: in adult patients
Intervention: rituximab

| Outcomes                  | Control | Death | Relative effect (95% CI) | No of Participants (studies) | Quality of evidence (GRADE) | Comments |
|---------------------------|---------|-------|-------------------------|------------------------------|-----------------------------|----------|
| Death                     | 6 per 1000 | 18 per 1000 (3 to 102) | RR 2.86 (0.51 to 16.15) | 401                          | ⊕⊕⊕⊕ high                 |          |
| Severe adverse events     | 384 per 1000 | 361 per 1000 (276 to 472) | RR 0.94 (0.72 to 1.23) | 401                          | ⊕⊕⊕⊕ high                 |          |

GRADE

High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

*The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).
Confidence interval; RR: Risk ratio.

In a total of 5 studies, 156 patients showed net changes in the BILAG score. The fixed-effects model was used, and compared to baseline, the BILAG score was obviously decreased in the “after rituximab” group (WMDfixed=−9.72, 95% CI=−10.42−9.01, I²=0%, P<0.00001, Fig 5C). Homogeneity was found among studies (I²=0%).
Figure 5
In 3 studies, the serum complement C3 data 13, 15, 18 were reported for a total of 45 patients. We selected the random-effects model, and in contrast to baseline, a significant increase in complement C3 was observed in the “after rituximab” group (SMDrandom=2.22, 95% CI=1.44-3.01, I²=48%, P<0.00001, Fig 5D). These studies exhibited moderate heterogeneity (I²=48%). Serum complement C4 data were reported in 2 studies 13, 21 including a total of 60 patients. We adopted the fixed-effects model and discovered that compared to baseline, a significant increase was observed in the “after rituximab” group (SMDfixed=1.87, 95% CI=1.43-2.30, I²=0%, P=0.00001, Fig. 5H). No heterogeneity was observed between the two studies (I²=0%). Serum anti-dsDNA antibodies data were reported in 2 studies including a total of 37 patients 13, 15. The random-effects model was adopted, and a remarkable decrease in anti-dsDNA antibodies was observed in the “after rituximab” group compared to baseline (SMDrandom=2.94, 95% CI=4.43–1.45, I²=78%, P=0.0001, Fig.5E). High heterogeneity was observed between the two studies (I²=78%). It was difficult to find the source of heterogeneity in the 2 studies.

Peripheral CD19+B-cell data were reported in 3 studies including a total of 36 patients 13, 14, 17. The random-effects model was used, and a significant reduction in the “after rituximab” group was observed compared to baseline (SMDrandom=1.46, 95% CI=–2.31–0.61, I²=54%, P=0.0008, Fig.5G). These studies had moderate heterogeneity (I²=54%). Serum creatinine data were reported in 5 studies including a total of 106 patients 13, 15, 16, 18, 21. The random-effects model was adopted, and serum creatinine levels did not differ between the two groups (SMDrandom=–0.51, 95% CI=–1.22–0.19, I²=82%, P=0.15, Fig.5F). High heterogeneity was observed among studies (I²=82%).

The 24-h urinary protein excretion data were reported in 2 studies including a total of 61 patients 15, 21. We adopted the random-effects model and observed that 24-h urinary protein excretion was significantly decreased in the “after rituximab” group compared to baseline (WMDrandom=–3.56, 95% CI=–4.41–2.70, I²=54%, P<0.00001, Fig.5B). Medium heterogeneity was found between the two studies (I²=54%).

Urinary protein-creatinine ratio data were reported in 2 studies including 36 patients 11,16. The fixed-effects model was used, and a marked decrease in the urinary protein-creatinine ratio was observed in the “after rituximab” group compared to baseline (SMDrandom=–1.04, 95% CI=–1.54–0.54, I²=7%, P<0.0001, Fig.5I). Low heterogeneity was found between the two studies (I²=7%).

**Discussion**

In recent years, SLE patients have received many biotherapies, and these biological agents presented encouraging results. Rituximab is a biological agent that selectively targets CD20+B cells. The earliest report of rituximab use in SLE patients was in 2001 25. Favorable responses and satisfactory tolerance for rituximab use for refractory SLE patients were revealed in clinical trials. Particularly, these refractory patients had symptoms involving the renal, hematological and nervous systems 26,27. A good therapy should control SLE activity and prevent more organs from being impaired by severe or fatal outcomes.

Borba previously reported the following efficacy outcomes for rituximab: clinical response, BILAG C score, time-adjusted AUCMB of the BILAG score and modification in the SF-36 PCS. Considering these results, significant variations were not found between the rituximab and placebo groups 11. Duxbury viewed rituximab can effectively control the activity of SLE in observational studies. Two RCTs did not display the benefit of rituximab by observing the complete response and the partial response rate 28. Nevertheless, in our meta-analysis, both RCTs and observational studies showed that rituximab had satisfactory efficacy and safety results.

The BILAG and SLEDAI scores were used to assess the disease activity. These assessments consider clinical symptoms, physical signs, laboratory results and physician judgments. A lower score indicates that SLE is controlled and indirectly reflects the curative effect. We observed that rituximab and a placebo exhibited no differences regarding changes in BILAG scores in RCTs. Observational studies indicated that both BILAG and SLEDAI scores were remarkably reduced in the “after rituximab” group compared to baseline. The results of both BILAG and SLEDAI scores are consistent with the observational studies of Lan LAN 29. The observational study outcomes suggest that rituximab is effective.

Higher anti-dsDNA antibodies and lower complement C3/C4 levels demonstrate the disease activity. We found a remarkable net increase in complement C3/C4 in the rituximab group compared to the placebo group. Net changes of anti-dsDNA antibodies were similar between the rituximab and placebo groups, and the P value was close to 0.05 (P=0.09); additional RCTs may make the results significant. In contrast to baseline, complement C3/C4 was significantly increased in
the “after rituximab” group, and a remarkable decrease in anti-dsDNA antibodies was observed in the “after rituximab” group in observational studies. Despite that a distinct improvement of anti-dsDNA and complement C3/C4 levels were not associated with Clinical outcomes, these changes correlated with the reduction of proteinuria in Lupus nephritis. Fervenza observed that rituximab is superior to cyclosporine in maintaining complete or partial elimination of proteinuria up to 24 months in membranous nephropathy. The complement C3/C4 results were reliable and showed that rituximab was efficacious in RCTs and observational studies, which suggested that the immune system was improved. The pathogenesis of SLE is attributed to the incidence of immune complexes that prompt supplementary pathway activation and complement consumption. Low complement C3/C4 levels are considered in the immunologic criteria of the Systemic Lupus International Collaborating Clinics (SLICC) when diagnosing and monitoring SLE. These results indicated that rituximab can control disease activity and improve the immune system, but further investigations are still needed.

B-lymphocyte dysregulation is the focus of SLE pathogenesis, and B cells act as antigen-presenting cells that present autoantigens to T cells; T cells activate and produce cytokines. T cells and B cells stimulate each other, and autoantigen-specific B cells produce autoantibodies. This mechanism is complex; the role of B cells is not only restricted to producing antibodies. Rituximab is a type of monoclonal antibody and targets CD20 on B cells, which exhausts B cells through different methods. CD19+ lymphocytes are B cells, and peripheral CD19+B cells were significantly decreased in the rituximab group compared to the placebo group. Patients who received rituximab over 52 weeks maintained good B-cell depletion. The peripheral CD19+B cells of the “after rituximab” group were remarkably decreased in observational studies. Both RCTs and observational studies demonstrated that rituximab can deplete peripheral CD19+B cells, and these results are reliable. Sfikakis reported that refractory lupus nephritis patients who received rituximab attained B-cell depletion and good clinical responses. The authors deduced that B-cell depletion was an effective therapy and that not only was an excessive production of autoantibodies avoided, but B cells were also hampered in presenting autoantigens to T cells, and the potential activation of T helper cells was quickly reduced. The B-cell depletion was similar between Sfikakis’s results and our analysis.

The 24-h urinary protein excretion extremely important for reflecting the activity and severity of renal impairment in chronic kidney disease. The spot urinary protein-creatinine ratio (Up/Ucr) may be more efficient, reliable and time-saving to diagnose proteinuria in patients who are not pregnant. The results of 24-h urinary protein excretion and the Up/Ucr were significantly decreased in the “after rituximab” group compared to baseline in the observational studies. Our 24-h urinary protein result was the same as that of Lan LAN in observational studies. Our analysis shows that rituximab may be effective in patients with refractory and severe lupus nephritis.

The possible reasons of failure of rituximab therapy in randomized placebo-controlled trials are explained below. Firstly, as a background therapy (e.g. high-dose corticosteroids and full-dose MMF), immunosuppressive therapy may have masked an obvious clinical benefit of rituximab. The composition of patients in the RCTs was different from that in the observational studies, as refractory patients were recruited in the observational studies but not enrolled in the RCTs. Moreover, factors of ethnic differences should be considered, with the African subgroup achieving a beneficial effect of rituximab in the RCTs. Secondly, we should pay more attention to background therapy. Ramos-Casals observed that the combination of rituximab and CYC may have synergistic effect and associated CYC with obvious benefits for complicated and refractory SLE. Other views including Duxbury showed that the number of patients in RCTs seemed too small (401 individuals) to reflect superiority of rituximab over placebo.

The safety results of RCTs included SAEs, deaths, infections, gastrointestinal disorders, any infusion-related SAEs and infusion-related AEs. Previously mentioned studies showed no significant variation between the rituximab and placebo groups, except for the occurrence rate of the 4th rituximab infusion, where infusion-related AEs were notably increased but did not affect the safety of rituximab applications. Our safety results are consistent with those of Borba, who concluded that rituximab is relatively safe for SLE patients. Another purpose of using rituximab is a reduction in steroids dose, which avoids the side effects of steroids. There is a significant correlation between higher doses of rituximab and a decreased rate of infection. However, it cannot be excluded from the findings that infections led to the termination of rituximab treatment or lower doses. Consequently, we recommend that rituximab...
is safe, but more high-quality long-term information is required. The reviewed safety outcomes of rituximab has been presented in a table using the GRADE profiler (Table 5)\textsuperscript{9,10}. The two most important safety outcomes of patient with SLE are displayed in the table. Patients with fewer immunosuppressive drugs previously low titers of complement C4 and severe disease may respond better. This indicates that the ideal candidates for rituximab may be patients without obvious refractory process\textsuperscript{40-42}. Relapses are related to increased damage. Thus, we should pay close attention to an appropriate balance between the dose and toxic risk of immunosuppressive drugs. As a maintenance treatment, Rituximab may be considered for refractory patients, for whom first-line immunosuppressive drugs are invalid. Moreover, there will be a high risk if these patients simply wait for symptomatic treatments after relapse\textsuperscript{42}.

**Conclusion**
We observed that rituximab treatment may be promising, especially for severe and refractory SLE. However, further investigation and discussion are required.

**Methods**
We conducted a meta-analysis to estimate the efficacy and safety of rituximab treatment for SLE and followed the Cochrane Handbook\textsuperscript{43}.

**Inclusion and exclusion criteria**
The inclusion criteria were as follows: (1) The SLE diagnosis satisfied the standards specified by the American College of Rheumatology\textsuperscript{44}. (2) The trials included rituximab as an intervention treatment for SLE. (3) Placebo group as control group in RCTs. Baseline group when patients did not receive rituximab as control group in observational studies. (4) The study included efficacy and safety results, and the parameters of efficacy were the BILAG score, SLEDAI score, complement C3/C4 levels, anti-dsDNA antibodies, peripheral CD19+B cells, serum creatinine, 24-h urinary protein and Up/Ucr. The safety results included the incidence of SAEs, deaths, infections, gastrointestinal disorders, infusion-related SAEs and infusion-related AEs. (5) Both RCT and observational studies that met the above conditions can be included in this study. Trials without clinical outcomes and articles that were merely obtainable as abstracts were excluded from the meta-analysis\textsuperscript{41}. No language restrictions were implemented.

**Authors’ contributions**
All authors made important contributions to this work.

Shanshan Wu and Yanhai Wang conceived and designed this research. Shanshan Wu and Jiaojiao Zhang searched articles and extracted data. Bo Han, Wanli Gao, Ning Zhang and Cheng Zhang verified and analyzed the data. All figures and tables were prepared by Yanhai Wang. Baishan Wang, Feng Yan and Zhijing Li wrote the manuscript. All authors reviewed and approved the manuscript.

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**Conflicts of interest**
The authors declare that they have no potential conflicts of interest regarding the research, authorship, and publication of this article.

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