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REVIEW

Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis?

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ABSTRACT

For patients with lupus nephritis, a 24-month course of intravenous cyclophosphamide has been advocated as the 'golden' standard of therapy. This regimen is associated with a high risk of persistent amenorrhoea in women or azoospermia in men. The risk of infertility is thus an important issue when discussing treatment options in patients with SLE. In this article I have summarised the information on cyclophosphamide-induced gonadal toxicity. In addition a brief overview is given of the literature on treatment of lupus nephritis. The data indicate that there is no hard evidence to support the superiority of long-term i.v. cyclophosphamide. Therefore, patients with SLE and the wish to have a baby should not be primarily treated with such a regimen.

Table 1
Treatment schedule of i.v. cyclophosphamide for Lupus nephritis

INDUCTION THERAPY: MONTHS 0-6
Six monthly pulses of i.v. cyclophosphamide 750 mg/m²
Prednisolone orally 1 mg/kg/day with gradual reduction to 15 mg/day
MAINTENANCE THERAPY: MONTHS 6-24
Cyclophosphamide 750 mg/m² every 3 months +
Prednisone orally 10 mg/day
MAINTENANCE THERAPY: MONTHS 24-48
Prednisone orally 10 mg/day +
Azathioprine 2 mg/kg/day

INTRODUCTION

Lupus nephritis is a common complication in patients with systemic lupus erythematosus (SLE). If left untreated outcome is poor, most patients progressing to end-stage renal disease (ESRD) or death. Treatment with oral prednisone lacks long-term efficacy.[1] The introduction of more aggressive immunosuppressive therapy has markedly improved the prognosis in these patients.[1,2] Treatment regimens typically consist of combinations of prednisone and azathioprine, or prednisone and cyclophosphamide. In recent years the so-called 'NIH regimen' consisting of pulses of i.v. cyclophosphamide and oral prednisone has become the standard of therapy in the Netherlands. The advocated treatment schedule is given in *table 1*.

Infertility is a common complication of cyclophosphamide therapy. Since SLE is typically a disease of young patients, issues related to fertility and reproductive ability have a prominent role in the discussion on treatment options. In the end, we must balance the risks and benefits of the various immunosuppressive regimens. For many patients, preserving fertility is worth some risk as indicated by the observations that many women with renal disease become pregnant and to a certain degree accept the associated risks, such as hypertension, premature delivery, dysmaturity and progression of renal failure.

In this commentary I will briefly address two questions:
What is the risk of infertility associated with cyclophosphamide therapy?
Is the superiority of long-term i.v. cyclophosphamide proven in controlled trials with hard endpoints?

GONADAL TOXICITY OF CYCLOPHOSPHAMIDE

Cyclophosphamide-induced amenorrhoea in women

In *table 2* an overview is given of six studies that have documented the risk of persistent amenorrhoea in patients with SLE after treatment with cyclophosphamide in cumulative dosages of 12 to 25 g.[1,3-7] The mean age of the patients was 28 years. The risk of amenorrhoea ranged from 27 to 60%. In general, amenorrhoea developed on average four months after starting cyclophosphamide therapy. Amenorrhoea may be transient, but in the studies mentioned above amenorrhoea was sustained in more than 80% of patients. These patients with sustained amenorrhoea have premature ovarian failure, and are characterised by elevated levels of gonadotropins and low levels of oestradiol. Risk factors for sustained amenorrhoea are the age of the patient at the start of therapy and the cumulative dose of cyclophosphamide. The effect of age on the incidence of sustained amenorrhoea can be appreciated from *figure 1*, which summarises the data published by Huong *et al.* and Mok *et al.*[6,8] The cumulative dose of cyclophosphamide in these studies was 12 and 18 g, respectively. In patients below 30 years of age the risk of amenorrhoea was 10%, as compared with 60% in patients above 40 years. Ioannidis calculated the risk of amenorrhoea for a standard dose of cyclophosphamide 15 g.[9] The incidence of amenorrhoea was 5 to 10% for patients <25 years, 30% for patients aged 25 to 31 years and 90% for patients >32 years. The risks of amenorrhoea are considerably less (and virtually negligible for young women) if the cumulative dose of cyclophosphamide is lower than 10 g. Mok *et al.* found no association between the route of administration and the risk of amenorrhoea.[8]

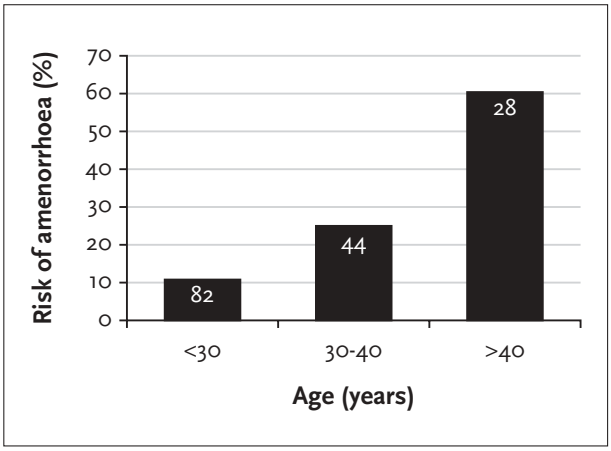

Figure 1
Risk of amenorrhoea in relation to age

Summary of data reported by Huong *et al.* and Mok *et al.*[6,8] Cumulative dose of cyclophosphamide was 12 and 18 g, respectively. The numbers in the bars indicate the total number of patients per age group included in the studies.

Cyclophosphamide-induced azoospermia in men

The incidence of SLE in male patients is low. Therefore, data on gonadal toxicity of cyclophosphamide in male SLE patients are lacking. Meaningful data can be derived from studies in patients who received courses of oral cyclophosphamide for idiopathic nephrotic syndrome or in patients with malignancies treated with cyclophosphamide. Several authors have evaluated the sperm count in men who had been treated in childhood or puberty due to idiopathic nephrotic syndrome.[10-14] Overall there was a clear relation between the duration of cyclophosphamide treatment or the cumulative dose of cyclophosphamide and the risk of azoospermia. *Figure 2* illustrates the reported findings. As the figure shows, the risk of azoospermia is particularly evident at cumulative dosages above 300 mg/kg, although even higher doses have been tolerated without a problem. One must realise that most patients involved in these studies were treated before puberty. Although the issue has not been settled, treatment started before onset of puberty may entail less risk of azoospermia.[15] Thus, the data may not be fully applicable to adult patients treated with cyclophosphamide. Based on the data provided, a cumulative dose of 168 mg/kg (equivalent to 12 weeks treatment at a dose of 2 mg/kg, or 12 g in total for a patient of 70 kg) is considered safe for adult patients. The latter conclusion is supported by data obtained in patients treated with i.v.

Table 2
Amenorrhoea after cyclophosphamide therapy

AUTHOR (REFERENCE)	PATIENTS (N)	AGE (YEARS)	DOSE OF CYCLOPHOSPHAMIDE	AMENORRHOEA (N/%)
Boumpas[3]	13	28	14 pulses of 0.5-1.0 g/m²	5 (38%)
Austin[1]	20	27	16 pulses of 750 mg/m²	9 (45%)
Illei[4]	20	28	14 pulses of 1 g/m²	12 (60%)
Illie[5]	23	28	14 pulses of 1 g/m²	12 (54%)
Huong[6]	84	29	3 pulses of 0.9 g	23 (27%)
Mok[7]	55	31	20 g	24 (43%)

Ages are average values.

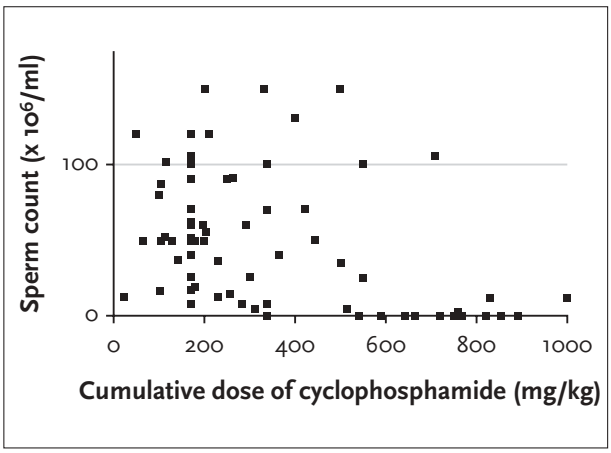

Figure 2
Sperm count in relation to the cumulative dose of cyclophosphamide

Data are derived from five studies that have evaluated sperm count in patients who received cyclophosphamide for idiopathic nephrotic syndrome.[10-14] Evaluation was performed many years after the end of treatment.

cyclophosphamide for malignancies.[16] Notably, these patients also received concurrent therapy with other chemotherapeutic agents and had often received radiotherapy. The study demonstrated that azoospermia developed approximately two to three months after the start of therapy, and was sustained during treatment. Recovery often occurred, and the rapidity and completeness of recovery was dependent on the cumulative dose. Recovery could take three years, but no further improvement was noted after five years. Recovery occurred in more than 70% of patients who received a cumulative dose <7.5 g/m^2; in contrast recovery occurred in less than 10% of patients who received >7.5 g/m^2.

MEASURES TO PRESERVE FERTILITY AFTER CYCLOPHOSPHAMIDE THERAPY

In recent years several options have become available to preserve fertility in women and men, as discussed in detail by Pendse *et al.*[17] Based on observations that the risk of gonadal dysfunction was lower in prepubertal girls, suppression of the ovarian cycle has been advocated as an option. The use of oral contraceptive agents has been claimed to lower the risk of amenorrhoea; however, this claim is based on an uncontrolled study reported in 1981.[18] No more reports have been published since, and well-documented data are lacking. In animal experiments loss of primordial follicles was attenuated by administration of gonadotropin-releasing hormone (GnRH) agonists. These drugs have been successfully used in two studies, one in patients with Hodgkin's disease and another in patients with SLE. These studies included a limited number of patients, and were not randomised.[17] Still, results look promising, sustained amenorrhoea occurring in 16 of 27 historical controls and in only one of 25 patients treated with a GnRH agonist. Controlled studies are needed to determine the benefits of these agents. Unfortunately, the use of these agents has been associated with flares of SLE disease activity.

Other strategies to preserve fertility in women include cryopreservation of primordial follicles, oocytes, embryos, and ovarian tissue. Thus far, these should be considered experimental therapies.

For male patients cryopreservation of sperm is a well-established procedure to preserve fertility. It is important to realise that the quality of the sperm is often low in patients with systemic diseases even before starting immunosuppressive therapy. Fortunately, newer techniques such as *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection may allow fertilisation with minimal amounts of viable sperm.

A recent study suggested benefits from drug treatment using testosterone to preserve fertility in men.[19] In this small randomised study that included 15 male patients, aged between 23 and 35 years, five received daily oral cyclophosphamide, five received monthly intravenous pulses of cyclophosphamide and five were treated with i.v. cyclophosphamide plus intramuscular testosterone. All patients developed azoospermia during therapy, after six months recovery was noted in all five patients treated with testosterone and in only one of ten untreated patients. Although promising, certainly more data are needed before such an approach can be routinely applied.

IS THE SUPERIORITY OF LONG-TERM I.V. CYCLOPHOSPHAMIDE PROVEN BEYOND DOUBT?

Which regimen is the golden standard?

The use of long-term i.v. cyclophosphamide has been propagated by controlled studies conducted by the National Institutes of Health (NIH).[1-5,20] This 'NIH regimen' has thus become the golden standard in the Netherlands as advocated by the Dutch SLE study group. However, is there a real 'golden standard'? *Table 3* provides an overview of the NIH studies. From the table it is evident that the NIH studies have not used one regimen consistently, rather each study has used a somewhat modified regimen. Thus it is clear that the currently advocated regimen has never been formally tested in controlled trials. Furthermore, it is of interest to note the actual number of patients involved in the NIH studies. In fact, conclusions on the risks and efficacy of long-term i.v. cyclophosphamide are based on data derived from 67 patients in total.

Table 3
The standard NIH regimen: one regimen? Many data?

STUDY	SCHEDULE	PATIENTS (N)
Austin/Steinberg[2]	0.5-1.0 g/m² every 3 months, duration of therapy median 4 years	20
Boumpas[3]	0.5-1.0 g/m² every month for 6 months; thereafter every 3 months for 24 months	20
Gourley/Illie[5]	1.0 g/m² every month for 6 months; thereafter every 3 months for at least 24 months (monthly administration repeated if no improvement after 12 months; quarterly administration continued for 24 months after reaching renal remission	27

Is the superiority of long-term i.v. cyclophosphamide proven?

Most investigators agree that oral prednisone monotherapy is insufficient for patients with SLE nephritis. Newer treatment regimens have, therefore, included alternative immunosuppressive agents such as azathioprine, cyclophosphamide or i.v. pulses of methylprednisolone. It is claimed that the NIH regimen consisting of six monthly pulses of i.v. cyclophosphamide followed by three-monthly pulses for two years, is superior; however, this claim is not supported by the data. In fact, even in a recent follow-up analysis of the NIH data, Illei *et al.* acknowledge that when comparing i.v. cyclophosphamide with i.v. methylprednisolone there were no differences among the treatment groups in risk for death or end-stage renal disease in an intention-to-treat analysis.[5] A difference only became apparent if the definition of failure was extended to include the need for additional immunosuppressive therapy, as more patients in the i.v. methylprednisolone group needed cyclophosphamide treatment at some time point in the course of their disease. Also in the other NIH studies long-term i.v. cyclophosphamide did not result in significantly higher renal survival rates when compared with azathioprine-based regimens or a regimen consisting of short-term i.v. cyclophosphamide (six i.v. pulses only).[1,3,20] Thus, the superiority of long-term i.v. cyclophosphamide is not proven on hard endpoints.

A recent meta-analysis published in the February issue of the American Journal of Kidney Diseases strengthens this conclusion.[21] The use of cyclophosphamide did not significantly reduce the risk of ESRD or death. Admittedly, there was a lower risk of doubling of serum creatinine in cyclophosphamide-treated patients. However, it is debatable whether doubling of serum creatinine is a reliable endpoint. Most controlled studies have used doubling of serum creatinine to define treatment failure and have allowed patients to switch to the alternative regimen at that point of time. If renal insufficiency can be prevented by switching to the alternative regimen, doubling of serum creatinine does not herald ESRD, and thus cannot be considered a hard endpoint. The conclusions of the studies should be read as follows: patients treated with long-term i.v. cyclophosphamide have a lower risk of needing additional courses of i.v. cyclophosphamide during follow-up. Interpretation of the above-mentioned meta-analysis is also hampered by the fact that the authors have piled the data of studies that used both oral and i.v. cyclophosphamide at dosages ranging from 3 to 50 grams.

Cohort studies including more patients than any of the NIH studies have provided compelling data to suggest that acceptable renal survival rates can be obtained by using regimens that contain no or only limited amounts of cyclophosphamide. Bono *et al.* recently reported an extended follow-up of patients with lupus nephritis treated by Cameron's group at Guy's Hospital.[22] All 110 patients were followed for at least ten years, 64 patients had lupus nephritis class III or IV, and the majority were treated with prednisone and azathioprine. The cumulative incidence of end-stage renal disease was 20% at ten years with no further events thereafter. On reviewing the literature, Bono and Cameron conclude that 'no data to date have demonstrated a superior effect of one immunosuppressive regimen over another when added to prednisone'.

The therapeutic efficacy of a regimen that contained a limited amount of cyclophosphamide is also suggested by Korbet, who has analysed the long-term outcome of patients who were included in a trial that studied the value of add-on plasmapheresis therapy.[23] A total of 86 patients were included in this study in the period 1981 to 1988. Patients were treated with prednisone with added cyclophosphamide in a dose of 2 mg/kg/day for approximately eight weeks. With this regimen a complete remission was obtained in 43% of patients. Notably, remission rate was higher in patients with an initial serum creatinine <124 μmol/l and in white patients. Renal survival was 94% at ten years in the remission group.

Finally, the efficacy of a low-dose cyclophosphamide regimen has recently been proven in two controlled studies.[24,25] The Eurolupus trial was a randomised, controlled study, comparing low-dose cyclophosphamide (six pulses of 500 mg cyclophosphamide every two weeks) with high-dose cyclophosphamide (eight pulses of 750 mg/m² in a one-year period).[24] All patients received azathioprine in the maintenance phase. The proportion of patients who reached a

remission (approximately 80% of patients at 48 months) or remained free of a relapse (approximately 60% at 48 months) was similar in both groups. In a recently published study Contreras *et al.* have compared i.v. cyclophosphamide as maintenance therapy with azathioprine or mycophenolate mofetil.[25] All patients received six monthly pulses of i.v. cyclophosphamide as induction therapy. Event-free survival was lowest in the patients who received cyclophosphamide maintenance therapy. Although the number of patients was small, this study certainly indicates that long-term i.v. cyclophosphamide is not superior.

HIGH-DOSE CYCLOPHOSPHAMIDE AND THE RISK OF RENAL FLARES

It has been proposed that the benefits of high-dose cyclophosphamide may only become apparent after very long follow-up (>10-20 years). Long-term cyclophosphamide may more effectively prevent slow ongoing fibrosis. Furthermore, renal flares were more common in patients receiving short-term i.v. cyclophosphamide.[3] Since the attainment of a complete remission was associated with an improvement in long-term renal survival, renal flares were considered to be early predictors of poor outcome. However, also in this regard the data do not allow such a conclusion. First, the above-mentioned study in which the patients received short-term i.v. cyclophosphamide can be criticised because the patients were not on additional immunosuppressive therapy with azathioprine, which is common practice in Europe. Moreover, in their analysis of the long-term follow-up of the NIH data Illei *et al.* conclude that renal flares do not necessarily result in loss of renal function if treated with additional immunosuppressive agents.[4]

CONCLUSIONS

Patients with lupus nephritis should be advised of the risk of permanent infertility associated with the use of cyclophosphamide. These risks are dependent on the age of the patient and on the cumulative dose of cyclophosphamide. Patients must be advised to seek additional counselling by an obstetrician/gynaecologist. Cryopreservation of sperm is well-established method of preserving fertility. Other measures are currently under study. The available data suggest that patients with lupus nephritis can be effectively treated with regimens that contain no or limited amounts of cyclophosphamide (<10 g). This is particularly so if patients are white and have moderately impaired renal failure. Patients should be warned that additional cyclophosphamide therapy may be needed if disease activity persists or if severe nephritic flares develop. Fortunately, this will only occur in a minority of patients.

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