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REVIEW ARTICLE

Comparison of the Efficacy and Safety of Leflunomide and Cyclophosphamide as Induction Treatment in Lupus Nephritis: Systematic Literature Review and Meta-Analysis

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ABSTRACT

Introduction: Although new agents are frequently being used in developed countries for lupus nephritis treatment, they are hard to access in low resource settings. Leflunomide may be an alternative agent in the treatment of lupus nephritis especially for patients who are intolerant or refractory to cyclophosphamide in these settings. Thus a systematic literature review was performed to analyze studies that compare leflunomide and cyclophosphamide in LN.

Method: Cochrane Reviews, MEDLINE, Ovid, Scopus, Web of Science were screened for the studies that compare leflunomide and cyclophosphamide as induction regimen in lupus nephritis. Meta-analyses for parameters of primary outcome (complete response) and secondary outcomes (partial response, total response – sum of complete response and partial response, change in albumin, creatinine, proteinuria, systemic lupus erythematosus disease activity index and adverse events) were performed.

Results: Eleven studies in the meta-analysis by Cao and the randomized controlled trial of Zhang M were included. Meta-analyses demonstrated that leflunomide was superior to cyclophosphamide in lupus nephritis patients in achieving complete response, total response and in reducing proteinuria. Both drugs had similar efficacy in achieving partial remission, maintaining creatinine and albumin levels, and in reducing disease activity index scores. Although a meta-analysis of adverse events was not possible, leflunomide was associated with less leukopenia and menstrual abnormalities but with more frequent hypertension.

Conclusion: Leflunomide may be a potential alternative to cyclophosphamide in the treatment of lupus nephritis patients with its comparable efficacy and acceptable safety profile. Larger studies of longer duration in lupus nephritis patients of different ethnicities is necessary before more robust conclusions can be reached.

Keywords: Cyclophosphamide, leflunomide, lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect multiple organs and systems with a highly variable clinical course and prognosis among different patients[1]. This disease has a complex pathogenesis which includes a combination of genetic factors, environmental triggers, hormonal factors and over production of various cytokines[2]. Lupus nephritis (LN) is one of the most common and severe organ manifestations of SLE, which is associated with significant morbidity and mortality with up to 20% of patients progressing to end stage renal disease[3]. Glucocorticoids, antimalarials, conventional immunosuppressive agents have been widely used in lupus nephritis patients with variable success[4]. With the addition of biologic agents into the therapeutic armamentarium of rheumatologists, agents such as rituximab and belimumab became available for treatment of LN[5]. Despite the improvements in therapeutic options, there is a significant proportion of refractory patients and a considerable amount of damage accrual, treatment associated morbidity and suboptimal health related quality of life even among patients that respond to the current treatment modalities[6]. There is an unmet need for new therapeutic options.

Lupus nephritis increases the economic burden of the patients on healthcare systems. In a retrospective cohort study, Garal-Pantelar et al reported that in Germany, patients with LN had greater healthcare resource utilization for most categories assessed and increased mean total costs per person-year versus controls(€15,115.99 versus €4,081.88 in the first year of follow-up)[7]. The novel effective treatment options for such as belimumab and voclosporin are likely to increase the cost of lupus nephritis treatments. A retrospective cohort study from Saudi Arabia reporting the cost consequence analysis of belimumab versus standard of care in management of SLE demonstrated that while belimumab demonstrated better effectiveness, it caused higher cost in 96% of the bootstrap cost-effectiveness distributions[8]. Study of Kennedy et al analyzing the cost effectiveness of voclosporin in treatment of lupus nephritis in the United States demonstrated the cost effectiveness model with inputs reflecting the latest and most relevant evidence. In this study, the incremental cost of voclosporin per quality-adjusted life-year was $88,076 and per equal value of life-years gained was $77,643[9].

Challenges of managing LN in low and middle income countries with low resources include non-compliance due to cost of therapy[9]. Due to high costs, treatment of glomerular diseases in adults and children in resource-poor settings largely is dependent on corticosteroids in combination with other immunosuppressive therapy, which often is cyclophosphamide because of its ready availability and low cost of treatment, despite significant adverse effects[10]. Globally, widespread access of lupus nephritis patients to novel and expensive treatment modalities such as belimumab and voclosporin in low resource settings in a short amount of time is unlikely. Therefore in these settings, alternative strategies including repurposing some existing immunsuprressive agents for use in LN treatment may be considered.

The pyrimidine antagonist leflunomide (LEF) is successfully used in the treatment of rheumatoid arthritis and psoriatic arthritis, with its place firmly established in the treatment guidelines of both diseases[11,12]. In daily rheumatology practice, for patients that encounter intolerance or lack of effcacy to methotrexate and/or azathioprine, there is a rationale to try off label use of leflunomide as a potential alternative agent[13]. There is an accumulating body of evidence, mainly from China, concerning the use of leflunomide in treatment of both treatment naive and refractory cases of LN[14-18]. There are also a number of studies that compare the efficacy and safety of cyclophosphamide (CYC) and leflunomide in the treatment of LN[19,20].

Leflunomide may present itself to the clinicians treating SLE patients as an alternative agent in the treatment of lupus nephritis especially for patients who are intolerant or refractory to CYC (or unwilling to use this agent due to infertility risk), especially in low resource settings. In order to analyze the accumulating evidence on this subject we decided to perform a systematic literature review and meta-analysis of the studies that compare the efficacy and safety of leflunomide and cyclophosphamide as remission induction agents in the treatment of lupus nephritis.

Method

This study is registered in PROSPERO database of systematic review protocols with the ID number of CRD42023415078. This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement for reporting of systematic reviews [21]. Due to the nature of the study, institutional ethics committee approval was not obtained.

Patient, Intervention, Comparator and Outcome

The patient, intervention, comparator and outcome (PICO) method was used to screen studies. P-"patient": SLE patients that are sixteen years or older who meet 1997 American College of Rheumatology with biopsy proven lupus nephritis according to International Society of Nephrology / Renal Pathology Society classification. I-"intervention": studies where leflunomide is used as "induction treatment" for lupus nephritis. C-"comparator": studies where cyclophosphamide is used as "induction treatment" for lupus nephritis, O-"outcome": primary outcomes- percent of patients achieving complete response (defined as proteinuria less than 500 mg/day and normal serum albumin and stable creatinine- percent change in creatinine less than 15% from baseline), secondary outcomes- percent of patients achieving partial response (defined as more than 50 % decrease in proteinuria with serum albumin>30 g/l and stable serum creatinine level), total response (defined as sum of complete and partial response), change in estimated glomerular filtration rate (eGFR), change in serum creatinine, change in proteinuria (spot urine sample or 24 hour urine sample), change in albumin concentration, change in systemic

lupus erythematosus disease activity index (SLEDAI) score, change in anti-dsDNA titers, change in serum complement 3 , complement 4 levels and adverse events associated with each drug.

Data Sources, Inclusion and Exclusion Criteria

Cochrane Reviews, MEDLINE, Ovid, Scopus, Web of Science were the included databases. Randomized controlled studies studies published until 1 January 2025 were included. Studies that were not randomized controlled studies, case series, case reports and studies that whose full text could not be obtained were excluded.

Search Strategy

Keywords that were used were "lupus" or "SLE" or "systemic lupus erythematosus" or "lupus nephritis" AND "leflunomide".

Data Extraction

Two reviewers (EST and EE) independently identified the studies to be included by first reading the abstracts and excluded the duplicates and then read the the full texts of the selected abstracts. From each of the selected studies they extracted the following information: First author, year of publication, study population characteristics, study design, pretreatment and posttreatment (or alternatively mean change in) serum creatinine, eGFR, albumin, proteinuria, anti ds DNA titer, serum complement 3, complement 4, systemic lupus erythematosus disease activity index, number of patients achieving complete response, the number of patients achieving partial response and the adverse events associated with each drug.

Risk of Bias and Certainty Assessment

Two reviewers (EST and EE) independently assessed risk of bias and certainty of each study. The methodological qualities of the included trials were assessed using the Jadad score, which judges descriptions of randomization, blinding and dropouts/withdrawals from trials (22).

Statistical Analysis

Begg and Mazumdar rank correlation tests were used to test for publication bias in the studies that were included in the meta-analysis.

Cochrane Q and I^2 index were used to determine the heterogeneity of the studies.

"Mean difference" was used as the effect size of the change of pretreatment and posttreatment mean albumin, creatinine, proteinuria, SLEDAI in leflunomide group and cyclophosphamide group. Meta-analyses of the mean values were reported under fixed effect and random effect models with weight coefficients and 95% confidence interval (CI). "Risk difference" was used as the effect size of complete remission, partial remission and total remission. Meta-analyses of the differences were reported under fixed effect models with ratio difference and 95 % confidence interval. Forest plots of the meta-analyses were demonstrated by the magnitude of the weight of each study with 95% CI. MedCalc v23.0.2 statistical software were used.

Certainty of Evidence Assessment

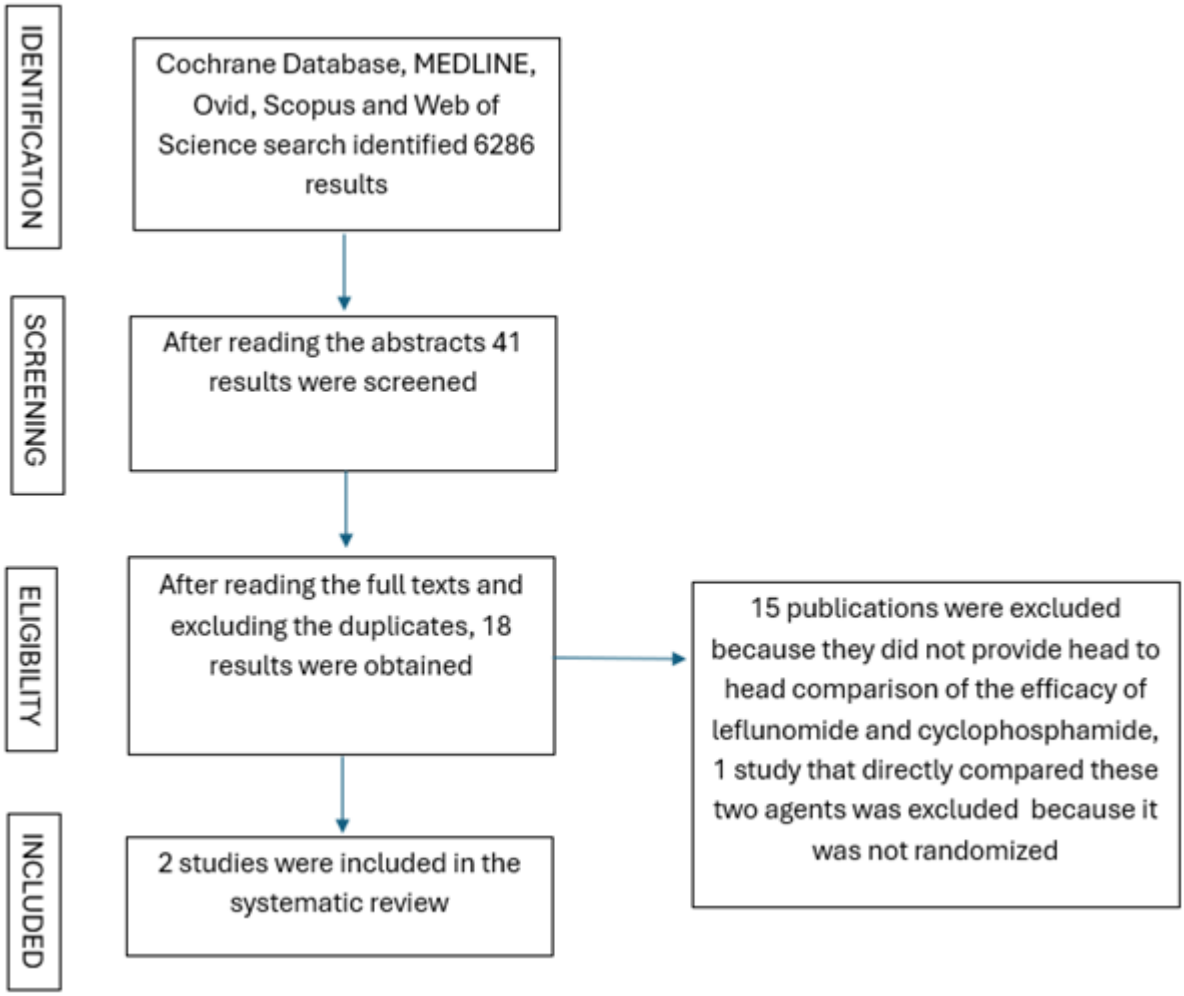
Certainty of the evidence of the meta-analysis for each outcome was planned to be assessed using the GRADE Handbook.

Results

Studies Selected

The flowhart is demonstrated in Figure 1. Two studies comparing the efficacy of leflunomide and cyclophosphamide in the treatment of LN were included in the meta-analysis: The randomized trial of Zhang M et al and the meta-analysis of Cao et al comparing the efficacy and safety of LEF and CYC in Chinese patients with lupus nephritis[20,23]. Meta-analysis of Cao included 11 studies that provided head to head comparison of the efficacy of leflunomide and cyclophosphamide in the treatment of LN[24-34].These studies were all in Chinese and their full texts could not be accessed but all the necessary information provided in the meta-analysis of Cao was utilized.

Figure 1: Flowchart of the study



Risk of Bias Analysis of Selected Studies

Risk of bias analysis of the included studies was performed. The randomized trial of Zhang M et al had a Jadad score of 3. According to the meta-analysis of Cao et al, among the 11 included studies, three studies[29,30,32] had a Jadad score of 4 and the remaining 8 studies[24-28,31,33,34] had a Jadad score of 3.

Parameters Included in Meta-Analysis

When the eleven studies included in the meta-analysis of Cao, and the study of Zhang M were analyzed, meta-analyses of the following 7 parameters were possible: Percent of patients with complete response, partial response and total response in ten studies[20,25,27-34], change in serum albumin of eight studies[20,24,25,28,29,30,32,34], change in SLEDAI score in seven studies[20,25,26,28,29,32,34], change in serum creatinine in ten studies[20,24,25,26,29,30,31,32,33,34] and change in proteinuria in eleven studies.[20,24,25,26,28,29,30,31,32,33,34]. Meta-analysis of the adverse events was not possible because the meta-analysis of the Cao et al did not report the number adverse events of each study separately (and their full texts could not be accessed).

Publication Bias Analysis

Then, the publication bias for each parameter was assessed. The results are demonstrated in Table 1. For all the parameters the p value was > 0.05, therefore no publication bias was present for these seven parameters.

Table 1: Results Concerning Publication Bias

	Kendall's Tau	p value	Comment
Change in serum albumin (2 studies)	0.286	0.32	There is no significant publication bias (p>0,05).
Change in SLEDAI (2 studies)	0.66	0.10	There is no significant publication bias (p>0,05).
Change in serum creatinine (2 studies)	0.02	0.93	There is no significant publication bias (p>0,05).
Change in proteinuria (2 studies)	0.66	0.10	There is no significant publication bias (p>0,05).
Complete remission	0.11	0.66	There is no significant publication bias (p>0,05).
Partial remission	0.20	0.42	There is no significant publication bias (p>0,05).
Total remission	0.24	0.35	There is no significant publication bias (p>0,05).

Heterogeneity Analysis

Cochrane Q and *I2* index were used to determine the heterogeneity of the studies. The results are demonstrated in Table 2. For change in serum albumin, change in SLEDAI and change in proteinuria p values were <0.05, therefore there was a heterogeneous distribution and random effect model was used. For change in creatinine, complete remission, partial remission and total remission p values were >0.05 thus, there was a homogenous distribution and fixed effect model was used.

Table 2: Results Concerning Heterogeneity

	I^2 (%)	p value	Comment
Change in serum albumin	76.37	0.001	There is heterogeneous distribution, therefore random effect model is utilized.
Change in SLEDAI	57.18	0.03	There is heterogeneous distribution, therefore random effect model is utilized. .
Change in serum creatinine	36.42	0.12	There is homogeneous distribution, therefore fixed effect model is utilized.
Change in proteinuria	94.04	0.001	There is heterogeneous distribution, therefore random effect model is utilized.
Complete Remission	0.00	0.65	There is homogeneous distribution, therefore fixed effect model is utilized.
Partial remission	0.00	0.95	There is homogeneous distribution, therefore fixed effect model is utilized.
Total Remission	0.28	0.18	There is homogeneous distribution, therefore fixed effect model is utilized.

Meta-Analysis of Efficacy Parameters

Table 3 demonstrates the meta-analyses of the mean serum albumin, mean SLEDAI scores, mean serum creatinine, mean proteinuria, complete response, partial response and total response. Meta-analysis of the mean difference in proteinuria demonstrated that change in proteinuria in leflunomide group was signifantly higher compared to that in cyclophosphamide group(p=0.02). Meta-analyses of the mean differences of serum albumin, SLEDAI scores and serum creatinine demonstrated no significant difference between CYC and LEF groups (p=0.92, p=0.40 and p=0.12 respectively. Meta-analyses of complete response and total response demonstrated that leflunomide group had significantly higher rates of complete and total response in comparison to cyclophosphamide group(p=0.02 and p<0.001 respectively). There was no significant difference between LEF and CYC groups in terms of partial response (p=0.42). Forest plots are demonstrated in Figure 2.

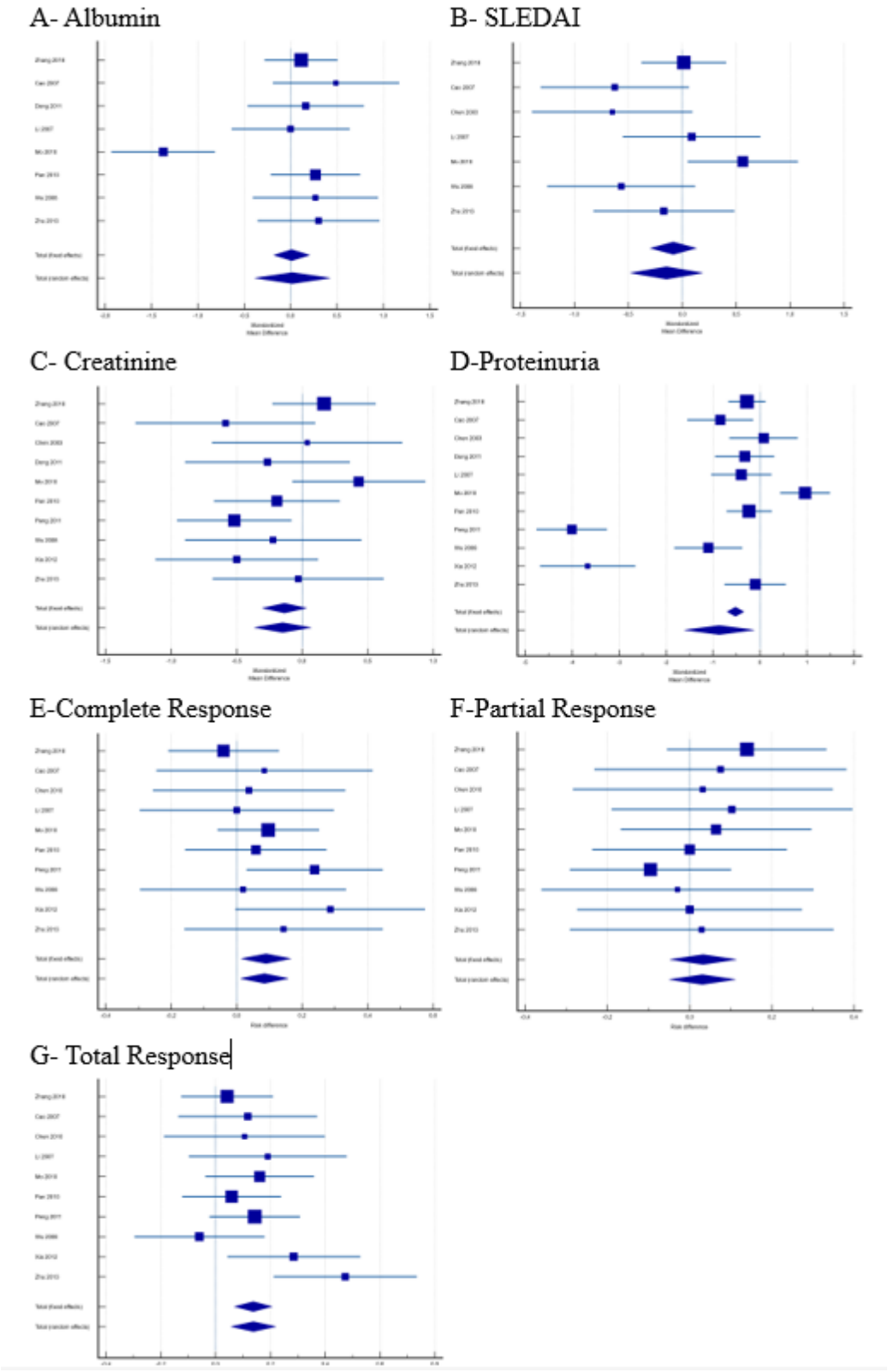
Table-3 Meta Analyses of the Studies

Meta Analysis Results of the Mean Difference in Serum Albumin Levels						
Study	**LEF**	**CYC**	**MD 95% CI**	**z**	**P**	**Weight (%)**
Zhang 2018	48	52	0.11(-0.28 0.51)			14.44
Cao 2007	17	18	0.49(-0.19 1.17)			11.58
Dong 2011	20	20	0.16(-0.46 0.79)			12.11
Li 2007	21	18	0.00(-0.64 0.64)			12.02
Mo 2010	31	31	-1.37(-1.93 -0.81)			12.77
Pan 2010	34	34	0.27(-0.21 0.75)			13.59
Wu 2008	17	18	0.27(-0.41 0.94)			11.66
Zhu 2013	19	18	0.30(-0.36 0.96)			11.83
Total (random effects)	**207**	**209**	**0.02(-0.39 0.43)**	**0.099**	**0.92**	**100**
Meta Analysis Results of the Mean Difference in SLEDAI Scores						
Zhang 2018	48	52	0.02(-0.38 0.41)			19.07
Cao 2007	17	18	-0.63(-1.32 0.06)			12.79
Chen 2003	12	20	-0.65(-1.4 0.10)			11.83
Li 2007	21	18	0.09(-0.55 0.73)			13.73
Mo 2010	31	31	0.56(0.05 1.08)			16.31
Wu 2008	17	18	-0.57(-1.25 0.12)			12.85
Zhu 2013	19	18	-0.17(-0.82 0.49)			13.42
Total (random effects)	**165**	**175**	**-0.15(-0.48 0.19)**	**0.852**	**0.40**	**100**
Meta Analysis Results of the Mean Difference in Serum Creatinine Levels						
Zhang 2018	48	52	0.17(-0.23 0.56)			18.7

Cao 2007	17	18	-0.59(-1.27 0.10)			6.49
Chen 2003	12	20	0.04(-0.69 0.77)			5.84
Dong 2011	20	20	-0.26(-0.89 0.37)			7.64
Mo 2010	31	31	0.43(-0.08 0.94)			11.49
Pan 2010	34	34	-0.19(-0.67 0.29)			12.89
Peng 2011	42	42	-0.52(-0.96 -0.09)			15.31
Wu 2008	17	18	-0.22(-0.89 0.45)			6.74
Xia 2012	21	21	-0.49(-1.12 0.12)			7.82
Zhu 2013	19	18	-0.03(-0.68 0.62)			7.15
Total (fixed effects)	**261**	**274**	**-0.14(-0.30 0.03)**	**1.568**	**0.12**	**100**
Meta Analysis Results of the Mean Difference in Proteinuria Levels						
Zhang 2018	48	52	-0.28(-0.67 0.12)			9.52
Cao 2007	17	18	-0.85(-1.55 -0.14)			9.04
Chen 2003	12	20	0.08(-0.65 0.80)			9
Dong 2011	20	20	-0.33(-0.96 0.31)			9.17
Li 2007	21	18	-0.40(-1.04 0.25)			9.15
Mo 2010	31	31	0.97(0.43 1.49)			9.33
Pan 2010	34	34	-0.23(-0.71 0.25)			9.41
Peng 2011	42	42	-4.01(-4.76 -3.26)			8.91
Wu 2008	17	18	-1.10(-1.83 -0.38)			9
Xia 2012	21	21	-3.67(-4.69 -2.66)			8.34
Zhu 2013	19	18	-0.10(-0.76 0.55)			9.13
Total (random effects)	**282**	**292**	**-0.87(-1.61 -0.12)**	**2.289**	**0.02**	**100**
Meta Analysis Results of the Risk Difference in Complete Response						
Zhang 2018	11/48	14/52	-0.04(-0.21 0.13)			18.78
Cao 2007	9/17	8/18	0.09(-0.24 0.42)			4.94
Chen 2003	6/19	5/18	0.04(-0.26 0.33)			6.23
Li 2007	7/21	6/18	0.00(-0.30 0.30)			6.11
Mo 2010	5/31	2/31	0.10(-0.06 0.25)			22.21
Pan 2010	11/34	9/34	0.06(-0.16 0.28)			11.53
Peng 2011	26/42	16/42	0.24(0.03 0.45)			12.48
Wu 2008	6/17	6/18	0.02(-0.30 0.33)			5.44
Xia 2012	31/21	7/21	0.29(-0.004 0.58)			6.43
Zhu 2013	8/19	5/18	0.14(-0.16 0.45)			5.85
Total (fixed effects)	**102/269**	**78/270**	**0.09(0.01 0.17)**	**2.314**	**0.02**	**100**
Meta Analysis Results of the Risk Difference in Partial Response						
Zhang 2018	27/48	22/52	0.14(-0.06 0.33)			17.31
Cao 2007	6/17	5/18	0.08(-0.23 0.38)			6.92
Chen 2003	8/19	7/18	0.03(-0.28(0.35)			6.53
Li 2007	8/21	5/18	0.10(-0.19 0.40)			7.60
Mo 2010	22/31	20/31	0.06(-0.17 0.30)			12.12
Pan 2010	18/34	18/34	0.00(-0.24 0.24)			11.60
Peng 2011	11/42	15/42	-0.10(-0.29)0.10)			16.88
Wu 2008	8/17	9/18	-0.03(-0.36 0.30)			5.96
Xia 2012	6/21	6/21	0.00(-0.27 0.27)			8.75
Zhu 2013	9/19	8/18	0.03(-0.29 0.35)			6.33
Total (fixed effects)	**123/269**	**115/270**	**0.03(-0.04 0.11)**	**0.799**	**0.42**	**100**
Meta Analysis Results of the Risk Difference in Total Response						
Zhang 2018	38/48	36/48	0.04(-0.13 0.21)			16.36
Cao 2007	15/17	13/17	0.12(-0.14 0.37)			7.20
Chen 2003	14/19	12/19	0.11(-0.19 0.40)			5.35
Li 2007	15/21	11/21	0.19(-0.10 0.48)			5.56
Mo 2010	27/31	22/31	0.16(-0.04 0.36)			11.69
Pan 2010	19/34	27/34	0.06(-0.12 0.24)			14.14

Peng 2011	37/42	31/42	0.14(-0.02 0.31)			16.92
Wu 2008	14/17	15/17	-0.06(-0.30 0.18)			8.20
Xia 2012	19/21	13/21	0.29(0.04 0.53)			7.83
Zhu 2013	17/19	8/19	0.47(0.21 0.74)			6.75
Total (fixed effects)	**225/269**	**188/269**	**0.14(0.07 0.21)**	**3.922**	**<0.001**	**100**

Figure 2: Forest plot of the meta-analysis of each parameter



Safety Parameters

Even though meta-analysis of adverse events was not possible. Table 4 provides the rates of each side effect in the meta-analysis of Cao et al and in the randomized trial of Zhang M et al. It is noteworthy that across the studies LEF was associated with less leukopenia and menstrual abnormalities compared to CYC and in the study of Zhang M et al, leflunomide was associated with more cases of hypertension. In the same study, 1 leflunomide patient died due to suicide, The frequency

of liver function test abnormalities differed across studies: in the meta-analysis of Cao , CYC group had higher rates of liver function test abnormalities, whereas in the study of Zhang M, LEF group had more patients with hepatotoxicity. Alopecia, gastrointestinal symptoms and total number of infections were more frequent in cyclophosphamide group than leflunomide group in Cao's meta-analysis, whereas in the study of Zhang M alopecia, gastrointestinal symptoms and total number of infections were in similar frequency in both groups.

Quality Assessment

Unfortunately the full texts of in the individual studies in the meta-analysis of Cao et al were not accessible, which prevented us from performing overall quality assessment for each parameter of meta-analysis according to GRADE Handbook.

Table 4- Adverse Events

Adverse event	Cao et al- 2015: Number of studies, Number of adverse event/Number of patients		Zhang et al- 2018: Number of adverse event/Number of patients	
	Leflunomide	Cyclophosphamide	Leflunomide	Cyclophosphamide
Liver function test elevation	9 studies, 16/225	9 studies, 32/222	9/48	5/52
Gastrointestinal symptoms	8 studies, 19/198	8 studies, 33/204	4/48	4/52
Alopecia	7 studies, 6/167	7 studies, 21/173	1/48	2/52
Leukopenia	6 studies, 1/134	6 studies, 11/129	1/52	16/48
Infection (Total)	5 studies, 11/121	5 studies, 23/121	16/48	18/52
Herpes Zoster Infection	2 studies, 0/65	2 studies, 2/65	1/48	3/52
Menstrual Abnormalities	4 studies, 0/106	4 studies, 6/106	0/48	2/52
Rash	7 studies, 9/176	7 studies, 6/171	N.A.	N.A.
Hypertension	N.A.	N.A.	5/48	1/52
Headache	N.A.	N.A.	1/48	3/52
Tachycardia	N.A.	N.A.	2/48	4/52
Death	N.A.	N.A.	1/48	0/52
Total	7 studies, 32/187	7 studies, 69/182	N.A.	N.A.

N.A.: Not available

Discussion

This meta-analysis compares the efficacy and safety of LEF and CYC in lupus nephritis patients, by assembling the data from 12 different studies with an acceptable risk of bias (a Jadad score of 3 and higher). Final results demonstrated a similar efficacy for both drugs in maintaining serum creatinine concentration, serum albumin concentration and in reducing SLEDAI scores as well as achieving partial remission. Leflunomide was significantly better than cyclophosphamide in reducing proteinuria and in achieving complete response and total renal response. Although a meta-analysis of the adverse events was not possible, LEF seemed to have an acceptable adverse event profile with less leukopenia and menstrual abnormalities compared to CYC but higher rates of hypertension. For other adverse events, even though results varied between the studies, leflunomide seemed to have an acceptable safety profile that is comparible with CYC (if not better in some aspects). This meta-analysis results agree with the meta-analysis of Cao et al[23], which is quite predicatable as it consists mostly of same studies that were included in that meta-analysis.

Leflunomide's best known mechanism of action is inhibition of de novo pyrimidine synthesis by blocking dihydroorotate dehydrogenase[35]. Activated lymphocytes demand a large amount of pyrimidine for ongoing DNA synthesis during proliferation which can not be sufficiently met by salvage of pyrimidine, which renders them susceptible to leflunomide[36]. Other mechanisms of action of leflunomide include inhibition of tyrosine kinases involved in T lymphocyte[37] and B lymphocyte[38,39] activation, suppression of interleukin-2 production[40], and blocking nuclear factor kappa beta activation[41]. In the animal study of Bartlett et al, when the MRL/lpr mice that spontaneously develop a SLE-like disease, treatment with leflunomide was associated with reduction in proteinuria, decrease in autoantibodies and better survival compared to prednisolone and cyclosporine A[42]. Study of Xu et al on the same animal model demonstrated that the main mechanism of action of leflunomide in this model is through tyrosine kinase inhibition rather than pyrimidine synthesis antagonism[43]. In their animal study, Popovic et al reported that leflunomide restored the suppressed T-cell response to the same level as found in healthy mice[44]. In the same

mouse model, He et al reported that leflunomide treatment could dramatically reduce the production of autoantibodies and immune complex deposition in the kidney, leading to relieved kidney damage and reduced mortality. The therapeutic effect of leflunomide on the lupus-prone mice was partially due to the inhibition of TLR9 signaling pathway[45]. In their animal study Qiao et al used MRL-lpr mice (female) and C57BL/6 mice model of SLE to demonstrate that LEF could inhibit development of lupus nephritis, attenuate the generalized autoimmune features by augmenting iTreg cells, which suppressed pathogenic interleukin-17 producing double negative T cells by an Akt dependent mechanism[46].

In their paper published in 2005, Cui et al conducted a prospective multicenter controlled trial on 51 systemic lupus erythematosus patients with biopsy proven proliferative lupus nephritis where treatment naive patients received either leflunomide (Group A) or intravenous cyclophosphamide (Group B), and relapsed patients who received immunosuppressives in the last three months received leflunomide (Group C). Forty-seven patients completed the six month treatment period. Total response rate, complete remission rate and SLEDAI improvement were statistically similar in both groups. In Group C total response rate was 60 % and complete remission rate was 6.7 %. Infection and alopecia were the major adverse events. This study proposed LEF as an effective alternative induction treatment in both treatment naive and refractory cases of LN[14].

In 2008, in their prospective multicenter non-randomized observational study of Wang et al enrolled 110 biopsy proven LN patients. Seventy patients were treated with LEF (with a loading dose of 1 mg/kg/day for 3 days followed by 30 mg/day.) and 40 patients were treated with CYC, with both groups receiving concomitant prednisone. At the end of 6 months 21% of the LEF patients were in complete remission and 52% were in partial remission, similar to the rates in CYC group (18% total and 55% partial remission). Both groups also had similar improvement in SLEDAI scores. Repeat kidney biopsies performed in 13 patients in leflunomide group demonstrated a very significant reduction in activity index but an increased chronicity index despite treatment. Both groups had a similar safety profile, with a higher incidence of gastrointestinal events in cyclophosphamide group[19].

In their study published in 2009, Zhang FS et al performed baseline kidney biopsy from 31 lupus nephritis patients, then treated them with LEF (initially a loading dose of 0.8–1.2 mg/kg per day for 3–7 days , followed by a maintenance dose of 0.4–0.8 mg/kg. Three of these patients priorly received CYC or mycophenolate for a long time. Initial biopsy revealed 5 patients with Class II, 11 patients with Class III, 10 patients with Class IV and 9 patients with Class V lupus nephritis. At the end of 12 months repeat biopsy was performed to all 31 patients. The posttreatment biopsies revealed 5 patients with Class II, 17 patients with Class III, 3 patients with Class IV and 6 patients with Class V LN. The rate of class transformation was 41.9%, with one patient transforming from Class IV to Class II, 6 patients transforming from Class IV to Class III, 3 patients from Class V to Class III and 3 patients from Class III to Class II. In patients without class transformation active lesions improved significantly. The chronic lesions did not aggravate under LEF treatment, unlike the study of Wang. SLEDAI scores of all patients improved significantly. There were no infections and side effects did not require leflunomide discontinuation[16].

The study of Zhang Y et al published in 2010 enrolled 43 lupus nephritis patients who had previously received cyclophosphamide but either relapsed under CYC or could not tolerate it. These patients received LEF for 12 months. At the end of first months proteinuria decreased significantly and by 3-6 months of treatment, serum albumin gradually increased and anti ds DNA titers as well as SLEDAI scores gradually decreased. Leflunomide was associated with less side effects compared to cyclophosphamide[17].

The latest study on this subject was published by Zhang S et al in 2023. This study enrolled 20 refractory lupus nephritis patients that met 2012 systemic lupus erythematosus classification criteria. Refractory disease was defined as " proteinuria and/or estimated glomerular filtration rate not improved or worsened for 4–6 months under two different induction regimens according to standards of care, based on the Kidney Disease-Improving Global Outcomes. and The European Alliance of Associations for Rheumatology / European Renal Association–European Dialysis and Transplantation Association guidelines". The previous immunosuppressive drugs these patients received included mycophenolate mofetil(19 patients), cyclophosphamide(18 patients), tacrolimus(10 patients), azathioprine(6 patients), cyclosporine A(5 patients) and methotrexate(4 patients). These patients received a daily dose of 20-40 mg LEF orally. Meanwhile, immunosuppressives were withdrawn, and corticosteroids were gradually tapered. The primary endpoint was the response of patient. The percentage of more than a 25% decrease in 24-hour urine protein quantity was calculated as the secondary endpoint. It was evaluated 3 months, 6 months, and 1 year after enrollment, with 13 patients continuing the treatment for 2 years. Eighteen patients (90%) completed the study. At 3 months, 80%(16/20) of the patients achieved more than a 25% decrease in 24-hour urine protein quantity. At 6 months, three patients(15%) achieved a partial response, and five patients(25%) achieved a complete response. However, by 12 months and 24 months, the complete response rate dropped to 15% and 20%, respectively. The overall response rates (sum of complete response and partial response) were 30%, 40%, 40%, and 30% at 3, 6, 12, and 24 months, respectively. As for the secondary endpoint, the ratio of patients with more than a 25% decrease in their 24-hour urine protein quantity was 80% at month 3, 65% at month 6, 60% at month 12, and 50% at month 24 in the intention-to-treat analysis. Treatment failure was observed in 9 patients(9/18) after 12 months, as well as in 7 of the remaining 13 patients after 24 months. Two patients withdrew from the study due to developing cytopenia

and leukopenia. This study also underlined leflunomide as be a promising treatment option for refractory LN[18].

Recent years have added numerous new and promising options to the therapeutic armamentarium of the clinicians that treat lupus nephritis. Addition of the new B cell depleting agent obinutuzumab[47], B cell activating factor inhibitor with belimumab[48] and the new calcineurin inhibitor voclosporin[49] to the current options of mycophenolic acid analogues, classical calcineurin inhibitors(cyclosporin A and tacrolimus) and B cell depleting agent rituximab, has enriched our thepeutic options. In fact in some developed countries, even cyclophosphamide which has for a very long time been the gold standard for remission induction therapy has been supplanted by the emerging combination immunosuppressive regimen[50]. When these options are considered, offering LEF as an option may not seem appealing especially in developed countries. However these new options are rather expensive. One must keep in mind that in low resource settings, access to even mycophenolic acid analogues is difficult. Thus, in those settings leflunomide can be offerred as a possible alterative to patients who respond inadequately to cyclophosphamide, can not tolerate CYC or do not want to utilize cyclophosphamide.

In most of these leflunomide studies that were included in the meta-analysis, patients used higher doses than the doses (10-20 mg/day) used in rheumatoid arthritis or psoriatic arthritis. In the randomized trial of Zhang et al, leflunomide tablets were given at an initial loading dose of 40 mg/day for 3 days and followed by 20 mg/day for 24 weeks. In the studies that were eligible for the meta-analysis of Cao et al, oral leflunomide was used at a dose of 20 to 50 mg/day for 6-12 months. Further studies with larger number of patients are necessary to determine the optimal dosing regimen of LEF in lupus nephritis patients.

The strength of this meta-analysis is the relatively large number of LN patients it assembles (it is the largest meta-analysis on this subject) and the acceptable quality of the included studies (with a Jadad score of at least 3) as well as the consistency and homogeneity of its design. This study was not without weaknesses. First of all, the inclusion criteria included all lupus nephritis patients, therefore the efficacy parameters account for all LN patients and not for specific subgroups such as proliferative or Class V lupus nephritis. Another important weakness is that the all the studies are all performed in China and therefore demonstrating the efficacy and safety of LEF in comparison to cyclophosphamide in lupus nephritis in only one ethnicity. Further studies on the efficacy of leflunomide in LN patients with different ethnicities are necessary. Other limitations include the lack of highest quality studies (with a Jadad score of 5), the presence of high heterogeneity observed in 3 parameters (namely serum albumin, SLEDAI score and proteinuria), the relative short duration of studies (mostly 6 months), different doses of LEF across different studies and the lack of post-treatment biopsies to assess histopathological improvement in activity indices. One other important limitation in our meta-analysis is our inability to access the full texts of the 11 studies in the meta-analysis of Cao, which prevented us from performing the meta-analysis of specific adverse events and certainty of evidence assessment according to Grading of Recommendations Assessment, Development and Evaluation Handbook. Finally, assessments concerning important outcomes such as mortality and end stage renal failure are lacking.

Conclusion

In this largest meta-analysis that compares the efficacy and safety of LEF and CYC in lupus nephritis patients, leflunomide has comparable (if not better in some parameters) efficacy in Chinese LN patients and an acceptable safety profile. LEF can be a possible alternative for lupus nephritis patients that respond inadequately to cyclophosphamide, relapse under CYC or are unable to tolerate cyclophosphamide, especially in resource poor settings where access to novel treatment regimen is not possible or sustainable. Larger randomized controlled trials with longer duration that determine the optimal dose of leflunomide in different classes of lupus nephritis in patients from different ethnicities are necessary before more robust conclusions can be reached.

Conflict of Interest: The authors declare no conflict of interest.

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Supplementary Material

Preferred Reporting Items for Systematic Reviews and Meta-Analyses Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 2
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Pages 2 and 3
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 3
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Not available
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 3
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 3
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 3
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Not available
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Not available
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 3
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not available
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not available
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Not available
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 3
RESULTS			

Section and Topic	Item #	Checklist item	Location where item is reported
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 3, Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Not available
Study characteristics	17	Cite each included study and present its characteristics.	Page 3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Page 4
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 3
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Not available because 11 studies were accessed secondarily
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Tables 2 and 3, Figure 2
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not available
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not available
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not available
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not available
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pages 8-10
	23b	Discuss any limitations of the evidence included in the review.	Page 10
	23c	Discuss any limitations of the review processes used.	Page 10
	23d	Discuss implications of the results for practice, policy, and future research.	Page 10
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 2
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 2
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not available
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Not available
Competing interests	26	Declare any competing interests of review authors.	Not available
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not available

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71