

## Original Article

# Risk assessment of infertility due to cyclophosphamide use for premenopausal women with systemic lupus erythematosus: a meta-analysis

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**Abstract:** Background: Recent studies suggested an increased risk of infertility due to cyclophosphamide (CYC) use in premenopausal women with systemic lupus erythematosus (SLE). However, the odds ratio (OR) reported are various according to different studies. Purpose: A meta-analysis was performed regarding the influence of CYC on fertility of premenopausal women with SLE, in an effort to obtain the accurate risk arising from CYC use. Methods: We conducted PubMed, Google Scholar and Ovid database search, as well as Cochrane Library search (up to November 2015) for studies assessing the association between CYC and infertility. We performed fixed effects meta-analysis of odds ratios (OR). Heterogeneity was evaluated by Q statistics and I<sup>2</sup> statistic. Results: After study selection, 4 unique studies (6 comparisons) including 239 patients were available for this meta-analysis. Pooled analysis of overall infertility risk of CYC group versus Non-CYC group showed a significant increase in risk of infertility (OR=5.97; 95% CI, 3.08-11.58; P<0.00001), without any heterogeneity (I<sup>2</sup>=0%). Stratified by study design, subgroup analysis with only randomized controlled studies revealed a significant increase of risk (OR=4.77; 95% CI, 2.17-10.48; P<0.0001) with little heterogeneity (I<sup>2</sup>=19%). And subgroup analysis with only retrospective studies also revealed a significant increase of risk (OR=9.37; 95% CI, 2.68-32.85; P=0.0005) without heterogeneity (I<sup>2</sup>=0%). Conclusions: This meta-analysis suggests a notably increased risk of infertility due to CYC use in the clinical practice. Physicians should carefully evaluate such risk factors for SLE patients before routinely prescribing CYC.

**Keywords:** Cyclophosphamide, CYC, infertility, MMF, SLE, systemic lupus erythematosus, meta-analysis

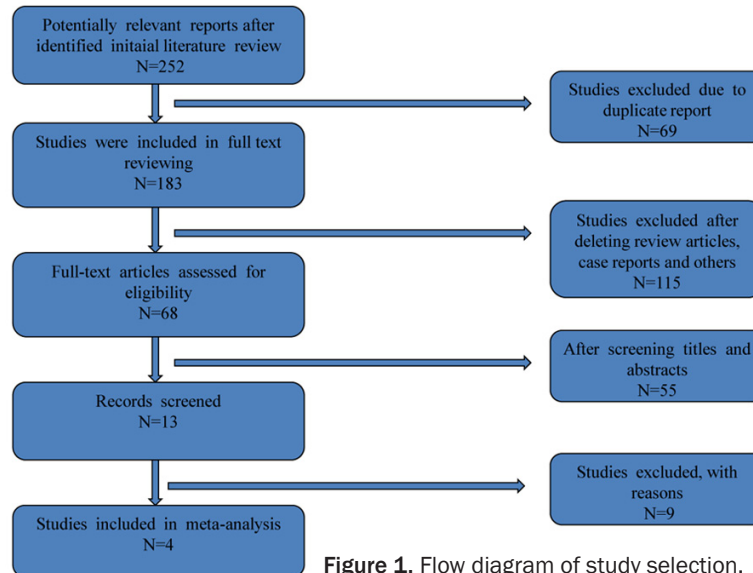
## Introduction

The alkylating agent, cyclophosphamide (CYC), is widely used for the treatment of various malignancies and autoimmune diseases. This cytotoxic agent reacts with DNA bases and damages DNA repair mechanisms, therefore inhibiting cellular replication [1]. The deleterious effect of CYC therapy on fertility began to be noted in the early 1970s [2], after amenorrhea secondary to CYC therapy was reported, with loss of menstrual cycles in 18 of 34 previously menstruating women who were treated with CYC for glomerulonephritis, nephropathy, and lupus nephritis (LN). The risk of sustained amenorrhea has been shown to develop in a dose-dependent and age-dependent manner, with older patients and patients receiving higher cumulative doses at an increased risk of

developing amenorrhea [3]. Despite long-standing concerns for these significant side effects and implications for fertility, CYC continues to be a key therapy for patients with SLE [4].

However, the risk for infertility arising from CYC therapy cannot be ignored especially for the premenopausal women patients with SLE, who are still needing CYC therapy. In this regard, it attracts the scientists, especially the physicians, to perform clinical research focused on the side effect of CYC use on fertility in the patients with SLE. Up to now, some studies have revealed the risk of infertility due to CYC use, but with inconsistent results [3, 5-7]. Given that a better understanding of the infertility risk resulting from CYC use is needed, we performed this meta-analysis in an effort to obtain the accurate risk arising from CYC use.

## Risk assessment of infertility



### Materials and methods

#### Search strategy

We conducted PubMed, Google Scholar and Ovid database search, as well as Cochrane Library search (up to November 2015) for studies assessing the association between CYC and infertility. Papers should be published in English. Potentially relevant studies included the word 'cyclophosphamide', plus at least one of the following terms: infertility, fertility, amenorrhea, sterility, ovarian failure; then plus at least one of the following terms: mycophenolate mofetil (MMF), azathioprine (AZA), methylprednisolone (MET), glucocorticoid, methotrexate, K562, hydroxychloroquine. In addition, we also manually searched the reference lists to detect additional eligible studies.

#### Selection criteria

We selected observational studies and randomized controlled studies that reported on the risk of infertility due to CYC use. For the observational studies, we selected case-control or controlled cohort (prospective or retrospective) studies that evaluated the risk of infertility due to CYC use. The specific inclusion criteria were that the studies had to report odds ratio/risk ratio/hazard ratio (OR/RR/HR) for the risk of infertility due to CYC use, or to report sufficient raw data to allow for calculation of OR. The excluded studies included reviews, case reports, editorials, comments, letters, abstracts

only, and studies with unavailable data.

#### Data extraction

Two authors scanned all titles and abstracts for studies that met the inclusion criteria, and excluded any articles that clearly did not fulfill the selection criteria. Full reports (where available) of potentially relevant trials and studies were retrieved and independently checked by these two authors. Three authors then independently collected information on study design. Outcomes of interest were infertility events, including infertility,

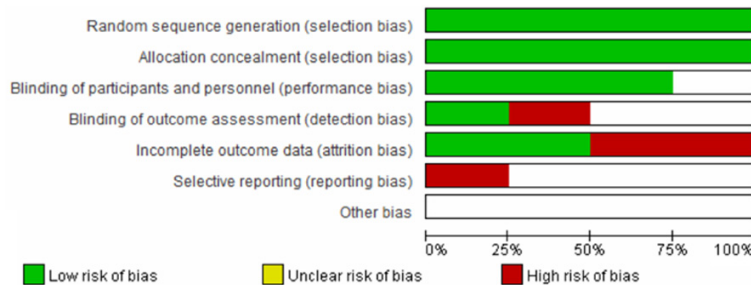
amenorrhea, sterility, ovarian failure, and others. The OR, RR or HR and 95% confidence intervals (95% CI) were extracted. When both crude and adjusted RR were provided, we used the most fully adjusted RR for all the included studies. We also extracted the following items from each individual study: author; year of publication; the country of study; duration of follow-up; the sample size, gender, and the mean age or age range of participants, and study design. Where there was any uncertainty or discrepancies, the article was discussed among the three authors to determine if the studies should be included. We also contacted authors if there were any areas that required clarification.

#### Statistical analysis

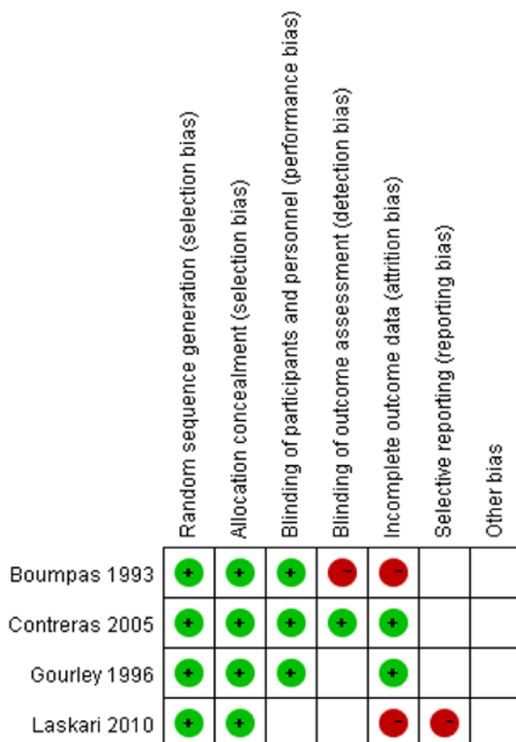
**Assessment of bias inclusion risk in the study:** To avoid inherent problems with scale validity [8], we did not use quality scale or checklists. We assessed the methodological quality as described by the Cochrane Reviews Handbook 5.3 (Methodological quality assessment scheme). The studies were classified into A: low risk of bias and each of the criteria was appropriate, B: medium risk of bias and most of the criteria were appropriate, and C: high risk of bias and most of the criteria were not appropriate.

**Measures of treatment effect:** Only dichotomous outcomes were mentioned in our study, so the OR and 95% CI were calculated for outcomes.

## Risk assessment of infertility



**Figure 2.** Risk of bias summary represented as percent chart. Review authors' judgments about each risk of bias item for each included study.



**Figure 3.** Summary graph of risk bias. Review authors' judgments about each risk of bias item for each included study. + is "yes", - is "no", null is "unclear".

**Assessment of heterogeneity:** Heterogeneity of the effect across studies was assessed by Q statistics, which is distributed as  $\chi^2$  statistics, with its *P*-values revealed by the forest plot. The heterogeneity test was considered statistically significant when  $P < 0.10$ , a conservative standard for meta-analyses. Simultaneously,  $I^2$  was used to estimate the size of the heterogeneity.  $I^2 > 50\%$  indicated considerable heterogeneity among the included studies and then a random effects analysis should be performed; or a fixed

effects analysis should be performed in metaanalysis.

**Test for risk of publication bias:** As a visual inspection of publication bias, funnel plot was performed. The funnel plot should be asymmetric when there is publication bias and symmetric in the case of no publication bias.

**Statistical software and *P* values:** All of the statistical

analyses were performed using Review Manager software (RevMan Version 5.3.5; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark). *P* values less than 0.05 were regarded as statistically significant. All *P*-values were presented as two-tailed.

## Results

### Literature search

After the application of search strategy, a total of 252 potentially relevant reports were identified in our initial literature search. Finally, 4 unique studies including 239 patients and 6 comparisons were available for this meta-analysis [3, 5-7]. All studies reported on amenorrhea including 6 comparisons. The study from Contreras et al. [6] and Gourley et al. [7] were included in this meta-analysis as randomized controlled studies. The others were included as retrospective observational studies [3, 5]. A flow chart showing the study selection is presented in **Figure 1**. No additional studies were identified through our hand search of references from published studies.

### Assessment of bias inclusion risk

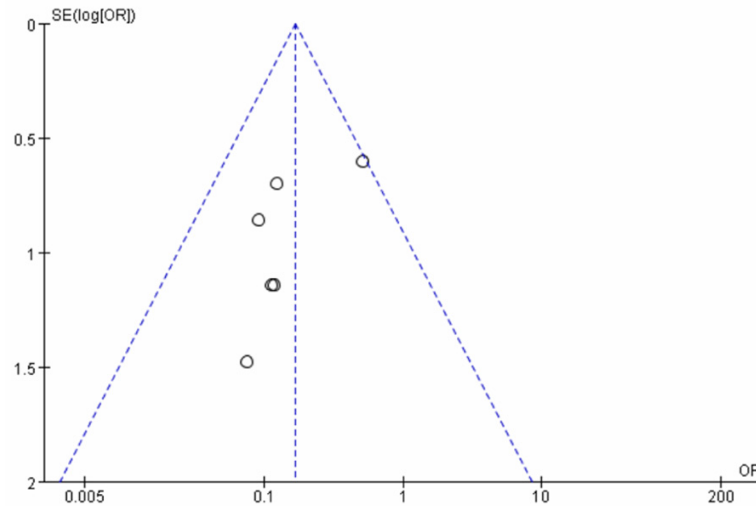
A summary of methodological item assessments for each included study is shown in **Figures 2** and **3**. Overall, the methodological quality of all included studies was found to be low risk of bias.

### Characteristics of included studies

The characteristics of the included studies are listed in **Table 1** and **Table S1**. The report from Contreras et al. [6] included two separate com-

**Table 1.** Characteristics of included studies

Author	Year	Race	Study design	Follow-ups	Gender	Average age (yr)	Events
Contreras et al [1]	2005	African-American, Hispanics	Randomized controlled study	28 months	Female	33	Amenorrhea
Gourley et al [2]	1996	American	Randomized controlled study	12 months	Female	30	Amenorrhea
Laskari et al [3]	2010	Caucasian	Retrospective study	12 months	Female	29	Amenorrhea
Boumpas et al [4]	1993	American	Controlled retrospective study	12 months	Female	26	Amenorrhea



**Figure 4.** funnel plot for publication bias.

parisons stratified by different immunosuppressants of MMF and AZA, respectively. Also, the study from Gourley et al. [7] reported two separate comparisons stratified by different immunosuppressants of MET and CYC+MET, respectively. Thus, 6 comparisons in total were included in the meta-analysis.

## Assessment of publication bias

Assessment of publication bias for all included studies was performed by funnel plot on visual inspection as shown in **Figure 4**. Obviously, the funnel plot is asymmetric and indicates the existence of publication bias.

## Overall risk of infertility

In this analysis, we have pooled all 6 included comparisons together. Pooled analysis of overall risk of infertility due to CYC use showed a significant increase (OR=5.97; 95% CI, 3.08-11.58;  $P<0.00001$ ) without any heterogeneity ( $I^2=0\%$ ), as shown in the forest plot **Figure 5**. In this analysis, a fixed effect model was used to perform this analysis. Because of no heterogeneity observed, it is no need to perform sensitivity analysis.

## Subgroup analysis for risk of infertility

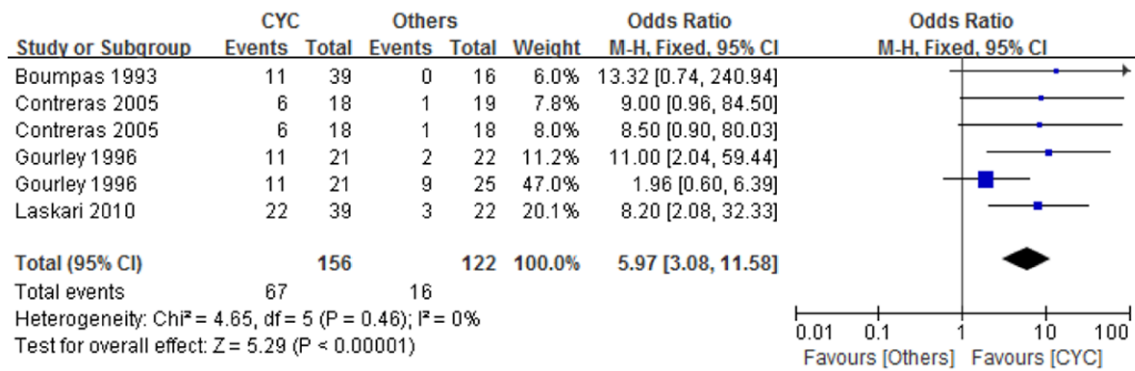
In an attempt to better understand the current analysis, subgroup analysis for risk of infertility due to CYC use was carried out, which was stratified by study design. As shown in **Figure 6**, subgroup analysis with only randomized controlled studies revealed a significant increase of risk (OR=4.77; 95% CI, 2.17-10.48;  $P<0.0001$ ) without little heterogeneity ( $I^2=19\%$ ). As shown in the forest plot **Figure 7**, subgroup analysis with only retrospective studies revealed a significant increase of risk (OR=9.37; 95% CI, 2.68-32.85;  $P=0.0005$ ) without heterogeneity ( $I^2=0\%$ ). In the analyses above, fixed effect models were used.

## Discussion

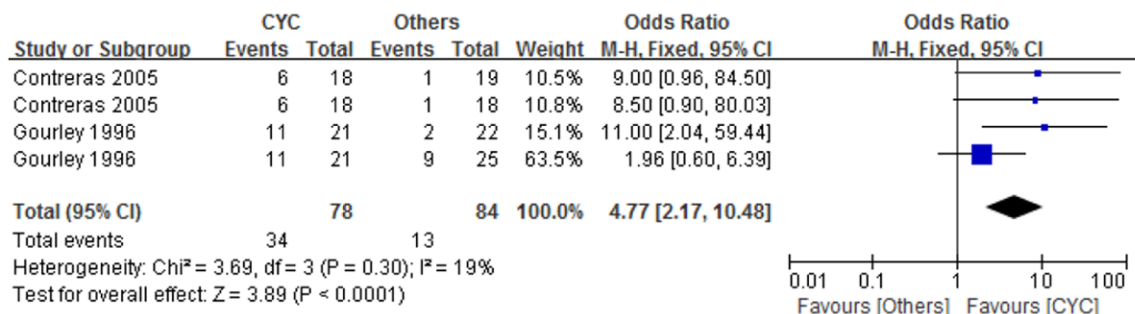
SLE is a chronic autoimmune disease with multisystem involvement. Renal disease is a common manifestation and is responsible for considerable morbidity and mortality. LN varies in severity and is an important predictor of poor outcome in SLE patients. The treatment of LN has improved substantially over the past 20 years and the proportion of patients going into end-stage renal failure much less [9]. In Asian patients, LN will be present in approximately 75% of patients with SLE during the course of the disease, a higher figure than that reported in Caucasian populations [10, 11].

The use of CYC for proliferative LN has resulted in the reduction of mortality from over 70% in the 1950s and 1960s to approximately 10% in recent years [12]. However, a serious problem is the potential significant toxicity of this regimen [4, 13, 14]. Amenorrhea is relatively

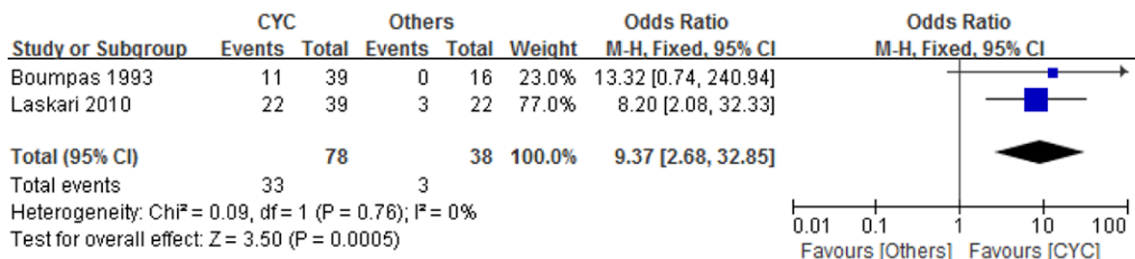
## Risk assessment of infertility



**Figure 5.** Forest plot of comparing risk of infertility in all included studies. OR, odds ratio; CI, confidence interval.



**Figure 6.** Forest plot of comparing risk of infertility in randomized controlled studies. OR, odds ratio; CI, confidence interval.



**Figure 7.** Forest plot of comparing risk of infertility in retrospective studies. OR, odds ratio; CI, confidence interval.

common and is associated with infertility when it involves women of childbearing age [1, 3, 15-18]. Amenorrhea is related to both age and cumulative CYC dose [19]. It suggests that sustained amenorrhea is very likely to develop in women aged  $\geq 32$  years old, even with very short CYC courses [15]. Patients at high risk are those who exceed the  $12 \text{ g/m}^2$  total CYC dose [15].

In the last decade, controlled studies have assessed the efficacy and safety of newer immunosuppressive regimens compared to

cyclophosphamide regimens [20-22]. Recent studies have also demonstrated the equivalent efficacy of MMF in inducing remission in LN. The less severe side effect profile of MMF has made it an attractive alternative therapeutic option, but long-term follow-up studies are needed [12]. The 2012 American College of Rheumatology Clinical Practice Guidelines for Lupus Nephritis established MMF as the preferred induction agent compared to CYC for patients in whom fertility preservation is a major consideration [23]. While the role of CYC in LN therapy may be evolving, at the present



time it continues to be an important drug in induction therapy for severe renal disease.

Most young girls with renal disease will not be treated with CYC doses that will place them at significant risk for acute ovarian failure (i.e.  $>10\text{--}15\text{ g/m}^2$ ) [3]. They may, however, be more likely to experience premature ovarian failure. Because acute ovarian failure is unlikely, fertility preservation methods at the onset of renal disease may not be necessary but should be discussed on a case-by-case basis so that patients and families can be informed of their risks and be able to consider their options.

The overall pooled estimates suggest that there is an increased risk of infertility associated with CYC exposure. This increased risk with CYC exposure is present irrespective of patient population. Since there is no heterogeneity surrounding the pooled estimate of overall infertility risk, the direction of effect shows a consistently elevated risk in all of the included datasets.

Moreover, the quality of individual studies varied. Also, a major limitation was the possibility of uncontrolled confounders, and the individual studies did not adjust for potential risk factors in a consistent way. The lack of adjustment for these confounding factors might have resulted in a slight overestimation of the OR. For instance, some diseases were potentially associated with elevated infertility risk, such as some gynecological diseases. This meta-analysis was subject to confounding factors within the included studies, which was an inherent limitation of all observational studies and meta-analyses. As not all of the included studies were adjusted for age, BMI, height, weight, smoking, alcohol intake, and other diseases sustained, confounders known or unknown may potentially have influenced the observed findings.

The asymmetrical funnel plot on visual inspection is not very accurate on account of just a few limited studies included. However, it can still indicate that there is a high risk of publication bias in all included studies in relation to infertility risk, as suggested by the asymmetrical distribution. However, this publication bias associated with infertility risk due to CYC use cannot be eliminated when the forest plot was generated in the analysis. Therefore, in the

pooled analysis, forest plot was created with the premise of existing publication bias. We must take this into account when the interpretation of the results is conducted.

With regard to the study design included in this meta-analysis, well-designed randomized controlled trials (RCTs) might minimize the selection bias in comparison with observational data. However, after a thorough search, only two RCTs were identified discussing infertility risk due to CYC use. Thus, more well-designed RCTs are needed to explore the side effects of CYC use on infertility risk in patients with SLE or LN.

There are still several potential limitations in our work. First, we only included the study in English, and some relevant studies reported in other languages might be not included in the review, due to a language limitation. Second, only four studies including six comparisons were included in this meta-analysis, thus reducing the power of the findings. Last but not the least, the sample size of patients included in the pooled analysis was not so big enough with only 239 cases, which cannot be neglected in interpretation of the results in this meta-analysis.

### Conclusions

In conclusion, the findings of this meta-analysis suggest that there is an increased risk of infertility arising from CYC therapy in premenopausal women patients with SLE or LN. Clinicians should carefully evaluate such risk before routinely prescribing CYC, and make a careful judgement as to whether CYC use may result in serious infertility events for young women patients with SLE or LN.

### Disclosure of conflict of interest

None.

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## Risk assessment of infertility

**Table S1.** Characteristics of included studies

CYC		MMF		AZA		CYC+MMF		MET		CYC+MET	
Events	Total	Events	Total	Events	Total	Events	Total	Events	Total	Events	Total
6	18	1	19	1	18						
11	21							2	22	9	25
22	39					3	22				
11	39							0	16		

Note: CYC, cyclophosphamide; MMF, Mycophenolate Mofetil; AZA, Azathioprine; MET, Methylprednisolone.