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Paediatric Update

Preventing organ-specific chemotherapy toxicity

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Abstract

Recent advances in treatment for pediatric cancers has increased overall survival rates. As more and more survive pediatric cancer, we continue to see the emergence of late effects of treatment within pediatric and the growing adult survivor population. The evaluation of late effects was initiated approximately two decades ago, and has become an extremely important facet of pediatric oncology. This review delves into several of the most serious organ-specific late effects of pediatric cancer treatment, outline what we know and what we do not currently understand about preventing or reducing them. Clinical and bench researach are necessary to develope interventions that will avoid or mitigate late effects and improve the health of pediatric cancer survivors.

Keywords: Late effects; Survivorship; Anthracyclines; Doxorubicin; Cisplatin; Alkylating agents; Ifosfamide; Fertility; Cardiomyopathy; Ototoxicity; Nephrotoxicity

1. Introduction

The increasing survival probability of childhood cancer patients is increasing the prevalence of late effects of treatment among children and adults. Where chemotherapy protocols were once designed only to maximise survival, we now must design them to minimise long-term toxicity as well. With some agents, there is sufficient knowledge to do this fairly effectively, but with other agents our knowledge is lacking. Paediatric oncologists often find themselves in an uncomfortable world of trade-offs as they attempt to balance anti-cancer efficacy against long-term toxicity.

We consider here the most serious organ-specific late effects of paediatric cancer treatment, and review what we know, and what we don't know, about how to prevent or ameliorate them. Exercising authors' prerogatives, in view of the vast literature and its highly technical nature, we are excluding myelosuppression and non-specific chemoprotection from our discussion.

2. Anthracycline cardiomyopathy

Anthracyline antibiotics, particularly doxorubicin, are widely used in paediatric oncology because of their broad spectrum of activity and high efficacy. They however, carry a worrisome late effect risk of cardiomyopathy that can appear during treatment or many years afterwards. Anthracycline cardiomyopathy appears clinically as congestive heart failure or arrhythmias, or may exist subclinically as asymptomatic abnormalities on electrocardiograms, Holter monitors, or tests of cardiac contractility. Sudden death from arrhythmia has occurred years after completion of doxorubicin treatment [1] and EKG abnormalities are both common and not correlated with echocardiographic findings [2].

The risk of symptomatic anthracycline cardiomyopathy is a complex function with many variables. Patients

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who are young at the time of treatment carry a higher risk than those who are older, and females appear to have a higher risk than males [3]. The risk increases with increasing cumulative dose, and doxorubicin appears to be more cardiotoxic than daunorubicin, which in turn is more cardiotoxic than epirubicin. Radiation therapy to the heart can also increase the risk of cardiomyopathy. Surveying a registry of 607 anthracycline-treated children, Kremer and colleagues found a 5% cumulative incidence of clinical congestive heart failure over 15 years, with a mean anthracycline dose of 461 mg/m^2 in the affected patients [4].

The trade-off between survival and toxicity is clear with doxorubicin. An analysis of early data evaluating doxorubicin for osteosarcoma concluded that the marginal benefit of an additional doxorubicin dose (increased probability of survival) exceeded the marginal cost (increased probability of cardiomyopathy) even at a cumulative dose of 540 mg/m^2 [5]. More recently, a comparison of 50 normal children with 120 survivors of acute lymphoblastic leukaemia (ALL) who had received 90, 180, or 270 mg/m^2 of daunorubicin revealed reduced fractional shortening and increased left ventricular end-systolic stress in all groups compared to controls, with a hint of a dose effect. However, the probability of disease-free survival increased with increasing cumulative doxorubicin [6]. The challenge is to maximise the benefit of the anthracyclines while minimising their long-term risks.

2.1. Continuous infusion

Continuous infusion clearly reduces the cardiotoxicity of doxorubicin, at least over the short-term in adults. It grew from the hypothesis that high serum levels lead to more myocardial damage. One of the first large studies testing continuous infusions of doxorubicin involved 21 adult patients with breast cancer or metastatic sarcomas who received the drug by infusions of increasing duration (24–96 h), and a comparison group of 30 patients (mostly with metastatic lung cancer) who received the drug over 15–20 min. All patients were followed with serial endomyocardial biopsies, and had treatment stopped when grade 2 changes were found (on a 0–3 scale). The continuous infusion patients received significantly more doxorubicin than the bolus group before developing grade 2 changes (medians of 600 *vs.* 470 mg/m^2, $P = 0.002$). Peak plasma doxorubicin levels were much lower during 24-h infusions than after bolus delivery (0.24 *vs.* 1.31 μg/ml), but prolonging the infusion to 48 or 96 h did not dramatically decrease the levels further (0.13 and 0.096 μg/ml, respectively) [7]. Since there was intra-patient prolongation of the infusions, the effects of different infusion rates on cardiotoxicity could not be compared.

Two randomised controlled trials in adults have demonstrated that doxorubicin is less cardiotoxic when given by infusion. Shapira and co-workers compared 15–20 min infusions (bolus group) to 6-h infusions in 62 patients with breast or ovarian carcinoma, with a dose of 50 mg/m^2 per cycle. The study revealed a large advantage for the 6-h infusion at a cumulative dose of 300 mg/m^2; the mean decline in left ventricular ejection fraction (assessed by radionucleotide imaging) was 17% in the bolus group and 4% in the 6-h group; at a cumulative dose of 400 mg/m^2, the declines were 21% and 6% ($P < 0.001$) respectively. Four patients developed congestive heart failure, all in the bolus group [8].

In the second randomised study, conducted in adults with soft tissue sarcomas, 82 patients were treated with 60 mg/m^2 doxorubicin every three weeks to a total dose of 540 mg/m^2 and assigned at random to a 5–10 min rapid "bolus" intravenous infusion or a 72-h continuous infusion. Defining cardiotoxicity as 10% or greater decline in the resting ejection fraction on a radionucleotide cineangiogram, Casper and colleagues found a statistically insignificant difference in cardiotoxicity between the two arms ($P = 0.11$). However, Kaplan–Meier analysis showed a significant difference ($P = 0.0017$) between arms in the cumulative dose of doxorubicin at the point where cardiotoxicity appeared. More infusion patients completed the entire course of therapy than bolus patients (64% *vs.* 37%, *P* not given). Freedom from metastases and survival did not seem to differ between the two groups [9].

The applicability of these findings to children is controversial. The adult studies focused on short-term cardiac toxicity (during therapy or at most a few months after therapy), while children are prone to cardiomyopathy for decades after treatment. The mechanisms of cardiac injury causing short-term heart failure may differ from those that cause arrhythmias and clinical cardiomyopathy 20 years after treatment.

Only one randomised controlled trial of infusion-*versus*-bolus doxorubicin has been published in paediatrics. Lipshultz and colleagues randomly assigned children with ALL to receive 30 mg/m^2 of doxorubicin every three weeks either as bolus (over 1 h) or as a 48-h infusion. The cumulative dose was 360 mg/m^2. Cardiac assessment at a median of 1.5 years after diagnosis revealed no difference between the two groups in a variety of echocardiographic measurements of left ventricular function [10]. Critics of this study argue that the individual doses of doxorubicin are much lower than those given to sarcoma patients, so that the results might not be applicable to them; and that the follow-up interval is too short to be meaningful.

Continuous infusion may however, not be the best approach for all anthracyclines. Idarubicin is very slowly metabolised, and its toxicity is increased by infusion.

Infusing daunorubicin may actually increase its cardiotoxicity, though clinical data are lacking [11].

2.2. Fractionation

The distribution of doxorubicin doses over time may affect the risk of cardiomyopathy. In the intergroup Ewing's sarcoma study (CCG-7881/POG-8850/INT-0091), patients received doxorubicin in 75 mg/m^2 bolus doses; the control arm received the drug every three weeks while the experimental arm received it every six weeks. Four cases of congestive heart failure have been reported, all in the control arm [Krailo, personal communication]. Small weekly doses of doxorubicin have been found to be less cardiotoxic than three-weekly doses in several studies in adults [12].

2.3. Liposome encapsulation

Liposome encapsulation of doxorubicin, with or without the addition of polyethylene glycol (pegylation), was developed to improve the therapeutic index by increasing the half-life (so that every dose is, in effect, a continuous infusion) and perhaps changing tissue distribution. Liposome-encapsulated, pegylated doxorubicin (Doxil®) is now commercially available in the United States. There are suggestions that its cardiac toxicity may be less than that of ordinary doxorubicin, [13,14], and that it may be more effective for some tumours, such as AIDS-related Kaposi's sarcoma [15].

PEG-doxorubicin has also been studied in Phase I in children [16]. Hypersensitivity reactions during infusion required extensive premedication and an increase in the infusion time from 1 to 4 h. The dose-limiting toxicity was mucositis (3 of 6 patients at 70 mg/m^2), leading to an maximum tolerated dose of 60 mg/m^2. Palmar-plantar erythrodysesthesia was not a dose-limiting toxicity, though it has been in some adult studies. No cardiac toxicity was seen, though most patients received only one cycle, and follow-up was short. There is thus no evidence to support its use in place of doxorubicin in paediatrics.

2.4. Drug substitution

Epirubicin is an epimer of doxorubicin developed as a less-toxic alternative, and is used widely in Europe to treat breast carcinoma and adult soft tissue sarcomas. It is not commercially available in the USA. An analysis of adult patients with a variety of cancers treated on Phase II trials and monitored with endomyocardial biopsy showed that 98 mg of epirubicin has about the same cardiotoxicity as 60 mg of doxorubicin [17]. However, therapeutically equivalent doses remain unclear, so the advantage would be with epirubicin only if less than 98 mg is equivalent to 60 mg of doxorubicin. No data comparing the two drugs are available in paediatrics.

2.5. Dexrazoxane

Doxorubicin appears to injure the heart through an interaction with myocardial iron, with the production of highly reactive hydroxyl radicals. Dexrazoxane (formerly ICRF-187 and ADR-529) chelates free iron to prevent its interaction with doxorubicin. In a randomised controlled trial in paediatric sarcoma patients, dexrazoxane proved effective in reducing doxorubicin cardiac toxicity, compared with bolus doses of 50–70 mg/m^2. Thirty-eight patients were treated with vincristine–doxorubicin–cyclophosphamide alternating with ifosfamide–etoposide, and randomised to receive dexrazoxane before each doxorubicin dose or not. Patients receiving dexrazoxane had a mean decrease in left ventricular ejection fraction on radionuclide angiography of 1.0% point per 100 mg/m^2 of doxorubicin, compared to 2.7% points for the group without dexrazoxane. Many fewer dexrazoxane-treated patients than control patients developed dose-limiting cardiotoxicity (4 of 18 *vs.* 10 of 15), and the total dose of doxorubicin delivered was higher (410 *vs.* 310 mg, $P < 0.05$) [18]. There was no apparent loss of anti-tumour efficacy, though the number of patients was small.

More recently, Lipshultz and colleagues showed in a randomised controlled trial, that paediatric ALL patients treated with doxorubicin plus dexrazoxane had lower cardiac troponin T levels, a marker of mycardial injury, than patients treated with doxorubicin alone. However, there were no differences in echocardiographic findings between the two groups, and much longer follow-up is necessary to determine whether late cardiac toxicity is affected, or whether dexrazoxane affects the anti-leukaemic efficacy of doxorubicin [19]. Current regimens only use dexrazoxane and doxorubicin in bolus doses. Lacking, but sorely needed, are studies comparing dexrazoxane with continuous infusion as a means to limit cardiac toxicity.

CarnitineL-carnitine is a trimethylated amino acid that participates in the transport of long-chain fatty acids across the mitochondrial membrane; such fatty acids are the main mitochondrial energy source. Several inborn metabolic abnormalities lead to carnitine deficiency or insufficiency, clinically apparent as dilated cardiomyopathy (reviewed in detail by Winter *et al.* [20]). Treatment with carnitine appeared beneficial in randomised controlled studies of adults with acute myocardial infarction [21] and chronic congestive heart failure from dilated cardiomyopathy [22], and in an infant with dilated cardiomyopathy from renal tubular carnitine wasting [23].

Carnitine is very effective in protecting mice against death from doxorubicin cardiomyopathy, reducing 120-day mortality from 53% to 8%. Heart-muscle homogenates from doxorubicin-treated mice had significantly lower carnitine concentrations than those from

doxorubicin + carnitine-treated mice [24], implying that doxorubicin causes carnitine depletion.

The mechanism of a doxorubicin-protective effect of carnitine is unclear. In isolated rat cardiac myocytes, doxorubicin inhibits palmitate oxidation, and carnitine reverses that inhibition [25]; in cardiac myocyte mitochondria the same pattern is observed with the oxidation of palmitoyl-CoA [26]. Heart homogenates from doxorubicin-treated rats have decreased carnitine palmitoyl transferase I and II activity, but carnitine supplementation does not restore it [27].

Human data are very limited. In a comparison of resting and exercised cardiac function in 38 anthracycline-treated paediatric ALL patients and 38 age and BSA-matched healthy controls, Hauser found that the 10 patients with ventricular dysfunction during exercise had markedly lower serum total and free carnitine levels than either the other patients or the controls ($P < 0.001$). The total anthracycline doses did not differ between the patients with normal and abnormal exercised ventricular function, and the carnitine levels in the patients with normal hearts were nearly normal [28]. Since this study did not involve pre-chemotherapy carnitine measurements, it is not clear whether doxorubicin causes carnitine depletion, or whether the hearts of patients with low carnitine levels to begin with are more sensitive to doxorubicin. Randomised controlled trials of carnitine to prevent doxorubicin cardiomyopathy are needed.

2.6. Post-treatment afterload reduction

The proportion of anthracycline-treated patients with symptomatic or subclinical cardiac abnormalities increases with time since treatment, raising the possibility that some variety of post-chemotherapy treatment might prevent the progression of cardiac changes. Afterload reduction with drugs such as enalapril are effective in other varieties of dilated cardiomyopathy, and may be useful in doxorubicin cardiomyopathy as well [29,30]. In a randomised placebo-controlled trial in 135 anthracycline-treated childhood cancer survivors, Silber and colleagues found that enalapril reduced left ventricular end-systolic wall stress, and six of the seven patients who were removed from protocol treatment because of deteriorating cardiac function were receiving placebo ($P = 0.11$). However, no effect on mean cardiac index, a variety of exercise variables, or quality of life was seen [31]. The ability of afterload reduction to prevent anthracycline cardiomyopathy thus remains unclear.

3. Cisplatin nephrotoxicity and ototoxicity

Renal and otic toxicity of cisplatin has limited its use, and burdened many treated patients, for over 20 years. The high-frequency hearing loss from cisplatin is irreversible, and may occasionally be progressive. The renal toxicity, best determined with isotopic GFR measurement [32], improves somewhat with time-off therapy, but rarely completely heals.

Cisplatin is usually given with large volumes of fluid, reflecting a belief that diuresis limits nephrotoxicity. Unfortunately, there are no randomised controlled trials showing that this is true. The nephrotoxicity of cisplatin is highly variable between patients [32], and attempts to associate toxicity with pharmacokinetics have failed [33].

Amifostine is a chemoprotective agent that reduces the acute hematologic toxicity of some chemotherapy. However, it did not reduce the renal or ototoxicity of cisplatin in a randomised controlled trial in osteosarcoma patients [34], nor did it reduce ototoxicity in a historically controlled trial in patients with high-risk germ cell tumours [Marina, personal communication]. Interestingly, carnitine appears to limit cisplatin's renal toxicity in rats, using histological changes and measurements of serum urea and creatinine as criteria, while not impairing its effectiveness against a transplantable hepatoma [35].

A promising new approach is to use the reducing compounds sodium thiosulfate (STS) and *n*-acetylcysteine (NAC). Both drugs have long been known to eliminate the cytotoxicity of cisplatin and carboplatin when used concurrently both *in vitro* and *in vivo*. Neuwelt and colleagues have separated carboplatin and STS spatially and temporally in patients with malignant brain tumours by first, opening the blood–brain barrier with intra-arterial mannitol; then giving intra-arterial carboplatin; allowing the blood–brain barrier to recover for 2 or 4 h; and finally treating systemically with intravenous STS. Compared with historical controls, high-frequency hearing loss is significantly delayed with STS (20 mg/m^2) either 2 or 4 h after blood–brain-barrier disruption, or intra-arterial carboplatin. After 6 cycles of treatment, the control group had an average 40 dB threshold at 4000 Hz, while the 2- and 4-h STS-treated groups had 18 and 10 dB thresholds, respectively ($P = 0.0075$ for the comparison of the historical controls to the combined treatment groups) [36]. Open questions are whether NAC can be used similarly, and whether temporal separation of platinum compounds and thiol compounds is sufficient without spatial separation.

4. Ifosfamide nephrotoxicity

Nephrotoxicity may complicate treatment of children with ifosfamide. Renal damage may be acute and reversible however, chronic toxicity can also develop. Proximal tubular dysfunction can lead to Fanconi syndrome with hypophosphatemic rickets, proximal renal tubular acidosis, electrolyte wasting, glycosuria and proteinuria.

A decrease in measured GFR may also occur. These abnormalities may occur singly or in any combination. Occasionally the abnormalities resolve fully, however very long-term chronic ifosfamide nephrotoxicity is also seen [37].

Several studies have evaluated potential risk factors for ifosfamide nephrotoxicity. Although extensive renal damage has occurred after low cumulative doses of ifosfamide, children receiving higher total dose (60–100 g/m^2) appear to be at greatest risk. A significant correlation has been found between a higher total ifosfamide dose and both glomerular and proximal tubular toxicity. Other risk factors for ifosfamide nephrotoxicity include young age at treatment, previous or concurrent cisplatin, previous renal irradiation, and having a single kidney [38,39]. Clinical risk factors for the development of chronic ifosfamide nephrotoxicity can be used to identify high risk patients. For instance, where possible one should avoid cumulative ifosfamide doses over 80 g/m^2, the drug should be used carefully in children younger than five years of age and in those patients who have previously received cisplatin or who already exhibit poor renal function [37]. Ifosfamide nephrotoxicity should be treated before the onset of chronic damage by stopping or modifying ifosfamide administration. Lastly one can use phosphate supplementation in ifosfamide induced hypophosphatemia in an effort to reduce the risk of rickets [37]. Amifostine, while protecting rabbit renal tubular cells from ifosfamide metabolites *in vitro*, does not appear to act similarly in paediatric patients, though data are sparse [40,41]. Mesna provides urothelial protection, but there is no evidence of renal parenchymal protection.

5. Osteoporosis

Skeletal manifestations, such as osteoporosis and fractures of the long bones and spine, have been described in children with malignancies. Therapy for childhood ALL may be harmful to the development of bone mass and density [42–44]. Additionally, patients whose therapy has included allogeneic bone marrow transplantation have been found to have decreased bone mineral density (BMD) and are at risk for osteopenia and osteoporosis [45]. Many factors relevant to paediatric oncology patients contribute to loss of BMD, including corticosteroids, inactivity, gonadal dysfunction, radiation therapy, chemotherapy, and cyclosporine use [42–46]. In addition, patients treated for osteosarcoma with high dose methotrexate have subsequently developed osteoporosis and some have exhibited therapy-related fractures [47].

5.1. Post-therapy interventions

Early identification of the risk for osteoporosis allows early therapeutic interventions that can minimise the loss of BMD. The simplest intervention is increasing calcium intake, which has been shown to correlate positively with increases in BMD and reduce the risk of fractures [48,49].

Bisphosphonates have an affinity for bone, are adsorbed onto the hydroxyapatite crystals, and suppress osteoclast-mediated bone resorption. Bisphosphonates have been found to be effective and well tolerated in various diseases in adults associated with increased bone resorption like Paget's disease of bone, hypercalcemia of malignancy and postmenopausal osteoporosis [50]. Moreover, in placebo controlled clinical trials in postmenopausal women with osteoporosis, bisphosphonates increase BMD in the lumbar spine, hip, and over the entire skeleton. A recent clinical trial with alendronate has demonstrated a beneficial effect on corticosteroid-induced osteoporosis, with a significant increase in BMD over the lumbar spine and femoral neck, and fewer new vertebral fractures in the treatment group compared to the placebo group [51]. Barr and colleagues completed a non-randomised pilot study of the use of pamidronate to ameliorate osteopenia in a cohort of ten children with ALL who were receiving maintenance chemotherapy. Pamidronate was given by intraveneous infusion, in a dose of 1 mg/kg on each of three successive days every three months. Patients were followed up 6 months after the first dose of pamidronate. Patients experienced gains in whole body BMD and lumbar spine BMD, averaging 5–10% over the 6 months study period [52]. Further studies evaluating the safety and efficacy of bisphosphonates in children are needed before this becomes standard of care for children with decreased bone mineral density. Specifically, the effects of bisphosphonates on the growing bones of children need to be examined as well as whether bisphosphonates will interfere with antineoplastic therapy.

6. Avascular necrosis

Avascular necrosis (AVN) is a disabling bony toxicity attributed to corticosteroid usage. Mattano reported a 3-year AVN cumulative incidence of 9% in children treated for higher risk ALL, with the highest frequency observed in adolescents [53]. AVN frequently involves multiple joints, and can lead to severe pain and loss of function. Options for treating AVN in the paediatric population are limited. Most patients undergo a prolonged course of non-operative care, including activity restrictions, protected ambulation with crutches, physical therapy and anti-inflammatory drugs. Sometimes, patients need total joint replacements [53–55].

6.1. Statins

The pathogenetic mechanisms underlying the development of steroid-related AVN includes marrow blood

flow stasis secondary to lipid infiltration and elevated intramedullary pressure [56,57]. The statin class of HMG-CoA reductase inhibitors may counter this by inhibition of lipid synthesis [58,59]. Animal models have shown that administration of lipid-lowering agents prior to steroid treatment abrogates the marrow changes that lead to AVN [60]. Statins have recently been reported to reduce the incidence of AVN in adults taking chronic steroids. The records of 284 patients who were taking statin drugs during their entire treatment with steroids were examined to determine whether osteonecrosis had developed. MRI scans were used to verify the osteonecrosis. After an average of 7.5 years (minimum follow-up, 5 years), only three patients (1%) from the group had osteonecrosis, much less than the 3–20% incidence usually reported for patients receiving high-dose steroids [61].

6.2. Bisphosphonates

Structural failure in AVN is the result of resorption of necrotic bone during revascularisation, before new bone has formed or consolidated enough for load-bearing [62]. If bone resorption associated with osteonecrosis can be delayed until sufficient new bone has formed, it is plausible that structural failure and its consequences could be avoided [62]. Bisphosphonates inhibit the resorptive action of mature osteoclasts, which may prevent progressive bone resorption and collapse. Agarwala and colleagues reported a study of 16 adult patients with AVN of the hip, 11 cases were steroid-related [63]. The mean age was 34 years (range 19–44 years), and the mean duration of AVN was 13.8 months (range 1–72 months). In 14/16 patients, both hips were involved. All patients received alendronate 10 mg/day plus calcium supplement of 1 g/day, with a mean period of follow-up of 24 weeks. After 12 weeks of treatment, there was a significant improvement in pain, disability, standing and walking capacity ($P < 0.0003$), which continued at 24 weeks. Concomitantly, there was significant improvement in the range of movement at the hip ($P < 0.05$). The MRI remained stable in 15 of 16 patients with resolution of edema in four, and resolution of osteoporosis and joint effusion in one patient each. The analgesic requirement declined considerably in all patients, 13 of 16 needing only an occasional analgesic after 6 weeks [63]. Further studies involving larger numbers of patients and a longer follow-up period are needed to determine the role of bisphosphonates in AVN.

7. Infertility

Cytotoxic chemotherapy and radiotherapy may damage gonadal tissue and result in permanent sterility. Cancer therapy may damage the developing gonad in children. However, the full impact of the deleterious effects generally remains latent in childhood and is manifest only in adulthood as infertility or premature menopause.

7.1. Males

The testes are extremely sensitive to chemotherapy, radiation and surgical interventions. Testicular dysfunction is among the most common long-term side effects of chemotherapy in men. All alkylating agents are gonadotoxic [64–66]. The extent and reversibility of cytotoxic damage generally depends on the agent and cumulative dose received, although significant individual variation has been observed.

7.2. Drug substitutions

Alkylating agents vary widely in their gonadal toxicity. Most data come from patients treated for Hodgkin's disease (HD), as these patients are often sexually mature and several alkylating agents have been used. HD patients treated with six or more courses of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) have demonstrated permanent azoospermia attributable to both the alkylating agents, mechlorethamine and procarbazine. Treatment of HD with combination chemotherapy regimens such as COPP (cyclophosphamide, vincristine, procarbazine and prednisolone) have also been reported in a number of studies to result in permanent azoospermia in 99–100% of patients treated with 6–8 courses [67,64]. Charak and coworkers found azoospermia in all 92 patients following treatment with six or more cycles of COPP; 17% of patients had been treated more than 10 years previously, suggesting that germinal epithelial failure is likely to be permanent [67].

Procarbazine appears to play a major role in this toxicity. Hassel and coworkers studied testicular function in boys treated for Hodgkin's disease without procarbazine, using OPA/COMP (vincristine, prednisone, doxorubicin/cyclophosphamide, vincristine, methotrexate, prednisone). These patients showed no major testicular damage, as assessed by measuring testosterone, basal and GnRH-a-stimulated LH- and FSH-levels. This was in contrast to boys who had received OPPA/COPP (includes procarbazine) in the DAL-studies HD-78 and HD-82 [68].

Efforts to reduce the risk of sterility after Hodgkin's disease include the use of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine), an effective combination that does not contain the alkylating agents chlorambucil or procarbazine [69,70], and other related regimens. Viviani and co-workers showed that while recovery of spermatogenesis after MOPP was rare, all

who experienced oligospermia after ABVD recovered completely by 18 months. [70]. Hybrid regimens (*i.e.*, alternating cycles of ABVD with ChlVPP or MOPP) are also less gonadotoxic than MOPP, ChlVPP or COPP given alone.

7.3. Assisted reproductive technology

The cytotoxic effect of chemotherapy on germinal epithelial function launched a search for possible fertility preservation strategies in men undergoing therapy. Cryopreservation of sperm has become standard practice, and should be offered to all newly diagnosed post-pubertal males at risk of infertility. Many improvements have been made in the techniques used to store sperm, and advances in assisted reproductive technology using intracytoplasmic sperm injection (ICSI) has increased the rate of successful pregnancies using banked sperm [71–73].

Ejaculatory azoospermia is not the same as testicular azoospermia [74]. The level of sperm production necessary for sperm to exist in the testis is far less than that required for sperm in the ejaculate [74]. Therefore with testis sperm extraction (TESE) followed by ICSI, it is now possible for some patients who did not sperm bank, and have azoospermia on semen analysis, to father children. A retrospective study by Damani and colleagues evaluated 23 men with ejaculatory azoospermia and a history of chemotherapy. They underwent TESE in search of usable sperm. Spermatozoa were found on TESE in 15 (65%) of 23 men. The subsequent fertility rate was 65% and pregnancy occurred in 31% of cycles [75]. Men with post-chemotherapy azoospermia must be fully evaluated before they are considered sterile.

Unfortunately, at this time there are no feasible options for pre-pubertal male patients. There has been no demonstrated protective effect of using GnRH analogues with and without testosterone to suppress testicular function during chemotherapy [76–78]. As paediatric oncologists, we must continue to attempt to reduce the gonadotoxicity of our treatment regimens while maintaining superior cure rates.

8. Females

Radiotherapy and chemotherapy may cause transient or permanent effects on hormonal, fertility and sexual function resulting in primary ovarian failure, impaired development of secondary sexual characteristics, menstrual irregularities including oligomenorrhea and amenorrhea, or premature menopause. The specific effects are dependent on the developmental status at the age of treatment, the ovarian dose of radiation, and the chemotherapeutic agents and their doses.

Radiation causes a decrease in the number of ovarian follicles, impaired follicular maturation, cortical fibrosis and atrophy, generalised hypoplasia, and hyalinisation of the capsule. Females treated before puberty have a greater number of ova than do older women and therefore ovarian function is relatively more resistant to radiotherapy in pre-pubertal as compared with post-pubertal females [79]. As is the case with radiotherapy, amenorrhea and ovarian failure occur more commonly in adult women treated with alkylating agents than in pre-pubertal females [80–85]. In a study of 2498 survivors and 3509 siblings treated between 1945 and 1975, there was a 7% fertility deficit among female survivors as compared with their siblings. Cancer survivors between the ages of 21–25 had a risk of early menopause four times greater than that of siblings. Significantly increased relative risks (RR) of menopause occurred after treatment with either radiotherapy alone (RR = 3.7), alkylating agents alone (RR = 9.2) or combination of both (RR = 27) [86].

8.1. Drug substitution

Reduction in the use and dose of alkylators and abdominopelvic radiotherapy is the most successful means of preserving ovarian function and promoting fertility. For example, in the treatment of paediatric Hodgkin's disease, mechlorethamine and procarbazine together are perhaps the most damaging agents to ovarian function. Substitution of cyclophosphamide for mechlorethamine appears to have significantly reduced the risk of ovarian dysfunction [87]. However, more time has to elapse before the impact of the substitution of cyclophosphamide for mechlorethamine on premature menopause can be evaluated.

8.2. GnRH agonists

Dividing cells are more sensitive to the cytotoxic effects of alkylating agents than are cells at rest, therefore it has been hypothesised that inhibition of the pituitary–gonadal axis by gonadotropin-releasing hormone agonists (GnRH-a) may protect the ovarian germinal epithelium from the cytotoxicity of chemotherapy. GnRH-a prevents follicular growth and mitosis by blocking gonadotrophin induction. The exact mechanism is unclear, but it may involve suppression of GnRH receptors in the ovary, and then subsequent inhibition of recruitment of small follicles into the proliferating pool as well as atresia of the already developed follicles [88].

A number of animal studies have demonstrated that GnRH-a inhibits chemotherapy-induced ovarian follicular depletion. Blumenfeld and colleagues, in a study of women treated with cytotoxic chemotherapy for lymphomas, demonstrated that 93% of the surviving pa-

tients receiving GnRH-a resumed spontaneous ovulation and menses, compared with 40% of the women in the control group who did not receive GnRH-a treatment [89]. In a subsequent study, a prospective clinical protocol was undertaken in 55 women aged 15–40 years with lymphoma, leukaemia, or non-malignant diseases such as systemic lupus erythematosus, treated with GnRH-a and cytotoxic chemotherapy. This group was compared with a concurrent control group of 55 women who were treated with similar chemotherapy without GnRH-a. All but 3 of the surviving patients within the GnRH-a chemotherapy treatment group resumed spontaneous ovulation and menses within 12 months, compared with less than half of the patients in the control group (chemotherapy without GnRH-a treatment) [90]. Thus, GnRH-a may provide some protection against alkylating agent based chemotherapy, but no advantage has been conferred against irradiation or high-dose chemotherapy such as conditioning therapy for bone marrow transplant, where ovarian failure is inevitable despite hormone suppression [88,91].

8.3. Assisted reproductive technology

Advances in assisted reproductive technology have resulted in the availability of several options for preserving fertility in patients about to receive potentially toxic chemotherapy or radiotherapy [84,85]. In pre and post-pubertal females, cryopreservation of ovarian cortical tissue is of potential clinical use. Most of this technology has been performed in laboratory animals [92–95]. However, recently Oktay have documented a pregnancy using cryopreserved ovarian tissue [96]. Another option available to the post-pubertal female is the stimulation of ovaries with exogenous gonadotropins and retrieval of oocytes for cryopreservation. Only a few oocytes can be harvested after stimulation of the ovaries [93]. Combining *in vitro* fertilisation and subsequent embryo cryopreservation has also been successful. These options may not be readily available to the newly diagnosed paediatric and adolescent patient, since a delay in cancer therapy is usually necessary for ovarian stimulation or in vitro fertilisation cycles [94,95].

9. Conclusion

During the last 30 years, multi-modality therapy for paediatric cancers has had a significant impact on survival rates. For example, the five-year survival for paediatric cancer is 75% for the period from 1985 to 1997 as reported by the National Cancer Institutes Surveillance and End Results section [97]. Studies of late effects began approximately two decades ago, and continue today as modifications in therapeutic strategies and increased survivorship have reinforced the focus on health-related outcomes. The results of these studies impinge upon clinical care, and assist in the development of clinical trials designed specifically to reduce long-term morbidity and improve the overall quality of survivorship [98].

As this brief review shows, in the prevention of late effects of therapy, the questions greatly outnumber the answers. There are few randomised trials, and there are virtually no cooperative group studies on such long-standing problems such as anthracycline cardiomyopathy and cisplatin ototoxicity. Research efforts to develop strategies to avoid or mitigate late effects are imperative if ultimately we are to improve the health of paediatric cancer survivors. The low incidence of some complications of therapy; the necessity for long follow-up periods; the transition of patients from paediatric to adult medical care; multispecialty involvement; and patient mobility and incomplete adherence to follow-up recommendations are large challenges to researchers in this field. Yet without such research, the victory over childhood cancer will remain incomplete.

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