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EDUCATIONAL REVIEW

Fertility preservation in patients receiving cyclophosphamide therapy for renal disease

Radha Gajjar · Steven D. Miller · Kevin E. Meyers · Jill P. Ginsberg

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Abstract Cyclophosphamide continues to have an important role in the treatment of renal disease, including nephrotic syndrome and lupus nephritis, despite known complications of gonadotoxicity and potential infertility in both male and female patients. It is important that the physician recommending this therapy mitigates the effect of the drug on fertility by adhering to recommendations on dosing limits and offering fertility-preserving strategies. In addition to well-established methods, such as sperm banking and embryo cryopreservation, advances in reproductive technology have yielded strategies such as oocyte cryopreservation, resulting in more fertility-preserving options for the pediatric patient. Despite these advances, there continues to be a significant barrier to referral and access to sperm banks and fertility specialists. These issues are further complicated by ethical issues associated with the treatment of pediatric patients. In this review we explore the development of recommended dosing limits and include a discussion of the available fertility-preserving methods, strategies for increasing access to fertility specialists, and the ethical considerations facing the pediatric healthcare provider.

S. D. Miller
Department of Pediatrics, The Johns Hopkins Hospital, Baltimore, MD, USA

R. Gajjar · K. E. Meyers
Division of Nephrology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

J. P. Ginsberg
Division of Oncology, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

R. Gajjar (✉)
Division of Nephrology, The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104, USA
e-mail: gajjara@email.chop.edu

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Introduction

In 1949, Chasis et al. published the first paper demonstrating that renal manifestations of glomerulonephritis could be reversed by the administration of a nitrogen mustard compound [1]. In 1952, patients with nephrotic syndrome (NS) who relapsed after standard therapy with steroids were also found to respond to treatment with nitrogen mustard [2]. Treatment of NS with nitrogen mustard fell out of favor until the introduction of cyclophosphamide (CPO), a less toxic nitrogen mustard derivative, in 1967. CPO was found to produce prolonged remission in patients with relapsing NS [3, 4] and was approved by the Federal Drug Administration as an anticancer agent. It continues to have significant clinical applications in nephrology, oncology, and rheumatology [5].

The deleterious effect of CPO therapy on testicular function began to emerge in the literature approximately 5 years after its introduction, with studies consistently demonstrating oligospermia or azoospermia in patients receiving CPO [6, 7]. In a 1992 study, Meistrich et al. followed sperm counts through the treatment course with CPO and demonstrated a drop from normal sperm count levels prior to treatment to azoospermic levels after 12 months of therapy [8]. Amenorrhea secondary to CPO therapy was also noted in the early 1970s, with loss of menstrual cycles in 18 of 34 previously menstruating women who were treated with CPO for glomerulonephritis, nephropathy, and lupus nephritis (LN) [8, 9]. The risk of sustained amenorrhea has been shown to develop in a dose-dependent and age-dependent pattern, with older patients and patients receiving higher cumulative doses at an increased risk of developing amenorrhea [10]. It is important

to note that a similar protective effect of age is not noted in male patients.

Despite long-standing concerns for these significant side effects and implications for fertility, CPO continues to be a key therapy for patients with steroid-dependent NS and rheumatologic diseases, including LN. A 2013 meta-analysis reaffirmed the role of alkylating agents, specifically CPO and chlorambucil, as the most effective second-line therapy in pediatric patients with steroid-sensitive NS who relapse despite steroid therapy [11]. Therapy for frequently relapsing NS with a combination of oral CPO and steroids for 8–12 weeks results in a significantly reduced risk of relapse at both the 6- to 12-month mark and the 12- to 24-month mark compared to prednisone alone [11]. The use of CPO for proliferative LN has resulted in the reduction of mortality from over 70 % in the 1950s and 1960s to approximately 10 % in recent years [12]. Recent studies have also demonstrated the equivalent efficacy of mycophenolate mofetil (MMF) in inducing remission in LN. The less severe side-effect profile of MMF has made it an attractive alternative therapeutic option, but long-term follow-up studies are needed [12]. The 2012 American College of Rheumatology Clinical Practice Guidelines for Lupus Nephritis established MMF as the preferred induction agent compared to CPO for patients in whom fertility preservation (FP) is a major consideration [13]. While the role of CPO in LN therapy may be evolving, at the present time it continues to be an important drug in induction therapy for severe renal disease.

While CPO has been used in patients with renal disease since the 1960s, advances in reproductive technology have resulted in FP receiving increasing attention during the treatment of patients with renal disease. A series of patient surveys have established that parenthood is important to young adults who have been treated with gonadotoxic therapy for oncologic disease, with up to three-fourths of respondents expressing a desire to have offspring in the future [14]. Patient preference and a perception of physician responsibility to mitigate the effects of gonadotoxic drug therapy have made FP a nationally accepted standard practice among pediatric oncologists [15]. While the majority of research on FP in the pediatric patient has been in the realm of oncology, a similar standard of care should be adopted for the pediatric patient with renal disease. The limitations of current data, including variable treatment regimens which cannot be directly compared and combination therapy with other gonadotoxic drugs in the setting of treatment for malignancy, must be taken into consideration. In this review, we address the risk of infertility associated with CPO therapy, fertility-preserving options for both male and female patients, and the importance of consistent and timely referrals to fertility specialists. Further, we discuss the unique challenges and ethical considerations facing pediatric nephrologists.

Dosing recommendations for treatment with CPO

Initially developed as an antineoplastic drug, CPO is classified as an alkylating agent. The mechanism of action involves the transfer of alkyl groups to cellular constituents and reaction with DNA bases, which damages DNA repair mechanisms and ultimately results in cell death [16, 17]. The mechanism of the effect on fertility, however, remains unknown [17].

Dosing limits for steroid-dependent NS range from 168–252 mg/kg of CPO, which corresponds to the gonadotoxic dose limit in males [18]. This dosing limit was initially established in 1981 in a long-term follow-up study by Trompeter et al. in which they evaluated adult males who had been treated with CPO as children, with doses limited to 3 mg/kg CPO for an 8-week course (168 mg/kg) [19]. These authors found that despite lower ejaculate volume and sperm densities and a higher percentage of immotile and abnormal spermatozoa compared to healthy controls, these abnormalities were not severe enough to suggest permanent infertility [19]. The treatment regimen recommended for steroid-dependent NS is a dose of 2–3 mg/kg/day CPO given orally for 8–12 weeks [18]. Ueda et al. recommend a dose of 2 mg/kg/day given orally for 8 weeks and demonstrated no significant benefit in extending the duration of therapy beyond 8 weeks, with comparable relapse-free rates at the 5-year follow-up and similar relapse-free intervals between the two durations of therapy [20]. However, in this report, these authors do include a discussion of one study that suggests benefit to extending therapy to a 12-week course, but outline a shorter duration of therapy as comparable to a 12-week course [20]. This regimen has been proven to be effective for steroid-dependent NS and also conforms to the limits recommended for FP.

Treatment of LN, in contrast to treatment of NS, employs gonadotoxic dose limits measured in gram per square meter (g/m^2) [8], which is the standard of care in pediatric oncology [15]. As discussed below, the recommended dosing limit in male patients is 7.5 g/m^2 based on sperm count recovery [8]. While this dosing limit is also accepted for the female patient, the protective effect of age allows younger patients to tolerate a higher cumulative dose, up to 10–15 g/m^2 [10]. Classically, LN treatment has involved a variable length course of 0.75–1 g/m^2 per month of CPO for up to 6 months, then every 3 months for up to 2 years [21]. Newer regimens include a shorter course of CPO for 3–6 months with conversion to MMF [22] or tapering to a low-dose CPO regimen [23]. The Euro-Lupus Nephritis Trial established that a treatment regimen consisting of induction therapy with a low-dose regimen of 500 mg administered every other week for a total of six doses (3 g total) followed by azathioprine for maintenance therapy is clinically comparable to a traditional high-dose regimen [23]. The authors reported no cases of secondary amenorrhea in the 14-year follow-up published. This regimen

was included in the ACCESS trial and was recently reported to produce a greater proportion of patients achieving complete remission in 24 weeks than prior trials of both CPO therapy and MMF. The study group included high-risk patients aged ≥18 years of whom 39 % were of African ancestry and 40 % Hispanic ethnicity [23].

Risk of infertility with the use of CPO in the male patient

While male patients develop profound oligospermia and eventually azoospermia over the course of therapy with CPO, this effect can be reversible over time in many patients, with a possibility of recovering fertility if dosing limits are maintained.

Dose-dependent recovery of fertility was demonstrated in a 1992 study by Meistrich et al. [8]. Sperm counts were monitored in patients receiving combination chemotherapy as part of a treatment regimen for either osteosarcoma or a soft tissue sarcoma. The data demonstrated that all patients who had sperm counts evaluated during chemotherapy were azoospermic after 12 months of treatment, despite being normospermic prior to the initiation of therapy. Recovery of normospermia, defined as sperm counts of at least 10 million cells/ml, was found to be dose-dependent. While only 11 % of patients who received CPO doses higher than 7.5 g/m^2 demonstrated normospermia, 72 % of patients whose cumulative dose was less than 7.5 g/m^2 were able to demonstrate recovery of the sperm count to a normospermic level. Although the dosing data in the oncology literature is based on combination chemotherapy, these limits are generally accepted, given the lack of data of therapy with CPO alone [8].

A recent survey of U.S. pediatric nephrologists showed that while 95 % of physicians monitor cumulative dose of CPO, variable dosing regimens are used, with 40 % of physicians dosing in grams per square meter, 23 % using milligrams per kilograms and 30 % using both regimens [24]. It is important to note that large adolescent males dosed in milligrams per kilogram can exceed the gonadotoxic limit in grams per square meter even if the former dose limits are respected [25]. Additionally, some patients may require a second course of CPO for refractory disease, resulting in very high cumulative doses with the attendant increased risk of infertility [25]. These concerns underscore the importance of appropriate counseling and intervention prior to initiation of CPO therapy.

Risk of infertility with use of CPO in the female patient

Infertility in the female patient is nuanced and more difficult to predict than that in male patients. The established dosing ranges cited above in both milligrams per kilogram and grams per square meter apply equally to males and females, but the limits of gonadotoxicity are somewhat different. The gonadotoxic effects on the ovary and oocytes are thought to be cumulative and irreversible given that the number of oocytes is determined in fetal life [26–29]. CPO therapy in female patients causes ovarian toxicity in an age- and dose-dependent manner, with progressively smaller doses resulting in sustained amenorrhea in older women [26–29]. The issues with different dosing regimens, including weight-based dosing and cumulative effects with repeat therapy, that are outlined above also apply to the female patient.

The difficulty in defining fertility in the female patient is a major limitation of studies of the gonadotoxic effects of CPO in female patients. Indicators of fertility found in the literature range from the presence or absence of menstruation to objective interpretation of serum levels of estradiol and gonadotropins to define premature ovarian failure, which makes a direct comparison between various studies difficult. In a 1993 study, Boumpas et al. evaluated the risk of sustained amenorrhea in patients with systemic lupus erythematosus (SLE) receiving a pulse-dose CPO treatment regimen. A group of 39 women aged <40 years were treated with either a short course (7 treatments) or long course (≥15 treatments) of pulse-dose CPO at a dose of 0.5–1 g/m^2 per treatment. The age- and dose-dependent risk of sustained amenorrhea was clearly demonstrated, with an increased incidence in older patients and those receiving higher cumulative doses of CPO. Sustained amenorrhea was noted in two of 12 (17 %) patients aged <25 years included in the long-treatment course, while none of the four patients in the short-treatment group in this age range demonstrated sustained amenorrhea. In contrast, among patients who were aged ≥31 years, 100 % in the long-treatment group were affected, compared to 25 % in the short-treatment group [10]. Intermediate risk was noted in patients aged between 26 and 30 years, with 43 % of patients in the long-treatment group and 12 % of those in the short-treatment course developing amenorrhea [10]. These results clearly demonstrate a protective effect of age that allows a higher cumulative dose in younger patients, with a small risk of sustained amenorrhea with doses of 10–15 g/m^2 [10].

A similar age-dependent effect was demonstrated by Ioannidis et al. in a cohort of 67 premenopausal women who received monthly pulse-dose steroids at a dose of 0.75–1 gm/m^2 for 6 months, followed by doses every other month for the following 12 months [26]. The patients were divided into a younger age group (≤31 years) and an older age group (≥32 years) and were monitored for sustained amenorrhea, defined as lack of menses lasting ≥12 months. In the younger age group, 5 of 44 women treated with CPO developed sustained amenorrhea compared to 16 of 23 women who were aged ≥32 years. These authors demonstrated that the dose resulting in sustained amenorrhea, i.e., amenorrhea lasting ≥12 months, in 50 % (D_{50}) of patients aged ≥32 years was 8 gm/m^2 and that the D_{90} was 12 g/m^2 [26]. Given the clear age-dependent and

dose-dependent effects on fertility in the female patient, FP methods should be considered in pediatric patients who are anticipated to receive treatment exceeding the 10–15 g/m^2 dosing limit.

Currently available fertility-preserving methods for males

Sperm banking is a well-established, effective, and accepted practice for collecting and storing male gametocytes in adolescent patients who are at Tanner III stage of development or greater [15, 30, 31]. In an adolescent male who is at the Tanner III stage of development or greater and can ejaculate after masturbation, it is a noninvasive procedure that can be accomplished prior to receiving therapy [31]. Adequate samples can be collected with only 24–48 h between ejaculations so that storage of one or two samples before treatment could be accomplished in all but the most emergent cases [14]. If a patient is unable to produce a sample, alternative sources of sperm include microsurgical epididymal aspiration, electroejaculation, or testicular biopsy. New methods for in vitro fertilization (IVF) include intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI), and these methods have improved fertility outcomes for men with low sperm counts or motility defects [28, 32].

A recent national survey of pediatric nephrologists showed that 56 % of respondents recommend sperm banking to some male adolescent patients and 53 % of respondents refer some male adolescent patients to a fertility specialist [24]. The low risk of collecting a sperm sample in an eligible male coupled with the benefit of avoiding potentially permanent infertility and the already broad utilization of sperm banking by pediatric nephrologists make sperm banking a highly feasible therapeutic option for patients with renal disease [25]. Our recommendation is that all teenage boys whose therapy will include CPO should be given the opportunity to cryopreserve sperm regardless of their planned dose of CPO. Doses of CPO are sometimes extended in refractory renal disease so it is not always possible to accurately predicate a cumulative exposure in these patients.

Options for FP in the prepubescent male are limited to experimental protocols. The goal of FP in patients who have not yet undergone spermatogenesis is preservation of spermatogonial stem cells (SSCs) [33]. SSCs can be isolated from aspirated cell suspension or from cryopreserved immature testicular tissue (ITT). The advantage of ITT cryopreservation is the maintenance of tissue architecture and cell-to-cell contacts between Sertoli cells and germinal stem cells, which eventually become important for the maturation of SSC [33]. Although there has been success with fertilization in animal models, preserved testicular tissue has not yet been demonstrated to be successful for spermatogenesis in humans. Ginsberg et al. showed that the collection of testicular tissue for cryopreservation is a feasible, safe, and acceptable procedure in a high-risk pediatric oncology population [34], but this experimental procedure is unlikely to be available to patients with renal disease in the near term.

Currently available fertility-preserving methods for females

Major advances in assisted reproductive technology for female patients over the past 20–30 years have allowed the field to begin to expand beyond the well-established practice of embryo cryopreservation and hormonal ovarian suppression. Emerging techniques include oocyte cryopreservation with vitrification of both mature and immature oocytes, thereby allowing the storage of an unfertilized egg. The introduction of cryopreservation of ovarian tissue through experimental protocols has the potential to expand the availability of assisted reproductive technology to premenstrual females. These exciting advances in technology have been greeted with enthusiasm by the medical community, which may partially explain why a recent national survey of pediatric nephrologists showed that 58 % of respondents had referred some female adolescent patients being treated with CPO to a fertility specialist [24]. Most young girls with renal disease will not be treated with CPO doses that will place them at significant risk for acute ovarian failure (i.e. >10–15 g/m^2) [10]. They may, however, be more likely to experience premature ovarian failure. Because acute ovarian failure is unlikely, FP methods at the onset of renal disease may not be necessary but should be discussed on a case-by-case basis so that patients and families can be informed of their risks and be able to consider their options.

Embryo cryopreservation is the best-established fertility-preserving technique in females, with proven pregnancy success rates of up to 60 % [35]. The major limitation of this technique is that the female patient must either identify a male partner or agree to the use of a sperm donor for fertilization of the ovum prior to cryopreservation [17, 36]. Further, the hormone therapy administered for ovarian stimulation may pose a risk for exacerbation of hormone-responsive conditions, such as lupus [30]. Although fertility specialists might be able to help eligible patients access this highly effective therapy, these limitations make embryo cryopreservation a problematic option in the vast majority of pediatric patients with renal disease. It is important for practitioners to maintain a working knowledge of recent advances and available techniques, even if still in an experimental phase [37].

Another commonly employed, albeit controversial, technique is the use of hormones to induce a quiescent state in the ovaries, which theoretically results in a protective effect. The use of gonadotropin-releasing-hormone agonists (GnRH-a) to induce hormonal suppression of activity in the ovary continues to be debated as conflicting results regarding their true protective effects have been reported [30]. A 2005 study

demonstrated a protective effect of monthly depot leuprolide injections against the development of premature ovarian failure in patients receiving intravenous (IV) CPO for severe SLE [38]. Hormonal suppression is induced by using either GnRH-a, such as leuprolide, or GnRH antagonists [30]. The suggested mechanism of protection in ovarian suppression is one of decreased blood flow to the dormant gonads through increased luteinizing hormone/follicle stimulating hormone release and decreased estrogen production, thus limiting exposure to gonadotoxic drugs [30]. Therapy with a GnRH analog can result in menopausal symptoms, including hot flashes, emotional lability, vaginal dryness, and decreased libido, but these generally resolve within 4–12 weeks after cessation of therapy [30]. It is important to note that GnRH-a treatment is also associated with an accelerated loss of estrogen-dependent trabecular bone mass [39], with contradictory results reported regarding reversibility [38]. Hormonal suppression is a therapy with limited efficacy but relatively low risk, making it a potentially suitable intervention on a case-by-case basis, with fertility specialists functioning as the gatekeeper for this therapy. A recent study involving triptorelin, a GnRH-a, has recently been conducted to test the safety of this medicine when used for the protection of the ovaries during cyclophosphamide therapy for SLE. The results of this study may impact the future use of ovary-protecting agents in this patient population (Hermine Brunner, Principal Investigator, ClinicalTrials.gov Identifier:NCT00124514).

Oocyte cryopreservation provides an opportunity for assisted reproduction when embryo cryopreservation is not an option [40]. When the decision is made to have children, harvested unfertilized oocytes are fertilized in vitro via ICSI and then implanted. A new technique called vitrification has resulted in safer freezing and thawing of cryopreserved oocytes with dramatically improved pregnancy rates, making oocyte cryopreservation a safe option [41], Recent guidelines acknowledge that more evidence is needed before mature oocyte cryopreservation can be routinely used in lieu of embryo cryopreservation [41]. The disadvantages in a pediatric nephrology population include the requirement that patients have achieved menarche and spontaneous ovulation and the need for an operative procedure to harvest the oocytes. Further, the relatively lower risk in younger patients of amenorrhea and infertility potentially tilts the risk/benefit ratio in favor of no intervention. At the same time, this technique is more likely to be realistic than embryo cryopreservation in a pediatric population, so referral to a fertility specialist may be indicated for interested patients and families.

At this time, the only available fertility-preserving method in prepubescent females is experimental ovarian tissue cryopreservation. This method has also been used for postpubertal females who cannot delay therapy for spontaneous ovulation or hormonal stimulation [42]. The procedure involves surgical removal of either a portion of ovarian cortical tissue or the whole ovary [43], followed by storage of the tissue in a tissue bank; it is then reimplanted when the patient is ready for reproduction. This procedure has resulted in a few successful pregnancies worldwide [44], but to date no live births have been reported in women who cryopreserved tissue before puberty. Although this is a promising new therapy, the experimental nature of the procedure is likely to serve as a major barrier in the near term for pediatric patients with renal disease.

Barriers to FP

A 2007 survey of oncology providers at major academic pediatrics centers revealed that while 92.8 % of physicians demonstrated a desire to consult with fertility specialists, only 34.6 % had actually successfully referred patients in their practice [37]. Similarly, in a national survey of pediatric nephrologists, 80 % of respondents agreed that referral for FP was a major concern for them, while only 53 % referred some male patients to a fertility specialist and 58 % referred some female patients. Nearly 50 % of those surveyed cited "patient is too ill to delay treatment" as a major barrier to referral for FP [24]. This is indeed a significant barrier in the case of patients with rapidly progressive glomerulonephritis or other critical illnesses, although creative solutions, such as sperm banking in the hospital, may sometimes be found. In the same survey, nearly 30 % of respondents cited lack of referral infrastructure as a major barrier to referral [24]. The relative rarity of lupus nephritis and steroid-dependent NS means that spending extensive time developing expertise in FP may not be feasible for pediatric nephrologists, making referral the best option. For male patients, the answer lies in referral to local sperm banks or consultation with colleagues in the department of oncology who may have a more established referral base. For female patients interested in fertility-preserving options, referral to a Reproductive Endocrinology and Infertility (REI) specialist is crucial for initial evaluation and workup, as well as recommendations regarding the most appropriate method of FP. Further, fertility specialists will likely assist with the use of preserved tissues when the decision is made to reproduce [29].

Sperm banking is an established component of oncology care in patients at risk for infertility in France, Norway, Japan, and the UK and is underwritten by the national healthcare system [45]. In the USA, coverage for FP varies by state, with insurance in some states covering initial costs of the harvesting and banking procedure but not always paying the annual storage fee [46, 47]. The estimated cost of storing three samples of sperm produced by masturbation for 3 years in a sperm bank in 2006 was close to $1,500 [42]. Oocyte retrieval, cryopreservation, and storage for 1 year costs $5,538, with an additional $3,162 incurred at the time of thawing, IVF, and

embryo transfer [42]. While these costs can pose a significant burden for a family facing the possibility of covering these costs out of pocket, it must be noted that patients do not cite cost as a significant consideration when making the decision whether or not to pursue fertility-preserving methods [31]. Patients with insufficient insurance coverage can be referred to nonprofit organizations such as Fertile Hope (www.fertilehope.com) that can help defray costs. Some patients may fall into a gray area in which they are ineligible for benefits but lack the financial means to access FP. Broader discussion and advocacy about access to FP could benefit these patients.

Ethical considerations

The concept of FP inherently raises ethical issues that are complicated by parental involvement in the consent process for pediatric patients. A fundamental concern is that families are asked to make a proxy decision regarding the possibility of childbearing for a patient who may be decades away from thinking about having a child. As with any treatment or procedure requiring informed consent, the discussion of FP procedures introduces the possibility of parents imposing their hopes or beliefs on their child. While the parents are ultimately responsible for providing consent for fertility-preserving interventions, the patient should be involved in the process to the extent of their ability in keeping with the American Academy of Pediatrics (AAP) 1995 Committee on Bioethics statement on Informed Consent, Parental Permission and Assent in Pediatric Practice [48]. The wishes of a child of assenting age should be taken into consideration over those of the family in the case of significant differences of opinion. The Ethics Committee Statement from the American Society for Reproductive Medicine clearly states that the procedure should not be performed if the patient objects [29].

The integrity of gonadal tissue, ova, and sperm stored for prolonged periods of time should also be considered, as children may not be of an age to use these tissues for many years [42]. The recent introduction of vitrification and other preservation techniques is likely to extend the survival of tissues to decades, but the empiric demonstration of this longevity remains to be demonstrated.

Conclusion and recommendations

Despite the risks of infertility, CPO remains an indicated treatment for pediatric patients with NS and LN. Prevention of gonadal failure should be the provider's foremost goal with regard to FP, with adherence to the established dosing limits for male patients, which is also accepted in female patients despite a lack of evidence for a definitive dosing threshold [30].

While adherence to dosing recommendations is largely protective against effects on fertility in the male patient, 28 % of patients in the study by Meistrich et al. did not regain normospermic levels after completion of CPO therapy [8]. This result suggests a certain level of unpredictability with respect to long-term effects on fertility, which obligates a discussion of FP with all patients receiving gonadotoxic therapy. Dedicated studies addressing the effects of current treatment regimens of CPO on fertility are sparse and these effects need to be addressed in order to move forward.

As the technology for preserving fertility in patients receiving gonadotoxic therapy continues to become more sophisticated, appropriate and timely referral to a fertility specialist or sperm bank by the prescribing physician has become increasingly important. Nephrologists should consult with local oncologists or REI specialists to familiarize themselves with the resources available in their community and develop relationships with fertility specialists. For those without local resources, a number of online sites, including that of the Livestrong Foundation, can provide information, financial assistance, and in some cases a mail-in sperm banking kit. The AAP recommends that the decision about candidacy for FP should be guided by an institutional policy that employs a multidisciplinary approach with a protocol integrated into each patient's care plan [42]. The use of CPO carries with it a responsibility to mitigate the toxic side effects of the drug, making FP a critical part of excellent care.

Key concepts

1. The gonadotoxic effects of CPO are well-known. Gonadotoxicity is dose-dependent in the male patient and has an age-and dose-dependent effect on female patients.
2. The recommended dosing limit in treatment for steroid-dependent nephrotic syndrome is 168 mg/kg, while the established limit in lupus nephritis is 7.5 g/m^2, up to 10–15 g/m^2 in young female patients. Low-dose treatment regimens for lupus nephritis have comparable clinical outcomes to traditional regimens and should be considered.
3. The unpredictable risk of developing permanent sterility obligates a discussion of FP in all patients.
4. The most common methods of FP are sperm banking and embryo cryopreservation.
5. Resources for more information include sperm banks, REI specialists, local oncologists, and the Livestrong Foundation.

Questions (answers to be found following the References)

1. In a recent survey, 80 % of pediatric nephrologists agreed that referral for FP was a major concern for them; however, only 53 % referred some male patients to a fertility specialist and only 58 % referred some female patients. Barriers to referral include:

   a. Lack of local access to appropriate specialists
   b. Patient is too ill to delay treatment
   c. Infrequent referrals resulting in poorly developed referral network
   d. Cost barriers to the family
   e. All of the above
2. What is the gonadotoxic dose limit of CPO for males?

   a. <2 g/m^2
   b. <4.5 g/m^2
   c. <7.5 g/m^2
   d. <10 g/m^2
   e. <15 g/m^2
3. A 12-year-old male with steroid-dependent nephrotic syndrome returns to clinic with persistent hyperglycemia. The decision is made to start the patient on CPO. At what Tanner stage will he be able to reliably use the sperm bank?

   a. Tanner 1
   b. Tanner 2
   c. Tanner 3
   d. Tanner 4
   e. Tanner 5
4. A 9-year-old girl at Tanner 1 stage of pubertal development is admitted to the hospital for urgent initiation of CPO therapy for lupus nephritis. What FP option might be available to the patient, and what is a major limitation of this intervention?

   a. Embryo cryopreservation; poor success rate when reimplanted
   b. Ovarian tissue cryopreservation; limited only to experimental protocols
   c. Oocyte cryopreservation; cryopreserved tissues are not stable over long periods of time
   d. Oocyte cryopreservation; limited only to experimental protocols
   e. Ovarian tissue cryopreservation; a male partner or sperm donor is required
5. The most appropriate place to refer pediatric patients interested in FP is:

   a. Reproductive Endocrinology and Infertility (REI) doctor
   b. Pediatric oncologist specializing in fertility preservation
   c. Local sperm bank
   d. The Livestrong Foundation
   e. All of the above

References

1. Chasis H, Goldring W, Baldwin DS (1949) Effect of febrile plasma, typhoid vaccine and nitrogen mustard on renal manifestations of human glomerulonephritis. Proc Soc Exp Biol Med 71:565–567
2. Kelley VC, Panos TC (1952) The nephrotic syndrome in children. I. Clinical response to nitrogen mustard therapy. J Pediatr 41:505–517
3. Kyrieleis HA, Levtchenko EN, Wetzels JF (2007) Long-term outcome after cyclophosphamide treatment in children with steroid-dependent and frequently relapsing minimal change nephrotic syndrome. Am J Kidney Dis 49:592–597
4. White RH (1967) Cytotoxic drug therapy in steroid-resistant glomerulonephritis. Proc R Soc Med 60:1164–1168
5. Emadi A, Jones RJ, Brodsky RA (2009) Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol 6:638–647
6. Fairley KF, Barrie JU, Johnson W (1972) Sterility and testicular atrophy related to cyclophosphamide therapy. Lancet 1:568–569
7. Penso J, Lippe B, Ehrlich R, Smith FG Jr (1974) Testicular function in prepubertal and pubertal male patients treated with cyclophosphamide for nephrotic syndrome. J Pediatr 84:831–836
8. Meistrich ML, Wilson G, Brown BW, da Cunha MF, Lipshultz LI (1992) Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. Cancer 70:2703–2712
9. Uldall PR, Kerr DN, Tacchi D (1972) Sterility and cyclophosphamide. Lancet 1:693–694
10. Boumpas DT, Austin HA 3rd, Vaughan EM, Yarboro CH, Klippel JH, Balow JE (1993) Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. Ann Intern Med 119:366–369
11. Pravitsitthikul N, Willis NS, Hodson EM, Craig JC (2013) Non-corticosteroid immunosuppressive medications for steroid-sensitive nephrotic syndrome in children. Cochrane Database Syst Rev 10: CD002290
12. Henderson L, Masson P, Craig JC, Flanc RS, Roberts MA, Strippoli GF, Webster AC (2012) Treatment for lupus nephritis. Cochrane Database Syst Rev 12:CD002922
13. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD, Karpouzas GA, Merrill JT, Wallace DJ, Yazdany J, Ramsey-Goldman R, Singh K, Khalighi M, Choi SI, Gogia M, Kafaja S, Kamgar M, Lau C, Martin WJ, Parikh S, Peng J, Rastogi A, Chen W, Grossman JM, American College of Rheumatology (2012) American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res 64: 797–808
14. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S (2002) Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol 20:1890–1897
15. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Beck LN, Brennan LV, Oktay K, American Society of Clinical Oncology (2006) American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 24: 2917–2931

16. Silva CA, Bonfa E, Ostensen M (2010) Maintenance of fertility in patients with rheumatic diseases needing antiinflammatory and immunosuppressive drugs. Arthritis Care Res 62:1682–1690
17. Elizur SE, Chian RC, Pineau CA, Son WY, Holzer HE, Huang JY, Gidoni Y, Levin D, Demirtas E, Tan SL (2008) Fertility preservation treatment for young women with autoimmune diseases facing treatment with gonadotoxic agents. Rheumatology 47:1506–1509
18. Latta K, von Schnakenburg C, Ehrich JH (2001) A meta-analysis of cytotoxic treatment for frequently relapsing nephrotic syndrome in children. Pediatr Nephrol 16:271–282
19. Trompeter RS, Evans PR, Barratt TM (1981) Gonadal function in boys with steroid-responsive nephrotic syndrome treated with cyclophosphamide for short periods. Lancet 1:1177–1179
20. Ueda N, Kuno K, Ito S (1990) Eight and 12 week courses of cyclophosphamide in nephrotic syndrome. Arch Dis Child 65: 1147–1150
21. Sauter M, Anders HJ (2007) Lupus nephritis. Minerva Med 98:749–758
22. Dooley MA, Jayne D, Ginzler EM, Isenberg D, Olsen NJ, Wofsy D, Eitner F, Appel GB, Contreras G, Lisk L, Solomons N, ALMS Group (2011) Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. N Engl J Med 365:1886–1895
23. Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido Ed Ede R, Danieli MG, Abramovicz D, Blockmans D, Mathieu A, Direskeneli H, Galeazzi M, Gul A, Levy Y, Petera P, Popovic R, Petrovic R, Sinico RA, Cattaneo R, Font J, Depresseux G, Cosyns JP, Cervera R (2002) Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. Arthritis Rheum 46:2121–2131
24. Miller SD, Li Y, Meyers KE, Miller VA, Caplan A, Ginsberg JP (2014) Fertility preservation in pediatric nephrology: results of a physician survey. J Renal Care. doi:10.1111/jorc.12079
25. Miller SD, Ginsberg JP, Caplan A, Meyers KE (2012) Sperm banking in adolescents and young adult cancer patients: Sperm quality, patient, and parent perspectives. Arch Dis Child 97:765–766
26. Ioannidis JP, Katsifis GE, Tzioufas AG, Moutsopoulos HM (2002) Predictors of sustained amenorrhea from pulsed intravenous cyclophosphamide in premenopausal women with systemic lupus erythematosus. J Rheumatol 29:2129–2135
27. Blumenfeld Z, Shapiro D, Shteinberg M, Avivi I, Nahir M (2000) Preservation of fertility and ovarian function and minimizing gonadotoxicity in young women with systemic lupus erythematosus treated by chemotherapy. Lupus 9:401–405
28. Mersereau J, Dooley MA (2010) Gonadal failure with cyclophosphamide therapy for lupus nephritis: advances in fertility preservation. Rheum Dis Clin North Am 36:99–108
29. Ethics Committee of the American Society for Reproductive Medicine (2005) Fertility preservation and reproduction in cancer patients. Fertil Steril 83:1622–1628
30. Dooley MA, Nair R (2008) Therapy Insight: preserving fertility in cyclophosphamide-treated patients with rheumatic disease. Nat Clin Pract Rheumatol 4:250–257
31. Ginsberg JP, Ogle SK, Tuchman LK, Carlson CA, Reilly MM, Hobbie WL, Rourke M, Zhao H, Meadows AT (2008) Sperm banking for adolescent and young adult cancer patients: sperm quality, patient, and parent perspectives. Pediatr Blood Cancer 50:594–598
32. Hsiao W, Stahl PJ, Osterberg EC, Nejat E, Palermo GD, Rosenwaks Z, Schlegel PN (2011) Successful treatment of postchemotherapy azoospermia with microsurgical testicular sperm extraction: the Weill Cornell experience. J Clin Oncol 29:1607–1611
33. Wyns C, Curaba M, Vanabelle B, Van Langendonckt A, Donnez J (2010) Options for fertility preservation in prepubertal boys. Hum Reprod Update 16:312–328
34. Ginsberg JP, Carlson CA, Lin K, Hobbie WL, Wigo E, Wu X, Brinster RL, Kolon TF (2010) An experimental protocol for fertility preservation in prepubertal boys recently diagnosed with cancer: a report of acceptability and safety. Hum Reprod 25:37–41
35. Gonzalez C, Boada M, Devesa M, Veiga A (2012) Concise review: fertility preservation: an update. Stem Cells Transl Med 1:668–672
36. Cohen CB (2009) Ethical issues regarding fertility preservation in adolescents and children. Pediatr Blood Cancer 53:249–253
37. Goodwin T, Elizabeth Oosterhuis B, Kiernan M, Hudson MM, Dahl GV (2007) Attitudes and practices of pediatric oncology providers regarding fertility issues. Pediatr Blood Cancer 48:80–85
38. Somers EC, Marder W, Christman GM, Ognenovski V, McCune WJ (2005) Use of a gonadotropin-releasing hormone analog for protection against premature ovarian failure during cyclophosphamide therapy in women with severe lupus. Arthritis Rheum 52:2761–2767
39. Matta WH, Shaw RW, Hesp R, Evans R (1988) Reversible trabecular bone density loss following induced hypo-oestrogenism with the GnRH analogue buserelin in premenopausal women. Clin Endocrinol 29:45–51
40. Brambillasca F, Guglielmo MC, Coticchio G, Mignini Renzini M, Dal Canto M, Fadini R (2013) The current challenges to efficient immature oocyte cryopreservation. J Assist Reprod Genet 30:1531–1539
41. Practice Committees of American Society for Reproductive Medicine; Society for Assisted Reproductive Technology (2013) Mature oocyte cryporeservation: a guideline. Fertil Steril 99:37–43
42. Fallat ME, Hutter J (2008) Preservation of fertility in pediatric and adolescent patients with cancer. Pediatrics 121:e1461–e1469
43. Dillon KE, Gracia CR (2012) Pediatric and young adult patients and oncofertility. Curr Treat Options Oncol 13:161–173
44. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, Pellicer A, Dolmans MM (2011) Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. Ann Med 43:437–450
45. Schover LR (2009) Patient attitudes toward fertility preservation. Pediatr Blood Cancer 53:281–284
46. Basco D, Campo-Engelstein L, Rodriguez S (2010) Insuring against infertility: Expanding state infertility mandates to include fertility preservation technology for cancer patients. J Law Med Ethics 38: 832–839
47. Quinn GP, Vadaparampil ST, McGowan Lowrey K, Eidson S, Knapp C, Bukulmez O (2011) State laws and regulations addressing third-party reimbursement for infertility treatment: Implications for cancer survivors. Fertil Steril 95:72–78
48. Committee on Bioethics, American Academy of Pediatrics (1995) Informed consent, parental permission, and assent in pediatric practice. Pediatrics 95:314–317

Answers

1. e: Each of these issues was cited in a national survey of pediatric nephrologists as a barrier to successfully referring patients to fertility preservation.

2. c: A dosing limit of <7.5 g/m^2 was established in the oncology literature based on results showing sperm count recovery after azoospermia caused with CPO treatment.

3. c: Tanner Stage III is the pubertal stage at which males can consistently and reliably produce a sperm sample sufficient for sperm banking.

4. b: FP options for prepubertal patients remain limited. Ovarian tissue cryopreservation is an experimental technique available to prepubertal females. However, a successful pregnancy using cyropreserved ovarian tissue from prepubescent females has not yet been reported. There have been successful pregnancies after ovarian tissue cryopreservation and reimplantation in postpubescent females.

5. e: Each of these resources may be helpful on a case-by-case basis to improve access to FP options.