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A systematic review and meta-analysis of the gonadotoxic effects of cyclophosphamide and benefits of gonadotropin releasing hormone agonists (GnRHa) in women of child-bearing age with autoimmune rheumatic disease

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Running title: Systematic review of CYC and GnRHa in autoimmune rheumatic disease

Abstract:

Objectives: To systematically review the risk of sustained amenorrhoea with intravenous (IV) cyclophosphamide in autoimmune rheumatic disease (ARD), and evaluate the efficacy of gonadotropin releasing hormone agonists (GnRHa) to reduce this risk.

Methods: Systematic search for papers reporting the incidence of sustained amenorrhoea ≥ 12 months in ARD following: IV cyclophosphamide; or GnRHa and IV cyclophosphamide compared to IV cyclophosphamide alone.

Results: From 31 articles and 1388 patients with a mean age of 27.7 years, sustained amenorrhoea occurred in 273 patients (19.7%). Of 56 patients (mean age range 23.9-25.6 years) receiving GnRHa and IV cyclophosphamide, and 37 controls (mean age range 25-30.1 years) given IV cyclophosphamide only, sustained amenorrhoea occurred in 2/56 (3.6%) patients treated with GnRHa, compared to 15/37 (40.5%) controls. Pooled odds ratio of sustained amenorrhoea with GnRHa and cyclophosphamide versus cyclophosphamide alone was 0.054 (95% CI 0.0115-0.2576 p<0.001), corresponding to a number needed to treat of 2.7 (95% CI 1.955-4.388) and absolute risk reduction of 36.95% (95% CI 35.6-38.4%).

Conclusion: Sustained amenorrhoea with IV cyclophosphamide was observed in patients with ARD, especially with increasing age and cumulative doses >5g. GnRHa reduced this risk and should be considered with IV cyclophosphamide in women of childbearing age with ARD.

Keywords: Sustained amenorrhoea, cyclosphosphamide, gonadotropin releasing hormone agonists, rheumatic disease

1. Introduction

Cyclophosphamide remains the gold standard treatment in many organ or life-threatening manifestations of autoimmune rheumatic disease (ARD) such as systemic lupus erythematosus (SLE) or primary systemic vasculitis. Use of oral cyclophosphamide in ARD has been largely superseded by intravenous (IV) administration because of its superior safety profile [1-3]. Gonadal toxicity however, with long-term consequences on fertility in women of reproductive age remains a significant concern [3], especially in women with ARD who already have reduced fertility compared to the normal population [4]. Therefore, attempts have been made to further reduce IV cyclophosphamide toxicity by lowering the cumulative dose in treatment protocols [5].

Throughout female reproductive life, there is a balance between ovarian follicles in quiescent (primordial) and growing stages, an equilibrium that depends on gonadotropins [6]. In chemotherapy-induced gonadal toxicity, there is damage to all ovarian follicular stages and cell types [6]. Gonadotropin releasing hormone agonists (GnRHa) are a treatment option alongside cyclophosphamide, to reduce ovarian toxicity and the rate of sustained amenorrhoea. There are various theories, some controversial, regarding their underlying mechanism including: i) GnRHa stimulate gonadotropin release which leads to desensitisation of GnRH pituitary receptors, inducing a transient hypogonadotropic pre-pubertal milieu and maintaining ovarian follicles in a quiescent state (the primordial stage) in which the follicles are less vulnerable to cyclophosphamide-induced gonadotoxicity; ii) GnRHa may decrease apoptosis [6, 7] and mitochondrial stress via its direct effect on GnRH receptors in the ovaries [6]; or iii) GnRHa decrease utero-ovarian blood flow and therefore cyclophosphamide dose to ovarian follicles [6, 8]. Furthermore, growing follicles normally produce anti-mullerian hormone (AMH), which negatively affects the recruitment of primordial follicles into the growing pool [6, 7]. Chemotherapy agents cause damage to growing follicles, a reduction in AMH, and subsequently more primordial follicles are recruited into the growing pool [6]. GnRHa prevent damage to growing follicles that normally produce AMH, limiting the gonadotoxic effect of chemotherapy [6, 7]. .

The ovarian protective effects of GnRHa in patients have been demonstrated in various meta-analyses of patients undergoing multidrug gonadotoxic chemotherapy for malignancies including breast cancer [11-13] and possibly in lymphoma [13]. Furthermore, in breast cancer patients undergoing chemotherapy, a systematic review found that GnRHa led to a 50% reduction in the risk of premature ovarian insufficiency [15]. Along with counselling about other fertility preservation methods such as embryo cryopreservation [16, 17], discussion about the use of GnRHa is now part of routine care in pre-menopausal breast cancer patients undergoing chemotherapy [15, 18]. GnRHa have limited, usually reversible side effects including hot flushes, headaches, sweating, and vaginal dryness [16]. However, the risk of cyclophosphamide induced ovarian dysfunction and the potential benefit of GnRHa found in patients with malignant diseases exposed to high dose regimes of cyclophosphamide, may not be directly comparable to patients with ARD. In

particular, patients with ARD may be exposed to lower cumulative doses of cyclophosphamide and fewer other gonadotoxins used in chemotherapy regimens. Additionally lupus patients may be particularly at risk, as they appear to have low ovarian reserve regardless of cyclophosphamide [19-21]. However, relevant information on the use of GnRHa and cyclophosphamide in patients with ARD has been thus far mostly been obtained from small cohort studies, and/or combined analyses with other patients receiving cyclophosphamide for malignancies.

Therefore, there is a need to more precisely categorise the risk of gonadal toxicity in patients with ARD, and determine whether GnRHa are effective in patients with non-malignant disease. Consequently, we performed a systematic review and meta-analysis with the aim of answering two questions: 1) What is the risk of sustained amenorrhoea in women treated with IV cyclophosphamide for ARD? and 2) Are GnRHa effective in reducing this risk?

2. Materials and methods

2.1 Publication search and selection of studies

Using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles [22], we performed a search of published English language papers in Pubmed, EMBASE, MEDLINE and the Cochrane Library from database inception until April 2018. The outcome measure used to assess premature ovarian insufficiency (POI) was sustained amenorrhoea, defined as more than 12 months of cessation of menstruation after treatment with IV cyclophosphamide.

Studies were identified by searching databases using combinations of the key MESH and free terms. On the advice from University College London Library staff, the search strategy used for Pubmed, EMBASE and MEDLINE was (cyclophosphamide) AND (gonadal toxicity OR premature ovarian failure OR amenorrhoea OR ovarian failure OR ovarian OR ovary OR infertility) AND (rheumatic OR rheumatology OR lupus OR SLE OR systemic lupus erythematous OR vasculitis OR autoimmune OR glomerulonephritis) and for GnRHa use we added AND (gonadotropin releasing hormone agonist OR GnRH OR GnRH-a). Due to its comparatively smaller database, the search strategy for Cochrane was kept broader to ensure studies were not missed and involved the search term (cyclophosphamide), and for GnRHa use the search term was (gonadotropin hormone releasing hormone agonist).

Two authors independently reviewed each study abstract identified from the searches and selected relevant papers based on the inclusion criteria of: ARD; IV cyclophosphamide; incidence reported of sustained amenorrhoea; and cohort more than 5 patients. Exclusions were: non-English language articles; abstracts; non-ARD patients unless their data could be separated from that of ARD patients; and use of oral cyclophosphamide unless this data could be separated from that of IV cyclophosphamide. To assess the use of GnRHa, the inclusion criteria were: GnRHa use in ARD during treatment with IV cyclophosphamide; an IV cyclophosphamide alone control group; and reported incidence of sustained amenorrhoea. Study

authors were contacted where further information was needed, which was included where received.

2.2 Data collection process

A data extraction sheet was developed, its reliability examined on 10 studies and then refined to ensure all relevant data were captured. Two authors extracted and independently checked the data. Disagreements were resolved by group discussion. Each selected article was systematically examined to note the following study characteristics.

2.3 Patient population: studies were selected that included more than five patients with ARD treated with IV cyclophosphamide +/- GnRHa. The mean cohort age was noted as well as study type and duration.

2.4 Intervention: information was gathered on the mean IV cyclophosphamide dose per cohort and in those patients who experienced sustained amenorrhoea.

2.5 Comparison: was made of patients with ARD treated with IV cyclophosphamide who experienced sustained amenorrhoea and those who did not, as well patients with ARD treated with IV cyclophosphamide +/- GnRHa.

2.6 Outcome: was sustained amenorrhoea (> 12 months) and variables used to assess this risk due to IV cyclophosphamide +/- GnRHa were: mean cohort age; mean IV cyclophosphamide cohort dose; incidence of sustained amenorrhoea; mean age of patients with sustained amenorrhoea; mean age of patients without sustained amenorrhoea; mean IV cyclophosphamide dose of patients with sustained amenorrhoea; and mean IV cyclophosphamide dose in patients without sustained amenorrhoea. Information was also gathered where available on: method of ensuring adequate menstruation pre-treatment; hormonal confirmation of sustained amenorrhoea; and pregnancy data post-treatment. Outcome bias was assessed at the analysis stage and is presented in the discussion. The strength of evidence of each study was assessed using the GRADE (Grading of Recommendations, Assessment, Development and Evaluations) framework [23].

2.7 Statistical analysis

Medcalc Version 15.8 was used to assess the odds ratio of sustained amenorrhoea compared to controls. R Version 3.5.1 was used for the meta-analysis using the metafor package. The random effects meta-analysis model was used to obtain the overall summary estimate of the proportion of sustained amenorrhoea across studies. Freeman-Tukey double arcsine transformation was used to stabilise the variance of individual studies where the proportion was close to the margin of 0 or 1. Heterogeneity was quantified by I^2 statistics. The evidence of bias was assessed through visual inspection of funnel plots and regression test. Meta-regression analysis was further performed to examine the possible sources of heterogeneity,

and the association between study factors and the proportion of sustained amenorrhoea.

3. Results:

3.1 Study and patient characteristics: An initial search revealed 1446 articles. After the removal of duplicate articles, 1158 articles were screened. Studies were excluded for various reasons including: study outcome not within the scope of this systematic review; article not available in the English language; or full text article not available to review. Ninety three papers were selected for full text review, from which 31 studies were identified that addressed the risk of sustained amenorrhoea with IV cyclophosphamide (Figure 1). These studies examined 1388 patients with ARD (Table 1) [5, 10, 19, 24-49]. The studies included patients with: SLE (n=1332); granulomatosis with polyangiitis (n=20); undifferentiated systemic vasculitis (n=12); polyarteritis nodosa, microscopic polyangitis or eosinophilic granulomatosis with polyangiitis (n=11, note no further breakdown available on diagnoses in this study) [25, 33]; idiopathic inflammatory myopathy (n=4); systemic sclerosis (n=3); Behcet's disease (n=1); juvenile idiopathic arthritis (n=1); mixed essential cryoglobulinaemia (n=1); panniculitis (n=1); relapsing polychondritis (n=1); and Takayasu's arteritis (n=1).

The studies consisted of: 13 retrospective cohort studies; 4 retrospective case-control studies; 2 retrospective cross-sectional studies; 6 prospective cohort studies; 1 mixed retrospective and prospective cohort study; and 5 randomised controlled trials.

3.2 Increasing patient age and total dose of IV cyclophosphamide determine risk of sustained amenorrhoea

Sustained amenorrhoea occurred in n=273 patients (19.7%, range across the studies 0-66.7% depending on age and cumulative cyclophosphamide dose). Twenty studies provided information on cumulative cyclophosphamide dose, usually reported as a mean dose. The rate of sustained amenorrhoea was positively correlated with increasing cumulative cyclophosphamide dose (Figure 2). The majority of cases of sustained amenorrhoea occurred in patients receiving a cumulative cyclophosphamide dose >5g.

In two studies, sustained amenorrhoea occurred at cumulative doses <5g. Baba et al found a 12.5% rate (4/29) of sustained amenorrhoea at a median cumulative cyclophosphamide dose of 1g [24]. However, all patients who developed sustained amenorrhoea in this study were above 40 years of age, thus confirming that age is an important risk factor [24]. Similarly Houssiau et al found a 4.9% rate (2/41) of sustained amenorrhoea with a mean cumulative cyclophosphamide dose of 3g; both of these affected patients were 44 years old [5]. Therefore, a low cumulative dose of IV cyclophosphamide (<5g) is less likely to induce sustained amenorrhoea in patients under 40.

Five studies, mainly comprising patients with SLE, found higher doses of cumulative cyclophosphamide (in grams) were associated with sustained amenorrhoea in patients with ARD, compared to those with ongoing menstruation (9.4g vs 8.4g $p<0.05$ [30], 16.8g vs 11.8g $p>0.05$ [32], 20.8g vs 13.4g $p<0.05$ [36], 18.9g vs 9.1g $p<0.05$ [47] and 18.6g vs 9.8g p value not provided [10]. Laskari et al found sustained amenorrhoea occurred in 51.2% of women receiving a mean cumulative cyclophosphamide dose of 20.1g, compared to 4.8% of those receiving 8.1g [28]. One study found sustained amenorrhoea developed in 12% of patients after seven monthly cyclophosphamide pulses of 0.5 to 1.0g/m^2 body surface area, and 39% of patients after fifteen monthly pulses of 0.5 to 1.0g/m^2 body surface area [26]. Another study found that a mean dose of 0.9g/pulse (range 0.5-1g) of cyclophosphamide produced rates of sustained amenorrhoea of: 0% in patients less than 26 years of age; 20% in patients aged 26-35 years (all ≥8 pulses); 50% in patients age 36-40 years receiving ≤7 pulses; and 100% in those greater than 40 years of age receiving ≥8 pulses [33].

In addition, several studies found an increased incidence of sustained amenorrhoea with increasing patient age (Figure 3). The most detailed evidence of this association came from three studies; one demonstrating sustained amenorrhoea in 12% of patients <25 years of age, 27% in patients age 26-30 years and 62% in patients age >31 years [26]. Two other studies reported incidence of sustained amenorrhoea according to age of: 27.3% in patients aged <30 years, 56.3% in patients age 30-39 years, and 87.5% in patients ≥ 40 years of age [32]; and 12.1% in SLE patients <30 years and 39.1% in SLE patients >30 years [33]. Several other studies also clearly distinguished the relationship between the incidence of sustained amenorrhoea and patient's age: 0% with a mean age of 13 years [37, 40], 11.4% ≤31 years and 69.6% ≥32 years [34]. In one study, 4/29 patients who developed sustained amenorrhoea were all ≥40 years [24].

The combination of increasing patient age and cumulative dose of IV cyclophosphamide increases the incidence of sustained amenorrhoea. In particular, one study found the risk of sustained amenorrhoea in patients >32 years of age to increase with total cumulative cyclophosphamide such that it was: 33% at >5g/m^2; 50% >8g/m^2; and 90% above 12g/m^2 [34]. A separate study found the incidence of sustained amenorrhoea to be: 0% at all ages with cumulative cyclophosphamide dose of <5g; 0% at age <20 years with cumulative dose of cyclophosphamide up to 15g; 16.7% age 21-30 years receiving >10g; 17% and 29% respectively for those aged 31-40 receiving 5-10g and 10-15g; and 33%, 100% and 100% respectively for those age>40 years receiving 5-10g, 10-15g and >15g [30]. One other study however, reported risk at a lower age in a 19 year old who developed sustained amenorrhoea after a cumulative dose of 14.4g of IV cyclophosphamide [28].

3.3 Meta-analysis

A meta-analysis of the proportion of sustained amenorrhoea was undertaken on 17 studies (n=671 patients) that provided the required data for meta-analysis purposes (Figure 4). The pooled estimate of sustained amenorrhoea based on a random effects model was 0.20 (95% CI: 0.14-0.28). There was considerable heterogeneity between studies (I^2=79%). This heterogeneity was not surprising as the studies had diverse methodology; patient cohorts were from different countries and ethnic backgrounds; and the majority of patients had SLE, which is a heterogeneous disease with different disease manifestations. Mixed effect meta-regression found that the rate of sustained amenorrhoea was positively associated with increasing mean age of study cohort ((β=0.02, 95% CI (0.005-0.04), p=0.01)) and higher IV cyclophosphamide dose ((β=0.03, 95% CI (0.01- 0.04), p<0.0001)). There was no significant association observed between the type of ARD and the rate of sustained amenorrhoea at a significance level of 0.05 (p=0.09), however, this result probably reflects the low numbers of patients with diseases other than SLE. Of the 671 patients, there were 664 SLE patients, but only 2 systemic sclerosis patients, 1 Behcet's patient, 1 juvenile idiopathic arthritis patient, 1 relapsing polychondritis patient, 1 granulomatosis with polyangiitis patient and 1 Takayasu's arteritis patient. The visual inspection of funnel plot (Figure 5) and regression test for asymmetry did not reveal significant asymmetry (z=0.2236, p=0.8231). This suggests a trend against reporting bias.

3.4 Successful pregnancy following IV cyclophosphamide

To further assess the potential impact of IV cyclophosphamide on fertility we considered pregnancy outcomes where stated in the selected studies. In their study, Boumpas et al assigned lupus nephritis patients randomly to either 7 or ≥15 monthly pulses of IV cyclophosphamide, and also included neuropsychiatric lupus patients who received a course of cyclophosphamide equivalent to the shorter course of cyclophosphamide [26]. The study reported 8 pregnancies (4 full term pregnancies with delivery of normal babies and 4 elective abortions) in a cohort with a mean age of 27 years treated with 7 monthly pulses of IV cyclophosphamide of 0.5 to 1.0 g/m^2 body surface area, and no pregnancies reported in the group who had 15 pulsed infusions at a similar dose [26]. Pregnancies reported in this study occurred after cyclophosphamide treatment, thus suggesting that women who do not develop amenorrhoea remain fertile [26]. Mitwalli et al found 3/39 patients had viable pregnancies following 24 pulses of treatment of 5mg/kg cyclophosphamide, in a cohort with a mean age of 30.3 years [29]. Langevitz et al noted 5 successful conceptions in 4 patients with a mean age at birth of 27.2 (range of cumulative cyclophosphamide dose in these patients 1.6-6.7g), resulting in the delivery of 5 full term healthy babies [41]. Park et al described 17/17 successful pregnancies in 13 patients, in a cohort with a mean age of 31.1 years and mean cumulative dose of cyclophosphamide of 9.0g [30]. However, no patients with sustained amenorrhoea who tried to conceive fell pregnant; notably the proportion of the overall cohort trying to conceive was not reported [30]. Blumenfeld et al reported 3 successful

pregnancies in a cohort with a mean age of 30.1, receiving a mean cumulative cyclophosphamide dose of 10.5g [50]. Somers et al found 3 successful pregnancies, in a cohort with a mean age of 25 and mean cumulative cyclophosphamide dose of 12.9g [51]. Massenkeil et al found 4/4 patients who tried to conceive were successful and gave birth to 6 healthy children. 2/4 patients had to use in vitro fertilisation, and 2/4 had 3 spontaneous conceptions [10]. Alarfaj et al found that 48/99 patients conceived, resulting in 90 pregnancies consisting of 29 foetal losses and 61 live births [45]. The mean cumulative cyclophosphamide dose and mean age was significantly lower in patients who conceived successfully, compared to those who did not fall pregnant (6.7g and 28.3 years vs 8.3g and 34.1 years respectively, $p<0.05$ for both dose and age) [45].

3.5 GnRHa co-treatment during IV Cyclophosphamide for Rheumatic Disease

Three studies examined the use of GnRHa with IV cyclophosphamide treatment in n=93 ARD patients with the following diagnoses: SLE (n=85), systemic sclerosis (5), mixed connective tissue disease (n=2), polyarteritis nodosa (n=1), juvenile idiopathic arthritis (n=1) and Behcet's Disease (n=1) (Table 2) [50-52]. These studies included: one retrospective case-control study [50]; one study with prospective data collection for cyclophosphamide plus GnRHa treated patients and retrospective cyclophosphamide only control data [51]; and one prospective cohort study [52]. Two of the studies were from the same centre, but presumably had different cohorts of patients based on the demographic and clinical data provided by the authors [50,52].

Of these patients, 56 received GnRHa plus IV cyclophosphamide and 37 received IV cyclophosphamide only. In all three studies, the mean age (years) in the GnRHa treated patients was lower in each of the three GnRHa groups than that of the controls (25.6±5.3 vs 30.1±5.5, $p=0.04$ [50]; 23.9±1.0 vs 25.6 ±0.9, $p>0.05$ [51]; and 25.6 vs 28.2, p value not provided [52]. The mean cumulative dose (in grams) used in the GnRHa group was lower than the controls in two studies (8.92 vs 10.52, p value not provided [50]; and 7.7 vs 13.3, p value not provided and data missing from 3 patients [52]). For the other study, cumulative cyclophosphamide dose was the same in both groups at 12.9±0.5 [51].

Sustained amenorrhoea occurred in 2/56 (3.6%) patients treated with GnRHa, compared to 15/37 (40.5%) of controls [50-52]. Somers et al found one patient developed sustained amenorrhoea despite GnRHa treatment [51]. However, this patient was in the 75th percentile for age (28.2 years) and 99th percentile for cumulative cyclophosphamide dose (33.5g) for their cohort [51]. The one patient on GnRHa who developed sustained amenorrhoea in Blumenfeld et al's 2011 study was aged 37 years [50]. The pooled odds ratio of ovarian dysfunction with GnRHa and cyclophosphamide compared to cyclophosphamide alone was 0.054 (95% CI 0.0115- 0.2576 $p=0.0002$, z-statistic 3.668), corresponding to a number needed to treat (NNT) of 2.7 (95% CI 1.955-4.388) and an absolute risk reduction of 36.95% (95% CI 35.6-38.4%).

4. Discussion

We found that the incidence of sustained amenorrhoea increased with increasing cumulative dose of IV cyclophosphamide and increasing age in patients with ARD. GnRHa significantly reduced the risk of sustained amenorrhoea in patients with ARD treated with mean cumulative cyclophosphamide doses ranging from 7.7 to 12.9g. This ovarian protective effect is in keeping with results achieved in patients receiving IV cyclophosphamide and GnRHa for malignancies, with various meta-analyses supporting its use in breast cancer (OR of POI = 0.36, 95% CI 0.23-0.57, p<0.001 [11]; and OR of POI = 0.38, 95% CI 0.26-0.57, p<0.001 [12] when comparing those who received GnRHa vs those who did not), although no significant benefit was seen in lymphoma patients (RR 0.70, 95% CI 0.20-2.47) [13]. .

The incidence of sustained amenorrhoea in our analysis became apparent at cumulative doses of IV cyclophosphamide of 1g or more, albeit in patients over 40 years, with most studies showing sustained amenorrhoea at doses >5g. The incidence of sustained amenorrhoea increased in direct correlation to increasing IV cyclophosphamide dose. A similar relationship was observed with increasing age, although several studies report sustained amenorrhoea in patients with ARD at 20-30 years of age [26, 27, 35, 51]. This finding is particularly relevant in SLE, since previous studies have identified low ovarian reserve in these patients [19-21]. Of further concern to younger patients is the possibility that they may require additional doses of cyclophosphamide to treat refractory or recurrent disease, which may further diminish already depleted ovarian follicle numbers. It remains difficult however, to risk stratify an ARD cohort by age that one can be certain is not at risk of sustained amenorrhoea when considering IV cyclophosphamide. This consideration is important because the consequences of gonadal toxicity and POI in young women include infertility, premature osteoporosis, atherosclerosis, mood disorders and cardiovascular mortality [42, 53-55]. Our findings however, do provide reassurance that cumulative doses of cyclophosphamide below 5g are unlikely to produce sustained amenorrhoea in patients with ARD under 40 years of age.

Findings of this systematic review are limited by the included studies that are mostly retrospective; lack disease controls; have few or no patients with diseases other than SLE; and report mean cohort age and cyclophosphamide dose with different cyclophosphamide dosing regimens that make direct comparison difficult. In addition, the effect of chronic disease on ovarian function was considered in some, but not all studies in multivariate analysis [30, 37].

Overall, we confirmed a protective effect of GnRHa in patients with ARD treated with IV cyclophosphamide. GnRHa are more convenient to administer than alternative ovarian preservation techniques, such as embryo and oocyte cryopreservation [56], and the latter techniques do not prevent the risk of chemotherapy-induced POI [6]. Hormonal treatment with the combined oral contraceptive has also been studied in conjunction with chemotherapy, but has failed to prove beneficial, despite its early

promise [57, 58]. One potential drawback however of GnRHa therapy is that it can take up to 22 days to achieve full ovarian suppression after an initial stimulatory effect on the ovaries, and ideally should be given well in advance of the administration of chemotherapy [59].

Limitations of the studies reporting the use of GnRHa include the small number of studies, small cohort numbers, and possible selection bias with older patients used in the control group raising doubt as to the magnitude of their efficacy. However, the mean age group in both GnRHa and control groups was 30 or under in all studies. Furthermore this finding may reflect the fact that older patients are not routinely being offered GnRHa, yet they are the age group most at risk of sustained amenorrhoea due to the effects of ageing on ovarian reserve. Indeed this finding is becoming ever more pertinent as the average age of women giving birth in the UK continues to rise, with the average age now 30.5 years in 2017 compared to 28.5 years in 2000 [60].

Another consideration when interpreting these studies is their use of menstruation as a surrogate marker of ovarian function, since functional impairment of ovarian function may exist despite normal menstrual cycles [20]. Therefore, reliance upon sustained amenorrhoea may underestimate or overestimate the protective effect of GnRHa on ovarian function. Furthermore, many of the studies were retrospective, so significant recall bias regarding return of menstruation may be a confounding factor. In addition, sustained amenorrhoea is not always indicative of permanent POI [61]. The gold standard outcome would be to measure hormonal markers of ovarian function such as follicular stimulating hormone or anti-mullerian hormone and carry out ovarian ultrasound to measure follicular reserve post IV cyclophosphamide [20]; this procedure however was only performed in only 2/31 studies included in our systematic review [50,52]. Although an additional 9/31 studies measured hormone levels, often to confirm POI in cases of sustained amenorrhoea, [10, 19, 27, 31, 32, 43, 47, 49, 51], only 4 of those studies provided specific results or exact levels (see Table 1) [10, 19, 47, 49] and is another limitation of our systematic review and meta-analysis. Another indication of fertility is the occurrence of a successful pregnancy and although we have included the available evidence, the reports are limited and do not state the proportion of patients trying to conceive.

Despite these limitations, the effectiveness of GnRHa is still very promising, with a large absolute risk reduction that cannot be fully explained by methodological or selection bias. A recent phase 3 randomised control trial of the GnRHa leuprolide undertaken by McCune et al unfortunately had to be terminated, presumably as only 7 patients were recruited [62]. Therefore, there still remains a pertinent need for larger, better quality studies assessing GnRHa use in ARD.

5. Conclusion

In conclusion we found a significant risk of ovarian toxicity manifested by sustained amenorrhoea, after therapy with IV cyclophosphamide in patients with ARD of various reproductive ages, particularly when receiving cumulative doses of greater than 5g. Concomitant therapy with GnRHa was highly effective in preventing sustained amenorrhoea, and should be offered to all premenopausal women with ARD before receiving IV cyclophosphamide treatment in non-urgent situations.

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Author, location, year	Cohort	Study type	Mean age, standard error and range in brackets (where available)`	Mean dose (grams); CYC protocol if available and reported as per study	Sustained amenorrhoea (% of total, n in brackets)`	Hormone levels measured	Strength of evidence using GRADE framework
Baba (Japan 2011) (24)	29 SLE	Retrospective case control study	31 (18-45)*	1*; 0.5g/body/pulse	12.5 (4)	No	Low
Martin-Suarez (UK 1997) (25)	43 SLE; 19 GPA; 12 USV; 11 PAN, MPA or EGPA; 4 IIM; 1 MEC	Retrospective cohort study	Not reported for females alone	Not reported for females alone; 0.5g weekly until complete/partial remission, then 0.5g monthly or switch to azathioprine	0 (0)	No	Low
Boumpas (short) + (USA 1993) (26)	16 SLE	Retrospective case control study	27±1.6	Not reported; 0.5 to 1.0 g/m^2 body surface area monthly x7	12.5 (2)	No	Low
Appenzeller (group B) + (Canada 2008) (27)	50 SLE	Retrospective cohort study	21.8±0.92 (15-36) group B	No data on whole cohort; 0.5mg/body surface and increased when necessary	0 (0)	FSH, LH, PRL, oestradiol measured but specific results not provided	Low
Laskari (group 2) + (Greece 2010) (28)	22 SLE	Retrospective cohort study	28±7.34 (14-45)	8.1±1.6; 1g/m^2 monthly x5-7	4.5 (1)	No	Low
Mitwalli (group 2) + (Saudi Arabia 2011) (29)	39 SLE	Randomised double blind controlled trial	Not reported for females alone	Not reported; 5mg/kg body weight monthly x6, then 2 monthly x18	15.4 (6)	No	High
Mitwalli (group 1) + (Saudi Arabia 2011) (29)	61 SLE	Randomised double blind controlled trial	Not reported for females alone	Not reported; 10mg/kg body weight monthly x6, then 2 monthly x6	41.5 (25)	No	High
Park (South Korea 2004) (30)	67 SLE nephritis	Retrospective cohort study	31.1±8.4 (range 17–46)	8.4±3.0 (menstruating) 9.4±2.2 (sustained amenorrhoea) 8.6 (combined); 0.5-0.75g/m^2 monthly x6, then 3 monthly x6	17.9 (12)	No	Low
Mok A (Hong Kong 2006) (31)	99 SLE	Retrospective cohort study	Not reported for females alone	Not reported for females alone; 0.5-1g/m^2 body surface area monthly x6, then 3monthly x6	13.1 (13)	FSH, oestradiol measured but specific results not provided	Low
Langevitz (Israel 1992) (41)	17 SLE	Prospective cohort study	29.4 (14-45)	6.3 (menstruating) 7.0 (premature menopause) 6.1 (combined); 10mg/kg monthly, or every 1-2wk followed by monthly pulses	23.5 (4)	No	Low
Blumenfeld (Israel 2011) (50)	8 SLE, 1 Behcet's, 1 JIA, 1 SSc	Retrospective case control study	29.4±5.82 (23-39)	10.4; monthly pulses x6 then 3 monthly pulses x8	45.4 (5)	FSH, LH, oestradiol, progesterone measured but specific results not provided	Low
Huong (France 2002) (33)	56 SLE	Retrospective and prospective cohort study	28±9 (13-53) 26±8 (13-45) (menstruating) 37±7 (30-53) (sustained amenorrhoea)	11.5; 0.5-1g/pulse with mean number of pulses 13+/-6.5, duration between pulses not reported	23.2 (13)	No	Low
Gonzalez-Crespo (Spain 1995) (40)	10 SLE	Retrospective case control study	13±2	13.0* (range 6.4 – 24); 0.5-1.2g/m^2 body surface with mean duration of 27+/-13 months of treatment, duration between pulses not reported	0 (0)	No	Low

Mcdermott (England 1996) (32)	35 SLE	Retrospective cohort study	36.1 (17-49)	11.8 (menstruating) 16.8 (sustained amenorrhoea) 14.5 (combined); 1g weekly x4, then fortnightly x4, then monthly x3	54 (19)	FSH, oestradiol measured but specific results not provided	Low
Somers (USA 2005) (51)	20 SLE	Prospective cohort study	25±0.9	12.9±1.5; 0.5g/m^2 body surface area monthly x6 with subsequent doses increased by up to 25% depending on WCC, then switched to azathioprine or mycophenolate mofetil or monthly CYC x4	30 (6)	FSH measured in patients with suspected POI but specific results not provided	Moderate
Ioannidis (Greece 2002) (34)	67 SLE	Retrospective cohort study	27* (22-35)	14.6*(IQR 9.0–21.75); 0.75-1g/m^2 monthly x6, then 2monthly x6, 3monthly x 4, then ceased or spaced at even longer intervals	31.3 (21)	No	Low
Appenzeller (group A) + (Canada 2008) (27)	57 SLE	Retrospective cohort study	20±7.8 (16-38)	No data on whole cohort 16.8g±2.8 (range 14-20) (Group A sustained amenorrhoea) 13.4g±1.8 (range 11-15) (Group A transient amenorrhoea; 0.75mg/ body surface and increased when necessary	17.5 (10)	FSH, LH, PRL, oestradiol measured but specific results not provided	Low
Bozzolo (Italy 2013) (36)	29 SLE nephritis	Retrospective cohort study	28.6±8.7 (menstruating) 32.7±4.7 (POF) 29.5 (combined)	13.4±5.7 (menstruating) 20.8±6.4 (POF) 14.9 (combined); 0.75-1g/m^2 monthly x6-11, then maintenance therapy of 3monthly pulses, azathioprine or mycophenolate mofetil	20.7 (6)	No	Low
Laskari (Group 1) + (Greece 2010) (28)	39 SLE	Retrospective cohort study	30.2±8.73 (14-46)	20.1±7; 1g/m^2 monthly x6, then 2 monthly x6, 3monthly x4 and then at even longer intervals based on response	51 (20)	No	Low
Boumpas (long) + (USA 1993) (26)	23 SLE	Retrospective case control study	25±1.5	Not reported; 0.5-1g/m^2 body surface area x15	39.1 (9)	No	Low
Belmont (USA 1995) (35)	27 SLE	Retrospective cohort study	Not reported	Not reported for females alone; 0.5-1g/m^2 body surface area monthly x6 then 3monthly until satisfactory response or irreversible deterioration of renal function	11.1 (3) – all females >27 years old	No	Low
Gourley (USA 1996) (39)	46 SLE nephritis	Randomised controlled study	Not reported	Not reported; 0.75g/m^2 monthly x6, then 3monthly x8	50 (23)	No	High
Contreras (USA 2005) (38)	18 SLE nephritis	Randomised controlled trial	Not reported for females alone	Not reported for females alone; 0.5-1g/m^2 body surface area monthly up to x7, then for maintenance therapy 0.5-1g/m^2 3monthly, azathioprine or mycophenolate mofetil	33.3 (6)	No	High
Knight (USA 2016) (42)	33 SLE	Retrospective cross-sectional study	Not reported	Not reported; protocol not reported	39 (13)	No	Low
Massenkeil (Germany 2016) (10)	5 SLE, 2 SSc, 1 RPC, 1 panniculitis, 1 TA, 1 GPA	Prospective cohort study	33.4±3.2 (22-48)	12.9±2.7; protocol not reported	27.3 (3)	FSH, LH, oestradiol measured – all 3 patients with sustained amenorrhoea had elevated FSH before CYC exposure	Low

Kaballo (Sudan 2016) (43)	75 SLE nephritis	Randomised control trial	Not reported for females alone	Not reported; 0.5g/m^2 monthly x6	0 (0)	FSH, LH measured but specific results not provided	High
Singh G (India 2016) (44)	34 SLE	Retrospective cross-sectional study	29.7±9.95 (8-67) for entire study (not reported for IV CYC exposed group alone or females alone)	Not reported for females alone; 0.5-0.75g/m^2 monthly x6, then 3monthly x6	17.6 (6)	No	Low
Alarfaj (Saudi Arabia 2014) (45)	99 SLE	Retrospective cohort study	29.8	7.1; 10mg/kg monthly x6, then 2monthly x6, or 5mg/kg x6 then 2monthly x18	13.1 (13)	No	Low
Houssiau (high dose) + (Europe 2002) (5)	43 SLE nephritis	Randomised control trial	Not reported for females alone	8.5±1.9; 0.5g/m^2 then increased by 0.25g/m^2 up to max 1.5g/m^2 monthly x6 then 3monthly x2	2.3 (1)	No	High
Houssiau (low dose) + (Europe 2002) (5)	41 SLE nephritis	Randomised control trial	Not reported for females alone	3; 0.5g fortnightly x6	4.9 (2)	No	High
Laskari (Greece 2010) (46)	28 SLE	Prospective cohort study	Not reported for females alone	Not reported; 1g/m^2 monthly pulses x5-7	4 (1)	No	Low
Medeiros MMC (Brazil 2001) (47)	26 SLE	Retrospective cohort study	28.5	12.5; 0.5-1g/m^2 monthly x7 then 1g/m^2 3monthly x6-8	34.6 (9)	FSH, LH, PRL, oestradiol, progesterone, testosterone measured but results not reported for those on IV CYC alone	Low
Medeiros PB (Brazil 2009) (19)	13 juvenile SLE	Retrospective cohort study	Not reported for IV CYC alone	Not reported; protocol not reported	0 (0)	FSH, LH, PRL, oestradiol, progesterone, testosterone measured but results not reported for those on IV CYC alone	Low
Singh (India 2007) (48)	35 SLE	Prospective cohort study	24.5±8.5	9.34±2.87; at least 6 pulses of CYC (dose/pulse not specified)	31.4 (11)	No	Low
Mok CC (Hong Kong 1998) (49)	16 SLE	Retrospective cohort study	Not specified for IV CYC alone	6.9±3.8 (SEM); 0.5-1g/m^2 monthly x6 then 3monthly x6	12.5 (2)	FSH, LH, oestradiol measured but results not reported for those on IV CYC alone	Low

Blumenfeld (Israel 2000) (52)	6 SLE	Prospective cohort study	28.2	13.3; 0.75 or 1g/pulse monthly x6	66.6 (4)	FSH, LH, oestradiol, progesterone measured but specific results not provided	Low

Table 1: Studies assessing the risk of sustained amenorrhoea after treatment with intravenous cyclophosphamide for autoimmune rheumatic disease

Table 1 showing the studies included in the systematic review, which assessed sustained amenorrhoea in patients with autoimmune rheumatic disease treated with intravenous cyclophosphamide.

Key: *, median; +, same study but cohort split in two by different cyclophosphamide dosing regimens; CYC, cyclophosphamide; EGPA, eosinophilic granulomatosis with polyangiitis; FSH, follicle stimulating hormone; GRADE, Grading of Recommendations, Assessment, Development and Evaluations; GPA, granulomatosis with polyangiitis; IIM, idiopathic inflammatory myopathy; IQR, interquartile range; IV, intravenous; JIA, juvenile idiopathic arthritis; LH, lutenising hormone; MEC, mixed essential cryoglobulinaemia; MPA, microscopic polyangiitis; PAN, polyarteritis nodosa; POF, premature ovarian failure; PRL, prolactin; RPC, relapsing polychondritis; SEM, standard error of the mean; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TA, Takayasu's arteritis; USV undifferentiated systemic vasculitis; WCC, white cell count.

Table 2: Studies assessing the effect of GnRHa treatment alongside intravenous cyclophosphamide (CYC) for autoimmune rheumatic disease on incidence of sustained amenorrhoea compared to intravenous CYC alone

Author and Location	Cohort	Study Type	Gnrh Dosing and Timing	CYC Dosing	Outcome Measure	Results and Follow Up	Additional Information and Grading of Evidence
Blumenfeld et al, Israel (50)	34 SLE, 5 systemic sclerosis, 2 mixed connective tissue disease, 1 polyarteritis nodosa, 1 Behcets, 1 juvenile idiopathic arthritis 31 GnRHa vs 11 control (excluding 2 patients lost to follow-up) Mean age 25.6±5.3 years (16-38) GnRHa group vs 30.1±5.5 years (23-39) control group	Retrospective case control	Decapeptyl CR, 3.75 mg monthly starting before CYC and continued during treatment, up to 6x	Monthly IV CYC ("standard regime") x6 – then 3 monthly x8. Further treatment dependent on disease e.g. SLE relapses treated with additional 4 monthly boluses of CYC. Mean cumulative CYC dose 8.92g GnRHa group vs 8.72g control group (after adjusting groups to include only patients who received up to 15g)	Premature ovarian failure (POF) based on amenorrhoea, hormonal profile (FSH, LH, estradiol, progesterone), ultrasound of ovaries and endometrium, and conceptions 1-10 years post treatment	POF in 1/31 GnRHa group vs 5/11 control group OR 0.0400 (95% CI 0.0039 to 0.4067, p=0.0065)	1/31 in GnRHa group who developed POF was aged 37 5/11 controls who developed POF had mean age of 32.2±7.2 When adjusted for age and dose differences, POF rate in control group remained 40%, significantly higher than in GnRHa group, p<0.05 7 successful pregnancies and 1 miscarriage in GnRHa group vs 3 successful pregnancies in control group Grade of evidence: low

Somers et al, USA (51)	SLE 20 GnRHa vs 20 control Age ≤35 years and no symptoms suggestive of POF. Mean age 23.9±1.0 years GnRHa group vs 25±0.9 years control group (range 17-32)	Retrospective analysis for controls, prospective for GnRHa group. Age (±5 years) and dose (±5g) matched	Monthly depot leuprolide acetate 3.75mg at least 10 days prior to CYC dose	IV monthly CYC x6 ±4 monthly boluses if still active Mean cumulative CYC dose 12.9g±1.5g in both groups	POF defined as amenorrhea ≥12months and FSH ≥40 mIU/ml	POF in 1/20 GnRHa group vs 6/20 control group OR 0.1228 (95% CI 0.0132 to 1.1384, p=0.0649)	Follow up minimum 3 years GnRHa treated patient with POF age 28.2 years and 33.5g Unknown how many women attempted conception after CYC treatment. 3/20 control patients (15%) and 7/20 GnRHa (35%) successful pregnancies. Grade of evidence: low
Blumenfeld et al, Israel (52)	SLE 5 GnRHa vs 6 controls Mean age 25.6 years GnRHa group vs 28.2 years control group (range 20-43)	Prospective cohort study	Monthly depot decapeptyl CR 3.75mg in parallel with CYC for up to 6 months	IV monthly CYC Mean cumulative CYC dose 7.7g GnRHa group (2 patients' data missing) vs 13.3g control (1 patient's data missing)	POF defined as amenorrhoea (4-15 years post treatment), estradiol <100pmol/L, FSH > LH, FSH >25IU/L	POF in 0/5 GnRHa group vs 4/6 control group OR 0.0505 (95% CI 0.0019 to 1.3450, p=0.0746)	Follow up minimum 4 years Grade of evidence: low

Table 2 comparing the three studies included in the systematic review that compared outcomes for patients on intravenous CYC and GnRHa treatment to those on intravenous CYC alone. The pooled odds ratio of ovarian dysfunction with GnRHa and cyclophosphamide compared to cyclophosphamide alone was 0.054 (95% CI 0.0115- 0.2576 p=0.0002, z-statistic 3.668), corresponding to a number needed to treat (NNT) of 2.7 (95% CI 1.955-4.388) and an absolute risk reduction of 36.95% (95% CI 35.6-38.4%).

Key: CI, confidence interval; CR, controlled release; CYC, cyclophosphamide; FSH, follicle stimulating hormone; GnRHa, gonadotropin releasing hormone agonist; LH, lutenising hormone; SLE, systemic lupus erythematosus; POF, premature ovarian failure; OR, odds ratio.

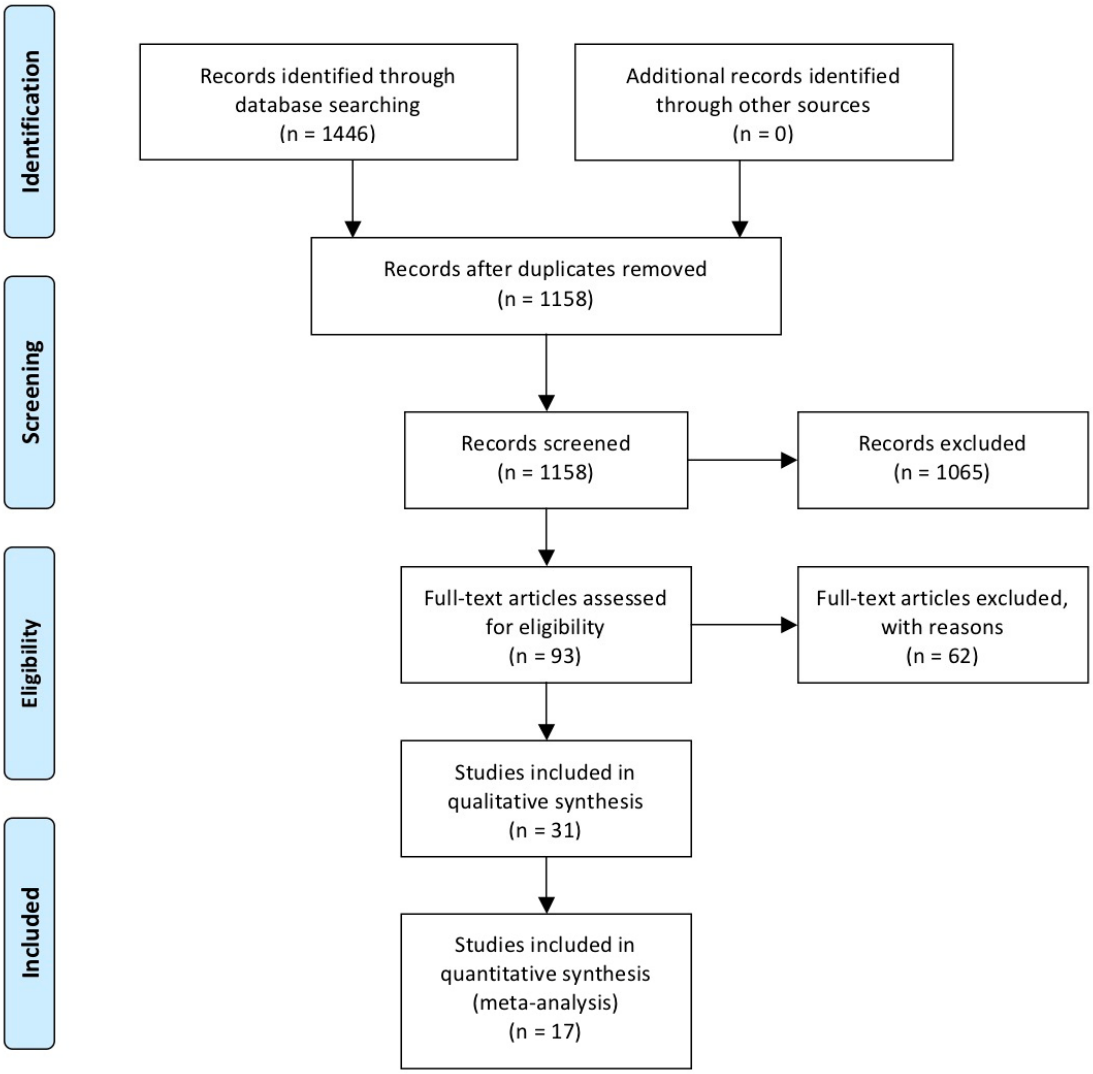

Figure 1. Flowchart of Study Selection for Systematic Review and Meta-Analysis of the Gonadotoxic Effects of Cyclophosphamide and Benefits of GnRHa in Women of Child-bearing Age with Autoimmune Rheumatic Disease

PRISMA diagram displaying the methodology of selecting studies for the systematic review and meta-analysis.

Adapted From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

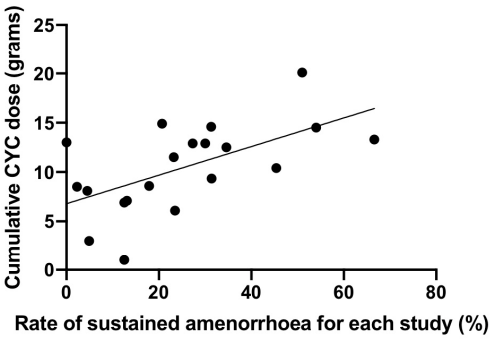

Figure 2. Rate of Sustained Amenorrhoea vs Cumulative Cyclophosphamide Dose.

The graph shows the rate of sustained amenorrhoea and the cumulative cyclophosphamide dose in individual studies.

Key: CYC, cyclophosphamide

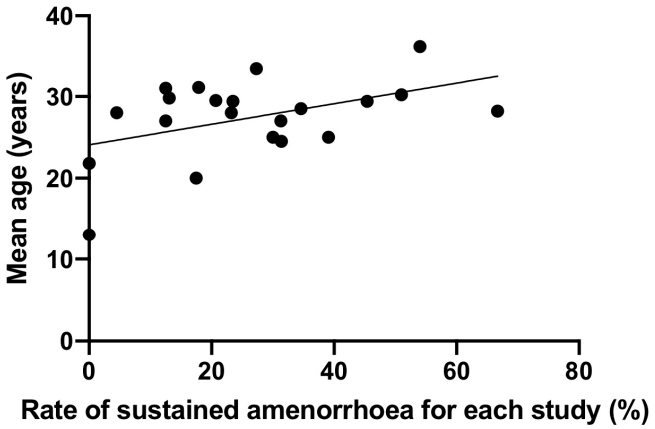

Figure 3. Rate of Sustained Amenorrhoea vs Mean Age.

Graph shows the rate of sustained amenorrhoea and corresponding mean age in individual studies included in the systematic review.

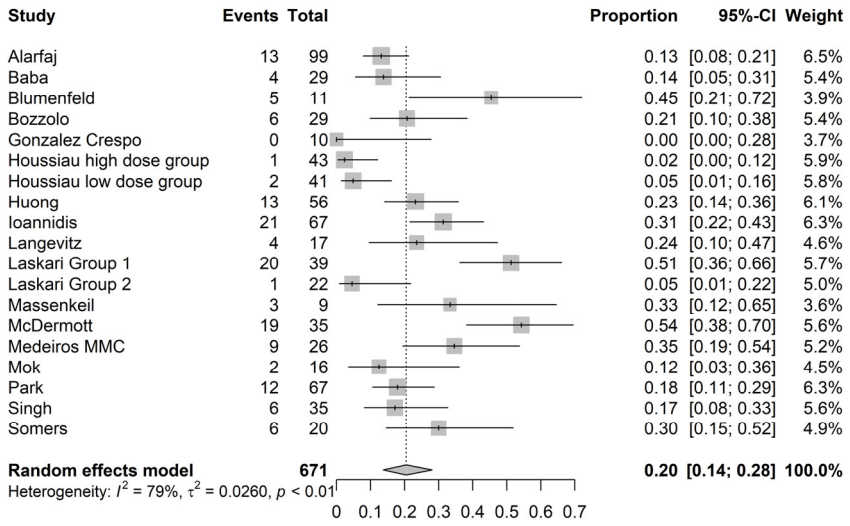

Figure 4. Forest Plot of Studies Included in Meta-Analysis Showing Proportion of Sustained Amenorrhoea.

Forest plot displaying the proportion of sustained amenorrhoea in individual studies included in the meta-analysis. Events refer to the number of patients who developed sustained amenorrhoea, and the total refers to the total number of patients in each study.

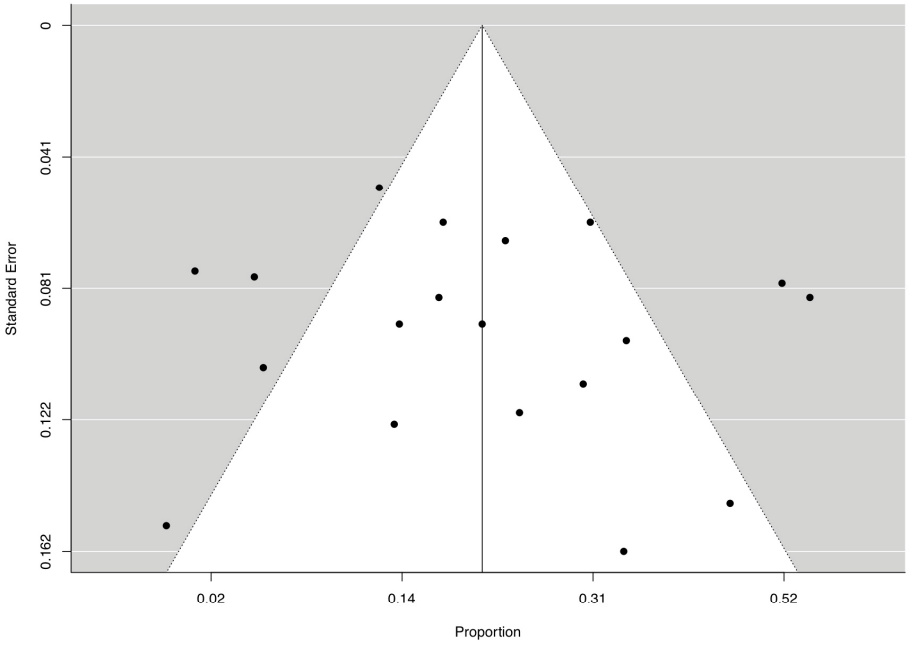

Figure 5. Funnel Plot of Studies Included in Meta-analysis.

Funnel plot with each dot representing a study (note two studies have two subgroups and therefore two dots each); the y-axis represents the study precision (standard error); and the x-axis shows the study's result (proportion of sustained amenorrhoea).