PROVINCIAL STANDARDS & GUIDELINES

BCRenal
Provincial Health Services Authority

Gonadal Toxicity with Cyclophosphamide

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Approved by the BC Renal GN Committee

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IMPORTANT INFORMATION

This BC Renal guideline/resource was developed to support equitable, best practice care for patients with chronic kidney disease living in BC. The guideline/resource promotes standardized practices and is intended to assist renal programs in providing care that is reflected in quality patient outcome measurements. Based on the best information available at the time of publication, this guideline/resource relies on evidence and avoids opinion-based statements where possible; refer to www.bcrenalagency.ca for the most recent version.

For information about the use and referencing of BC Renal guidelines/resources, refer to http://bit.ly/28SFr4n.

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1. BACKGROUND

Infertility is a major concern for patients and providers when considering cyclophosphamide (CYC) therapy. Although CYC can be life/organ saving, it is toxic to both the ovaries and testes amongst other side effects.

According to the published literature, the incidence of gonadal dysfunction is believed to be dependent upon the patient's:

1. Sex
2. Age
3. Cumulative CYC dose

Severity of disease, duration of therapy and route of administration has also been associated with gonadal toxicity, although data is either minimal or conflicting.

We would like to remind the reader that patients of any age may have baseline deficiencies in semen quality or have subclinical ovarian damage/ diminished ovarian reserve, and that fertility in females declines with certainty in the last 2 decades prior to menopause (according to the American Society of Reproductive Medicine, most women in their mid-40's are unable to have successful pregnancies)[1]; in addition, amenorrhea or azoospermia may result from stress to the body, such as during acute illness. For example, 54% of female patients (age 18 to 39) with systemic lupus erythematosus (SLE) in one study experienced amenorrhea without receiving any CYC.[2] Thus, our literature review focused on data in patients with GN from controlled studies, although much of the available data is from non-controlled, retrospective, observational studies - we clearly indicate the study design from where the data is derived (see Appendix A for methodology).

2. PHARMACOLOGY OF CYC

CYC is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis.[3] By suppressing the rapidly dividing cells of the immune system, it may place the patient's glomerulonephritis (e.g. Lupus nephritis, ANCA-associated vasculitis, or membranous nephropathy) into remission.

3. PATHOPHYSIOLOGY OF FEMALE GONADAL TOXICITY WITH CYC

Given CYC's ability to prevent cell division, growing ovarian follicles are more vulnerable to its cytotoxic effects than primordial follicles. In animal models, it has been demonstrated that after a dose of CYC, serum estradiol levels and the number of granulosa cells expressed will decrease (i.e. follicles have been destroyed); this leads to up regulation of follicle-stimulating hormone secretion, which accelerates further follicle recruitment that perpetuates a vicious cycle leading to an accelerated depletion of ovarian follicles. The end result is amenorrhea and therefore infertility.[4] Depending on the study, the cumulative incidence of ovarian failure when used to treat lupus nephritis (LN) varies between 12 and 83%.[5]

If menses continues during CYC therapy, or it resumes after CYC therapy is finished, the patient should be considered fertile,[5–7] although one study from Saudi Arabia (N=535) found that patients with SLE previously exposed to CYC had a higher risk of pre-term deliveries compared to those who didn't (41 vs. 21%).[8] Another retrospective observational study from France (N=84) found premature deliveries

to occur in 62% of LN patients who had previously been treated with IV CYC,[5] while Park et al. found no increase in premature deliveries or low birth weights in patients with LN and ANCA-associated vasculitis (AAV) at a single center in Korea (N=67).[9] For patients of childbearing age, the use of contraception is recommended during therapy to prevent pregnancy while exposed to this teratogen; there is insufficient evidence/controversy over whether oral contraceptives or the use of Gonadotropin-releasing hormone will reduce the risk of CYC-related amenorrhea.

Lastly, longitudinal studies are required to determine if patients who maintain menses are at an increased risk of premature menopause.

4. RISK FACTORS FOR FEMALE GONADAL TOXICITY WITH CYC

Women treated after the age of 30 are at a significantly higher risk of infertility compared to younger patients; in addition, the total cumulative dose is an independent risk factor for ovarian toxicity regardless of how the medication is administered.[4–6,10,11] Duration of therapy and baseline severity of disease may also influence risk of amenorrhea, although this data is not as robust.

Age at onset of CYC therapy:

The highest quality data demonstrating age as a risk factor comes from a retrospective controlled clinical study of women treated for LN (N=39) with IV CYC (0.5 to 1 g/m^2 monthly x 7 to 15 doses). Sustained amenorrhea occurred in 12% of women < 26 years, in 27% of those aged 26 to 30, and in 62% of those > 30 compared to no observed amenorrhea in patients who only received of IV methylprednisone (1 g/m^2 x 9 doses).[4]

There are also 3, uncontrolled, retrospective single center chart reviews addressing the issue of age and gonadal failure in patients who received CYC. In the first review, LN patients treated with IV CYC (0.5 to 0.75 mg/m^2 monthly x 6 months then q3months x 18 months) developed sustained amenorrhea in 0 women < 20 years, 6% of women 21 to 31 years, 23% of women 31 to 40 years and in 75% of women > 40 years.[4] In the second study, patients with LN and AAV who received IV CYC (0.9 ± 0.14 g per pulse x 13 ± 6.5 doses) developed sustained amenorrhea in 0 patients < 25, in 45% of patients > 31 and in 83.3% of patients > 40.[5] In the final study of LN patients (N=92) treated with oral CYC (1 to 2 mg/kg/day with duration unspecified), the risk of sustained amenorrhea was 4% in women < 21 years, 29% in women 21 to 30 years and 54% in women > 30 years.[6] The mean age of patients who developed amenorrhea was 30.8 ± 7.4 vs. 24.3 ± 6 ($p<0.01$) in women who did not develop amenorrhea.[6]

In summary, younger women have greater ovarian reserve and are therefore less likely to experience ovarian failure with CYC exposure. According to the limited data available, it appears women < 20 are unlikely to experience ovarian failure with an initial course of CYC (0 to 4%), whereas the risk is significant in women > 30 (23 to 54%) and > 40 (75%).

Cumulative exposure to CYC:

Given the relationship between age and ovarian reserve, it is unsurprising that the cumulative exposure associated with onset of amenorrhea depends on age. The most widely cited study on the effects of cumulative exposure on gonadal toxicity is in breast cancer patients (N=18) who took CYC 100 mg/day orally. The average dose of CYC given before

the onset of amenorrhea was 20.4 g in women aged 20 to 30, 9.3 g in women aged 30 to 40, and 5.2 g in women who were in their 40s.[12]

The data from GN patients is more difficult to interpret. In a retrospective chart review of patients with LN (N=157) with a median age of 32, the cumulative dose of CYC that resulted in transient amenorrhea (defined as absence of > 3 menstrual cycles < 1 year) was 13 to 17 g, whereas patients with an exposure of 7.5 to 13 g were unaffected.[13] In another study of patients with SLE (N=92) with a mean age of 30, a cumulative dose > 10 g was the only risk factor for premature ovarian failure (defined as sustained amenorrhea > 1 year, with a low estradiol level and a high FSH level).[14] Wang et al., in a single center retrospective chart review found that patients with a mean age of 26 (N=92) could tolerate much higher doses of CYC - the mean cumulative dose causing amenorrhea was 32.6 ± 21.8 vs. 22.4 ± 16.7, (p=0.018) in patients who did not experience amenorrhea.[6] In this study, cumulative doses < 36 g did not result in amenorrhea in patients aged 14 to 20, but for patients with > 36 g of total exposure the relative risk of sustained amenorrhea was 2.1 (1.46 to 4.02), regardless of age.[6] The final study, which reported total incidence of ovarian failure in all age groups (mean age 31.1 ± 8.4) found the risk to be 0 with < 5 g of exposure, 8% with 5 to 10 g, 25% with 10 to 15 g and 66% with > 15 g of cumulative exposure.

Duration of CYC therapy:

Based on the pathophysiology of ovarian toxicity, a longer duration of therapy should correlate with ovarian failure. However, published data on this risk factor is conflicting;[6,13] therefore, we recommend the calculation of cumulative dose when assessing risk of ovarian failure, as this accounts for both length of treatment and dosage administered.

Severity of disease:

There is only one study identified in our literature review that assesses baseline severity of disease as a risk factor for amenorrhea. In this retrospective review (N=67), SLE disease activity was rated using the SLE-DAI and damage was rated using the Systemic Lupus International Collaborating Clinics / American College of Rheumatology damage indices. Using logistic regression analysis, the investigators found the damage index at the initiation of IV CYC was independently associated with cessation of menstruation for > 4 months (β = 0.6, SE=0.28, P=0.029) and cessation of menstruation for > 12 months (β = 1.1, SE=0.55, P=0.02).[9] It is worth noting that high disease activity has been associated with menstrual irregularities independent of CYC exposure.[2,15]

5. PATHOPHYSIOLOGY OF MALE GONADAL TOXICITY WITH CYC

Often, more attention is paid to the infertility risk of CYC exposure in female patients, but men are expected to have a higher severity and incidence of gonadal damage due to greater mitotic activity of the testis.[16]

In the testis, the cells within the seminiferous tubules of the germinal epithelium have the highest mitotic and meiotic indices, and are thus most vulnerable to the toxic effects of CYC. While sperm counts begin to decline within a few weeks of CYC, it typically takes 2 to 3 months for azoospermia to occur, in keeping with the known kinetics of spermatogenesis. Because antineoplastic agents act on the sperm cells during cell division, they are most toxic to the

rapidly proliferating type B spermatogonia, which can be reproduced from the germinal stem cell layer. However, the severity and duration of gonadal damage induced by cytotoxic agents correlates best with the number of stem cells (type A spermatogonia) that are destroyed. If the stem cells within the tubules remain intact, spermatogenesis may begin to show recovery approximately 12 weeks after treatment.[17]

Less commonly, cytotoxic agents such as CYC can damage Leydig cells, the site of testosterone production within the testis. However, Leydig cell dysfunction in this setting is typically subclinical, characterized by testosterone levels that are at the lower end of the normal range in association with elevated luteinizing hormone levels. The clinical significance of this state of "compensated hypogonadism" is still unclear.[17]

6. RISK FACTORS FOR MALE GONADAL TOXICITY WITH CYC

As with females, the severity and risk of gonadal toxicity from CYC exposure is dependent on gonadal activity at the time of treatment (prepubertal vs. sexually mature males), and total cumulative dose. Autoimmune diseases such as SLE also influences fertility independent of CYC exposure. In one study comparing patients with SLE to healthy age-matched controls (N=70), the patients with SLE had a lower sperm volume (2.3 ± 1 vs. 3.2 ± 1.7 mL, p=0.015), a lower median sperm count (70 x 10^6/mL vs. 172 x 10^6/mL; p=0.02), a lower median motile sperm count (32 x 10^6/mL vs. 119 x 10^6/mL; p=0.004), a lower percentage of normally formed sperm (11.2 ± 8.3 vs. 17.4 ± 11.3%; p=0.011) and were less likely to bear children after disease onset (20 vs. 80%; p=0.0001);[18] outcomes were even worse if patients received CYC. Even though the severity and incidence of gonadal toxicity is greater in males, this population is not as well studied, and data is derived from a small number of studies, mostly from oncology indications.

Prepubertal males and cumulative CYC dose:

It may come as no surprise that prepubertal children with inactive gametogenesis have a lower incidence of damage compared to similarly treated sexually mature adults. In one meta-analysis of 30 studies (N=150) of patients with renal disease, Hodgkin's disease and leukemia, the incidence of gonadal failure defined by a combination of semen analysis, LH/LHrH levels, FSH levels was zero up to an exposure of 400 mg/kg (e.g. 30 g in a 75 kg male), after this point, the incidence of gonadal failure increased to 30%. This was sustained toxicity, given time from end of therapy to evaluation was 4.2 ± 2.7 years.[16]

Sexually mature males and cumulative CYC dose:

In the same meta-analysis that examined prepubertal males, the incidence of gonadal failure in sexually mature males was found to be 20% with exposure as low as 100 to 200 mg/kg (e.g. 7.5 to 15 g in a 75 kg male), 50% with 200 to 300 mg/kg of exposure (e.g. 16 to 20 g in a 75 kg male), 80% with 300 to 400 mg/kg of exposure (e.g. 21 to 30 g in a 75 kg male) and almost 100% with over 400 mg/kg of exposure (e.g. > 30 g in a 75 kg male). This was sustained toxicity, given time from end of therapy to evaluation was 3.8 ± 2.6 years.[16]

However, there are numerous studies suggesting a lower cumulative exposure to CYC may lead to significant, sustained gonadal toxicity. In 1972, Qureshi et al. published a case series of patients with GN (N=14); they observed that a total CYC dosage of > 11 g led to azoospermia, whereas smaller doses

led to a severe reduction in sperm count.[19] Next a prospective case control study of patients with Behcet's disease (mean age 31 years) treated with PO CYC and/or colchicine (N=31) found that 91% of patients who received > 10 g of CYC developed azoospermia or severe oligospermia that did not recover during the duration of follow up (up to 37 months).[20] Lastly, a prospective cohort study of patients (N=58) who received IV CYC as part of their chemotherapy regimen +/- radiation for Ewing and soft tissue sarcomas, found that even after 5 years of stopping CYC, only 10% of patients recovered normal sperm levels when a total dose of 7.5 g/m^2 was exceeded (e.g. 13 g for a 1.73 m^2 male), whereas, 70% of patients who received doses < 7.5 g/m^2 recovered normal sperm levels.[21]

7. SUMMARY

In summary, the cumulative exposure to CYC that results in ovarian failure varies by age. To avoid amenorrhea, female patients < 20 years old should likely receive < 15 to 20 g of total exposure, patients aged 20 to 30 should receive < 15 g of total exposure and those > 30 should likely receive < 10 g of total exposure. We did not make a recommendation for maximum exposure to prevent infertility in patients > 40 as this group already has a low baseline fertility rate, worsened by disease activity. In males, the maximum CYC exposure for patients who still wish to conceive is lower at approximately 10 g.

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APPENDIX A: Flow Diagram of Search Results

Search Strategy:

Medline 1946 to Present

Search executed on Aug 9, 2016 (LN ovarian failure), September 29, 2016 (AAV ovarian failure), October 24, 2016 (AAV and LN azoospermia)

Search Terms:

Cyclophosphamide AND ovarian failure AND lupus

- These are MESH headings, automatically mapped and exploded by Medline

Cyclophosphamide AND azoospermia AND lupus

- These are MESH headings, automatically mapped and exploded by Medline

Cyclophosphamide AND ovarian failure AND vasculitis

- These are MESH headings, automatically mapped and exploded by Medline

Cyclophosphamide AND azoospermia AND vasculitis

- These are MESH headings, automatically mapped and exploded by Medline

Limits:

- Human
- English Language

Results:

LN ovarian failure

- Number of articles identified through database search: n=46
- Number of articles identified through review of reference: n=15
- Full-text papers selected for appraisal and analysis: n=19

LN azoospermia

- Number of articles identified through database search: n=5
- Number of articles identified through review of reference: n=0
- Full-text papers selected for appraisal and analysis: n=2

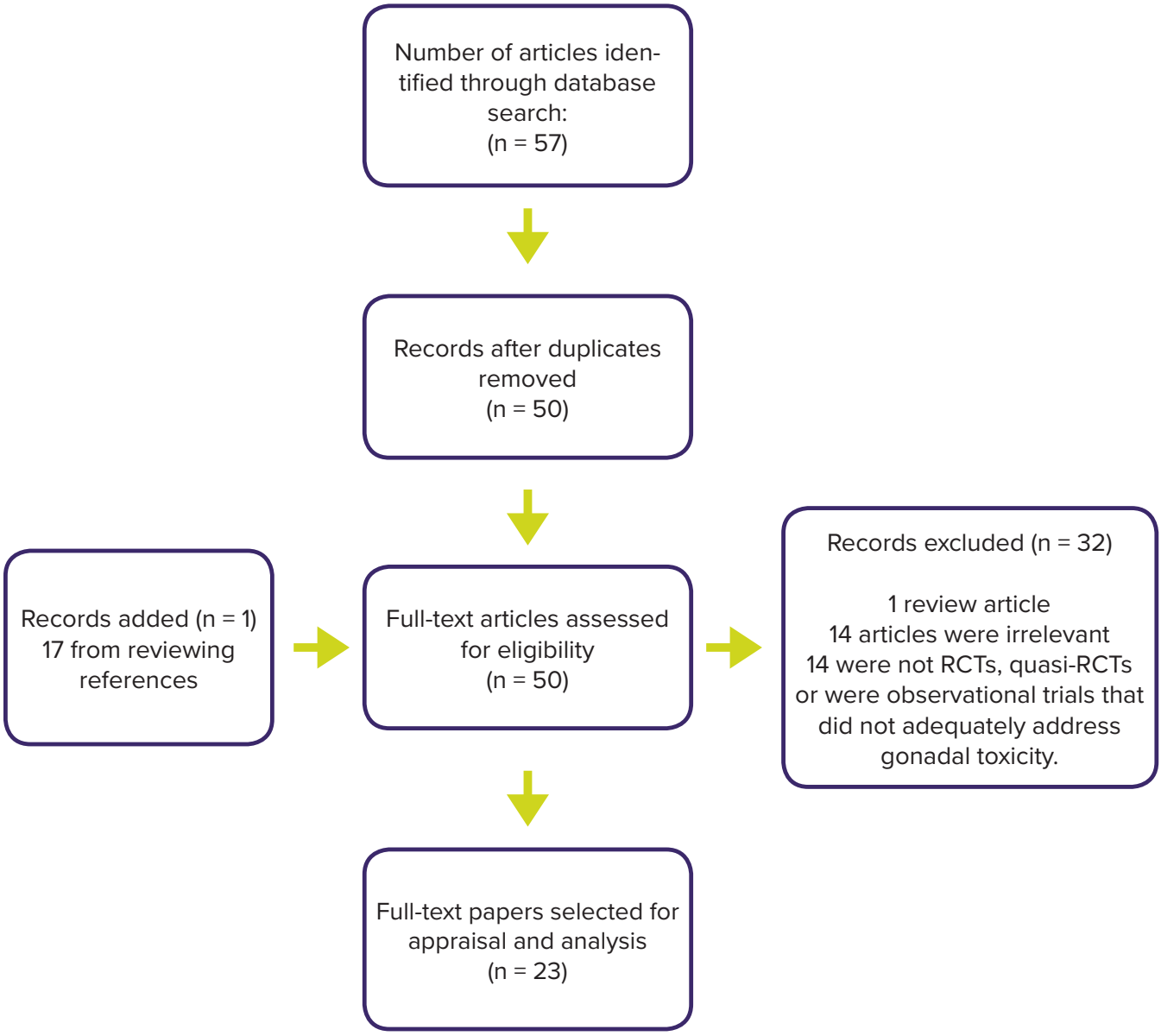
AAV ovarian failure

- Number of articles identified through database search: n=5
- Number of articles identified through review of reference: n=2
- Full-text papers selected for appraisal and analysis: n=2

AAV azoospermia

- Number of articles identified through database search: n=1
- Number of articles identified through review of reference: n=0
- Full-text papers selected for appraisal and analysis: n=0

Figure 1. Flow Diagram of Search Results



APPENDIX B: Summary of Included Studies

Table 1: Risk of premature ovarian failure (POF) with cyclophosphamide in **lupus patients** according to **meta-analysis data from randomized controlled trials**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Henderson et al. Treatment for lupus nephritis *Cochrane Database of Systematic Reviews 2012, Issue 12. Art. No.: CD002922.*	Systematic review and meta-analysis of literature published until April 15, 2012* RCTs and quasi-RCTs only	N=2846 Biopsy-proven lupus nephritis in both adult and pediatric patients with class III, IV, V +III and V +IV lupus nephritis	All immunosuppressive treatments	**POF (2 studies, n=498)** IV CYC vs. MMF RR 0.15, (95% CI 0.03 to 0.8)	Based on high quality data (GRADE), the risk of POF in a patient with LN is reduced by 85% if MMF is used instead of IV CYC	

** There are no relevant RCTs published after 2012*

Table 2: Risk of premature ovarian failure (POF) with cyclophosphamide in **lupus patients** according to **randomized controlled data**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Appel et al. Mycophenolate Mofetil versus Cyclophosphamide for Induction Treatment of Lupus Nephritis *Journal of the American Society of Nephrology 2009, 20, 1103-1112*	Multinational randomized controlled trial Duration: 24 weeks	N=370		Impact on women of childbearing age as a result of the potential of IV CYC to cause ovarian dysfunction was not objectively addressed in this study because of the length of follow-up and limited health economic assessments undertaken.		
Bao et al. Successful Treatment of Class V+IV Lupus Nephritis with Multitarget Therapy *Journal of the American Society of Nephrology 2008, 19, 2001-2010*	Randomized controlled trial Duration: 6 mo to 9 mo	N=40	20 pts (16 female) – randomized to multitarget therapy (mycophenolate mofetil, tacrolimus, steroids). 20 pts (18 female) – randomized to CYC therapy (monthly 0.75 g/m^2 of BSA for 1st month, then adjusted between 0.5 and 1.0 g/m^2 BSA monthly on the basis of nadir white cell count of ≤2.5 x 10^9/L 7 to 10 d after the infusion).	**Irregular menstruation:** Multitarget group – 1 (5%) CYC group – 4 (20%)	Numerically lower incidence of irregular menstruation in the multitarget therapy group.	

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Boumpas et al. Risk for sustained amenorrhea in patients with SLE receiving intermittent pulse CYC therapy. *Ann Intern Med 1993, Sep;119(11):366-9*	Controlled, retrospective clinical study. Amenorrhea = no menses for at least 4 months Sustained amenorrhea = no menses for at least 12 months	N=39 < 40 yrs old Active LN or neuropsychiatric lupus	(N=16) pulse CYC (0.5 - 1 g/m^2 BSA monthly for a total of 7 doses (short-CYC), and 23 patients received ≥ 15 doses (long-CYC). Control patients were treated with monthly pulses of methylpred (1 g/m^2) for a total of 9 doses	12% of short-CYC developed sustained amenorrhea 39% in the long-CYC developed sustained amenorrhea < 25 yrs: 12% 26 to 30 yrs: 27% > 30 yrs: 62% Amenorrhea was not observed in patients who received methylpred	Intermittent pulse CYC in patients with SLE is associated with amenorrhea, which is related to both age and number of doses	
Yee et al. EULAR randomized controlled trial of pulse cyclophosphamide and methylprednisolone followed by azathioprine and prednisolone in lupus nephritis *Ann Rheum Dis 2003, 63, 525-529*	Multicentre randomized controlled trial	N=32 Biopsy-proven proliferative lupus nephritis	16 pts – randomized to intermittent pulse CYC; IV 10 mg/kg three times weekly for four doses, then orally same dose split over two days at four weekly intervals for nine months, then at six weekly intervals for 12 months. 16 pts – randomized to continuous CYC plus AZA (3 pts excluded – recruited mistakenly); orally 2 mg/kg/day for three months, after three months changed to oral azathioprine at 1.5 mg/kg/day	**Permanent amenorrhea:** Continuous CYC therapy (16 pts) – 1 pt 40 y.o. (6.3%) Pulse CYC therapy (13 pts) – 1 pt 40 y.o. (7.7%)	None other than a trend observed toward less severe adverse effects in the pulse regimen compared with continuous regimen	
Tak Mao Chan et al. Efficacy of Mycophenolate Mofetil in Patients with Diffuse Proliferative Lupus Nephritis *The New England Journal of Medicine 2000, 343, 1156-62*	Randomized controlled trial	N=42 Biopsy-proven proliferative lupus nephritis	21 pts (group 1) – randomized to oral MMF (1 g twice/day) + prednisolone (0.8 mg/kg/day) 21 pts (group 2) – randomized to oral CYC (2.5 mg/kg/day) + prednisolone (0.8 mg/kg/day)	**Permanent amenorrhea:** Group 1 – 0 Group 2 – 3 (23%; 95% CI)	Amenorrhea occurred only in the group treated with CYC	
Ginzler et al. Mycophenolate Mofetil or Intravenous Cyclophosphamide for Lupus Nephritis T*he New England Journal of Medicine 2005, 353, 2219-28*	Multicentre randomized controlled trial	N=140	71 pts (group 1) – randomized to oral MMF (initial dose, 1000 mg/day, increased to 3000 mg/day) 69 pts (group 2) – randomized to monthly IV CYC (0.5 g/m^2 BSA, increased to 1.0 g/m^2)	**Permanent amenorrhea:** Group 1 – 0 Group 2 – 2	Amenorrhea occurred only in the group treated with CYC	

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Gourley et al. Methylprednisolone and cyclophosphamide, alone or in combination in patients with *Lupus Nephritis Annals of Internal Medicine 1996, 125(7) 549-557*	Single center RCT Follow-up: 5 yrs	N=82 Mean age = 30	Methylprednisone 1 g/m^2 BSA IV monthly x 1 year (N=20) Vs. CYC 0.5 to 1 g/m^2 BSA IV monthly x 6 months and then quarterly (N=21) Vs. Methylprednisone + CYC with above doses (N=24)	**Sustained amenorrhea (definition not given):** Methylpred = 2 (7.4%) CYC = 11 (41%) CYC + Methylpred = 12 (43%)	Monthly bolus therapy with methylpred was less effective than monthly bonus therapy with CYC. A trend towards greater efficacy with combination was seen. CYC causes amenorrhea.	Amenorrhea was a secondary endpoint.
Sabry et al. A comparative study of two intensified pulse cyclophosphamide remission-inducing regimens for diffuse proliferative lupus nephritis: an Egyptian experience *Int Urol Nephrol 2009, 41, 153-161*	Randomized controlled trial Duration: 1 year	N=46 Biopsy-proven	20 pts (2 male, 18 female) – randomized to 6 fortnightly fixed low-dose CYC (500 mg/dose). 26 pts (5 male, 21 female) – randomized to 6 monthly fixed high-dose CYC + 2 quarterly doses (max 1 g/dose).	**Menstrual abnormalities:** None recorded	Study referred to other studies, citing ovarian failure as a function of cumulative dose of CYC	
Mitwalli et al. Comparison of High and Low Dose of Cyclophosphamide in Lupus Nephritis Patients: A Long-term Randomized Controlled Trial *Saudi Journal of Kidney Diseases and Transplantation 2011, 22(5), 935-940*	Randomized controlled trial Duration: 10 Yrs	N=117 Biopsy-proven de novo lupus nephritis	73 pts (group 1, 61 females) – randomized to high-dose CYC (10 mg/kg/ month for 6 months then bi-monthly for 36 months). 44 pts (group 2, 39 females) – randomized to low-dose CYC (5 mg/kg/ month for 6 months then bi-monthly for 36 months).	**Sustained amenorrhea w/o pregnancy:** Group 1 – 34.6% (abortion – 1.4%) Group 2 – 13.6% (abortion – 0%)	Significantly higher risk of amenorrhea under high-dose CYC regimen	

Table 3: Risk of premature ovarian failure (POF) with cyclophosphamide in **lupus patients** according to **observational data**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Alarfaj et al. Fertility, ovarian failure, and pregnancy outcome in SLE patients treated with intravenous cyclophosphamide in Saudi Arabia *Clinical Rheumatology (2014) 33, 1731-1736*	Retrospective study 26 years SLE was diagnosed according to 1982 revised criteria of ACR. Demographic data, treatment modalities, data pertaining to amenorrhea, pregnancy, and its outcome were recorded from medical charts.	N = 535 N = 188 received IV CYC N = 99 type of amenorrhea assessed in IV CYC receiving married women	The high-dose regimen consisted of IV CYC given as 10 mg/kg doses, monthly for 6 months, thereafter bimonthly for another 12 months (12 doses in 18 months), and the low-dose regimen consisted of doses given as 5 mg\kg doses, monthly for 6 months, thereafter bimonthly for another 36 months (24 doses in 42 months)	**Amenorrhea (transient or sustained):** 53 of 188 (28.2%) SLE patients treated with CYC developed amenorrhea **POF:** Of the 13 women with sustained amenorrhea, 6 (6.1 %) experienced POF before the age of 40 years	28.2% SLE patients treated with CYC developed amenorrhea (transient or sustained) 6.1 % experienced POF before the age of 40 years	
Appenzeller et al. Ovarian failure in SLE patients using pulse CYC: comparison of different regimes *Rheum Int (2008) 28:567-571*	Retrospective chart review	N = 157 Had to be < 40 years old	Group A: CYC 0.75 mg/m2 BSA (N=57) Group B: CYC 0.5 mg/m2 BSA (N=50) Group C: No CYC (N=50)	Group A: 7 (12.3%; p=0.001) had transient amenorrhea and 10 (17.5%, 0.017) had sustained amenorrhea • 5/6 patients who used drug > 10 months had transient amenorrhea • cumulative dose for transient amenorrhea = 16.8 g (14-20; SD=2.8) vs. 13.4 g (11-15; SD=1.8) in unaffected patients, p=0.013) Group B: 10 (20%; p=0.017) had transient amenorrhea and 0 had sustained amenorrhea • 7/10 patients who used drug > 10 months had transient amenorrhea • cumulative dose for transient amenorrhea = 13.2 g (11-15; SD=1.2) vs. 7.5 g (5-9; SD=2.1) in unaffected patients, p=0.01) Median age of amenorrhea onset = 32 years	The most important risk factors were duration of treatment and cumulative dose.	
Akawatcharangura et al. Prevalence of premature ovarian failure in systemic lupus erythematosus patients treated with immunosuppressive agents in Thailand *Lupus (2016) 25, 436–444*	Observational study 14 months (Jan 2010 to March 2011) POF was diagnosed in those who had sustained amenorrhea with the level of estradiol 30pg/mL and follicular stimulating hormone (FSH) 40IU/L	N = 92 Female SLE patients in Bangkok, Thailand Baseline Characteristics (mean +/- SD): Age at enrolment: 30.4 +/- 6.9 (18–40) Age at diagnosis: 21.8 +/- 6.8 (9–38) Disease duration (months) 103.2 +/- 67.5 (6.9–314.3)	All patients had received at least one of the following immunosuppressive agents: CYC, AZA, MMF, chlorambucil or cyclosporine for more than 6 months.	**POF:** 11 of 92 (12%) SLE patients developed POF. A cumulative dose of CYC of more than 10 g was the only risk factor for POF (hazard ratio 17.0, 95%CI 1.96–147.72, p 1⁄4 0.01).	12% of SLE patients developed POF. A cumulative dose of CYC of more than 10 g was the only risk factor for POF	

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Ghaleb et al. Anti-Müllerian hormone: A marker for ovarian function in systemic lupus erythematosus patients treated with cyclophosphamide *Joint Bone Spine (2013) 80, 434-435*	Observational study 18 months (January 2010 to October 2011) POF was defined as secondary amenorrhea for more than 3 months duration documented with serum level AMH less than 1.26 ng/m	N = 96 Female SLE patients in Egypt Mean Baseline Characteristics (POF vs. menstruating patients): Age at enrolment 32.5 ± 3.9 vs. 28.3 ± 5.6 Age at onset: 26.2 ± 3.6 vs. 23.5 ± 4.6 Disease duration (months) 6.2 ± 2 vs. 4.8 ± 2.7	This was a study evaluating AMH as a predictor of POF. The cohort that did not receive CYC was given corticosteroids or azathioprine.	**POF:** 12 of 52 (23.1%) of SLE patients treated with IV CYC developed POF whereas none of the patients who did not receive CYC developed POF	CYC treated SLE patients having a frequency of POF of 23.1%. So, for older SLE patients in whom the use of CYC is warranted, a shorter course and lower dosage could be considered. Assessment for AMH assay and AFC can be done before initiating CYC therapy as a better step towards counselling women at risk for ovarian failure.	
Mayorga et al. Prevalence of premature ovarian failure in patients with systemic lupus erythematosus *Lupus (2016) 25, 675-683*	Cross-sectional study POF prevalence was estimated in the study sample and in a subgroup of patients aged <40 years at interview. Associations between POF and selected variables were assessed by logistic regression analyses. POF was defined as amenorrhoea of at least 12 months duration and elevated concentrations of FSH in women <40 years old	N = 961 Consecutive patients with SLE diagnosed according to the ACR criteria and younger than 60 years N = 652 patients <40 years N = 287 patients exposed to CYC Age at first exposure, mean (SD): 29.2 (6.9)	Cumulative CYC dose, mean (SD), g: 33.2 (49.7)	**POF:** 48 of 287 (16.7%) all SLE patients treated with CYC had POF 10.1% SLE patients <40 years treated with CYC had POF	16.7% all SLE patients treated with CYC had POF 10.1% SLE patients <40 years treated with CYC had POF	

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Park et al. Risk of ovarian failure and pregnancy outcome in patients with lupus nephritis treated with intravenous CYC pulse therapy. *Lupus (2004) 13, 569-574*	Single center retrospective chart review Amenorrhea = no menses x 4 months Sustained amenorrhea = no menses x 12 months	N=67 SLE class III or IV Mean age at CYC initiation = 31.1 ± 8.4 Mean dose of CYC administered = 988.1 ± 268.8 mg per pulse and 8.8 ± 2.4 times	IV CYC 0.5-0.75 mg/m^2 x 6 months then q3months x 18 months	Amenorrhea occurred in 25 women (37.3%) Incidence of ovarian failure by age group: < 20 yrs: 0% 21-31 yrs: 6% 31-40 yrs: 23% > 40 yrs: 75% Incidence of ovarian failure by cumulative dose: < 5 g: 0 5-10 g: 8% 10-15 g: 25% >15 g: 66% 15 women had 19 pregnancies in the 45.8 months following. 2 pregnancies were terminated because baby was not wanted. The rest were successful and without preterm or LBW.	Age, disease severity and cumulative dose are related to ovarian failure. Successful pregnancies can result after exposure.	
Takada et al. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis *Arthritis & Rheumatology (2004) 50, 2202-2210*	Observational They examined the association between genotypes (CYP2B6, 2C19, 2C9, and 3A5) and the following clinical end points: development of POF, end-stage renal disease (ESRD), doubling of serum creatinine level, and achievement of complete renal response. POF was defined as sustained amenorrhea occurring before 45 yrs of age.	N = 62 LN III or IV treated with CYC **Mean Baseline Characteristics (group 1 vs. 2):** Age in 24 to 51 year category: 4 vs. 40 patients Disease duration (> 6 mo): 6 vs. 47 patients	Patients were given 1 of 2 regimens 1) IV CYC targeting 1 gm/m^2 of BSA, given monthly for the first 6 months then quarterly for a total duration of at least 12 months 2) a combination of regimen 1 and IV methylprednisolone, 1 gm/m^2 of BSA, administered as a monthly bolus for 6 or 12 months	N = 48 (14 were excluded because they were male or had explained menopause prior to the study) 28 (58%) developed POF after a median of 12 cycles of pulse CYC therapy. Of all the CYP enzymes studied, only the genotype of *CYP2C19* was associated with the development of POF	Determination of selected cytochrome P450 enzyme genotypes may be valuable for predicting the risk of premature ovarian failure in lupus nephritis patients treated with CYC. The association of these genotypes with renal response needs further validation	

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Wang et al. Ovarian failure in oral CYC treatment for SLE *Lupus (1995) 4,11-14*	Single center retrospective chart review LN patients resistant to oral prednisone x 12 weeks Permanent amenorrhea defined as > 12 months of no menses	N=92 Mean age at initiation of CYC = 26.3 (+/-7) years	Oral CYC 1-2 mg/kg/day. Length not provided	18 (19.6%) had oligomenorrhea and 33 (36%) had amenorrhea during therapy 11 (12%) had oligomenorrhea and 25 (27.2%) had sustained amenorrhea after therapy Mean age of amenorrhea vs. no amenorrhea 30.8 ± 7.4 vs. 24.3 ± 6 ($p<0.01$) < 21 yrs: 4% 21-30 yrs: 29% > 30 yrs: 54% Mean duration of treatment, amenorrhea vs. no amenorrhea 13.2 ± 7.4 vs. 9.33 ± 6.34 ($p<0.018$) Mean cumulative dose, amenorrhea vs. no amenorrhea 32.6 ± 21.8 vs. 22.4 ± 16.7 g ($p=0.018$)	Cumulative dose was associated with amenorrhea whereas duration was NOT after adjusting for age.	

Table 4: Risk of premature ovarian failure (POF) with cyclophosphamide in **AAV patients** according to **RCT data**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Clowse et al. Ovarian reserve diminished by oral cyclophosphamide therapy for Granulomatosis with polyangiitis (Wegener's) *Arthritis Care & Research. 2011;63(12) 1777-1781*	RDBPCT Diminished ovarian function measured by AMH levels	N=40 females but ONLY 12 entered the study with normal ovarian reserve at baseline (based on AMH levels) Mean age = 35.2 ± 9.2 (all < 50 years old)	All patients received etanercept 25 mg SC 2x/week in addition to: CYC 2 mg/kg/day + prednisone if severe disease Vs. MTX (or AZA if renal dysfunction) and prednisone if limited disease	6 of 8 (75%) who received CYC developed diminished ovarian reserve. - mean AMH declined from 4.2 ± 2.3 ng/mL to 0.6 ± 0.5 ng/mL 0 of 4 (0%) who did not receive CYC developed diminished ovarian reserve [p < 0.05] - mean AMH increased from 3.3 ± 1 ng/mL to 4 ± 1.4 ng/mL POF occurred predominantly in women > 35	Daily oral CYC, even when administered for < 6 months caused diminished ovarian reserve. Change in AMH correlated inversely with cumulative CYC dose (p < 0.01), with a 0.74 ng/mL decline in AMH level for each 10 gram	

Table 5: Risk of premature ovarian failure (POF) with cyclophosphamide in **AAV patients** according to **observational data**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Huong et al. Risk of ovarian failure and fertility after IV CYC. A study in 84 patients *Journal of Rheumatology 2002:29(12) 2571-2576*	Single center observational chart review Amenorrhea = lack of menses x 4 months Sustained amenorrhea = lack of menses x 12 months	N=84 56 patients had SLE 28 had other diseases (mainly AAV) Mean age = 20 ± 10 All had to be < 55 years old	IV CYC pulse - mean CYC dose was 0.9 ± 0.14 g per pulse Mean number of pulses: 13 ± 6.5 Mean follow-up duration: 5.1 ± 3.7 years	23 (27%) women developed amenorrhea (1 to 12 months following CYC) 19 (23%) women developed sustained amenorrhea Mean age at ovarian failure onset = 40 ± 7.6 yrs The rate of sustained amenorrhea was 0 at 25 years, 45% at 31 years and 83.3% over 40 yrs. 16 pregnancies occurred in 9 women after CYC • 2 were aborted due to morphological abnormalities • 1 was aborted due to SLE relapse • 3 spontaneous miscarriages occurred • 10 premature deliveries occurred • 3 full term deliveries occurred	The risk of ovarian failure depends on age. Pregnancy may occur during and after CYC with favourable outcomes in 2/3 of cases.	

Table 6: Risk of premature ovarian failure (POF) with cyclophosphamide in **LN patients** according to **observational data**

	Design	Patient(s)	Intervention	Outcome	Conclusion	Limitations
Soares et al. Gonad evaluation in male systemic lupus erythematosus *Arthritis & Rheumatism 2007 56(7) 2352-2361*	Single center (Sao Paulo) case control study Gonadal failure based on urologic evaluation (hormone profile, semen analysis, anti-sperm antibodies) and testicular US Azoospermia = no spermatozoa in ejaculate Oligozoospermia = sperm concentration < 20 million/mL Asthenozoospermia = normal sperm motility in < 50% of sperm Teratozoospermia = normal sperm morphology in < 30% of sperm by WHO criteria Oligoastenoteratozoospermia = presence of oliogozoospermia, asthenozoospermia and teratozoospermia	N=70 35 SLE patients 35 age matched healthy controls Mean age = 28.9 ± 8.8 yrs	35 consecutive SLE male patients were prospectively evaluated for demographic and clinical features as well as previous and current treatment.	SLE vs. controls: Lower sperm volume (2.3 ± 1 vs. 3.2 ± 1.7 mL, p=0.015) Lower median sperm count (70 x 10^6/mL vs. 172 x 10^6/mL; p=0.02) Lower median motile sperm count (32 x 10^6/mL vs. 119 x 10^6/mL; p=0.004) Lower % of normally formed sperm (11.2 ± 8.3 vs. 17.4 ± 11.3%; p=0.011) Having children after disease onset (20 vs. 80%; p=0.0001) SLE patients who had IV CYC: Lower median sperm concentration (2 x 10^6/mL vs. 82 x 10^6/mL; p=0.0001) Lower median sperm count (6 x 10^6/mL vs. 10 x 10^6/mL; p=0.0001) Lower median motile sperm count (2.5 x 10^6 vs. 94 x 10^6; p=0.0001) Lower % of normally formed sperm (7.5 ± 7.3 vs. 15 ± 8.2%; p=0.011) Elevated FSH (42.9 vs. 9.5%; p=0.038)	Gonadal function is severely affected in male SLE patients. The persistence of abnormal testicular function after 5.11 ± 3.72 years since IV CYC treatment demonstrates by elevated FSH levels and lower testicular volumes support the notion of long-lasting damage to primordial sperm cells and reinforces the need for sperm cryopreservation. Anti-sperm antibody was not involved.	
Silva et al. Gonadal function in male adolescents and young males with juvenile onset SLE *Journal of Rheumatology 2002 29(9) 2000-2005*	Clinical and laboratory evaluation of 4 young men with SLE (with renal involvement) at a single center in Sao Paulo Brazil	N=4 Age 16 to 22	Clinical evaluation – SLE disease severity Testicular ultrasound Sperm concentration and motility according to WHO criteria Hormonal levels Anti-sperm antibodies	Patient 1 (SLE class V) • 8 g (140 mg/kg) exposure to IV CYC • azoospermia • high FSH (19.9 IU/l) and LH (11 IU/L) Patient 2 (SLE class IV) • 24.6 g (396 mg/kg) exposure to IV CYC • Oligoastenoteratozoospermia at presentation (while on CYC) and teratospermic 6 months later (no CYC) Patient 3 (SLE class IV) • 11.1 g (185 mg/kg) exposure to IV CYC, 6.5 yRs earlier • teratospermic Patient 4 (SLE class V) • No exposure to IV CYC • teratospermic	Fertility is decreased in SLE patients.	