

review

Fertility preservation methods for female neoplastic patients

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Background. The ability to have biological children is of great importance to cancer patients and fertility preservation before the oncological treatment is nowadays not rare. Oncologists have the responsibility to inform patients about the risks that their cancer treatment will permanently impair fertility and about the ways to limit this iatrogenic damage. Methods of fertility preservation are evolving quickly, yet the medical oncology literature is still poor regarding this topic. Indications and contraindications, limits and controversies of the fertility sparing techniques are reviewed in order to help the oncologist to counsel patients.

Conclusions. Any oncologist seeing reproductive-aged patients should discuss the fertility implications of the oncological treatment and the fertility preservation options. A referral to appropriate fertility specialists as early as possible is recommended. People attempting fertility preservation in the context of cancer treatment are encouraged to enrol in clinical trials.

Key words: neoplasms; fertility; ovary; oocytes; embryo; cryopreservation; fertilization in vitro

Introduction

Nearly 25% of cancers affect women who have not had a child or who have delayed childbearing. The number of women surviving cancer is increasing, but at the same time the long-term fertility adverse effects of the treatment that they received are growing. This iatrogenic damage substantially impairs the quality of life of the cancer survivor, leading to premature ovar-

ian failure and infertility in the majority of these patients.

These women can experience persistent sexual problems, fertility concerns, and related adverse psychosocial sequel even many years after their cancer treatment. Reproductive concerns are significant mostly for those who »very much« desire children prior to cancer, had none prior, and are unable to reproduce subsequently. Cancer diagnosis reduces the desire to have children in 6-13% of patients, but it increases such desire in 19-24% of them. Among cancer survivors, 76% of those without children and 31% of those who are already parents, desire to have children in the future.¹

This suggests that there may be a need for more formalized intensive counselling

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both prior to and after cancer treatment to aid patients in resolving or managing psychosocial sequel resulting from the unplanned infertility (Table 1).^{2,3}

Table 1. Percentages of cancer survivors having specific reproductive concerns³

Reproductive Concerns Scale	%
Loss of control over reproductive future	30
Discontent with number of children	27
Inability to talk openly about fertility	18
Illness affected ability to have children	15
Sad about inability to have children	13
Frustrated ability to have children affected	11
Angry ability to have children affected	11
Mourned loss of ability to have children	11
Concerns of having children	8
Guilt about reproductive problems	8
Less satisfied with life because of problem	6
Less of a woman	6
Blame self for reproductive problems	4
Others are to blame for reproductive problems	40

Discussing fertility issues at the time of diagnosis provides the patient and her family with the reassurance that the oncology team believes in a future of survival and even of acceptable quality of life. While none of the fertility preservation options currently available provide total reassurance regarding the future fertility, for many young women, both the counselling involved in discussions of fertility preservation and the potential for optimisation of the chances of fertility in the future, together have a positive psychological impact during a very traumatic time in their lives.^{4,5}

Oncologists have traditionally focused more on providing the most effective treatments available, and less on the patient's post treatment quality of life. Physicians treating younger patients for cancer should now be aware of the adverse effects of treatment on fertility and of ways to minimize those effects. If gonadal toxicity is unavoidable,

they should be knowledgeable about options for fertility preservation and must discuss with patients the following options.^{2,6}

Gonadotropin-releasing hormone agonist (GnRHa) co-treatment with chemotherapy

The results of gonadoprotective hormonal therapy are considered contradictory and the controversy will only be resolved by prospective randomized clinical trials. Following encouraging findings in animal models, nonrandomized studies with a short-term follow-up suggested a protective role for GnRHa co-treatment,⁷⁻¹¹ but these studies were criticized for their lack of randomization, different follow-up periods for treatment and control groups, and the use of ovarian failure as the endpoint, which may not reflect the decrease in primordial follicle count in response to chemotherapy in young women.¹²

The mechanism by which GnRHa co-treatment may protect against chemotherapy-induced gonadal damage is still debated, as is the presence of follicle-stimulating hormone (FSH) receptors in primordial follicles.^{7,12}

Mechanisms by which GnRH-a could minimize chemotherapy-associated gonadotoxicity:⁷

1. the hypogonadotropic state generated by the GnRH-a creates a prepubertal hormonal milieu that decreases the activity and so the rate of follicular apoptosis and degeneration.
2. the hypoestrogenic state may decrease utero-ovarian perfusion, resulting in a decreased total cumulative exposure of the ovaries to the chemotherapeutic insult.
3. gonads contain GnRH-I and GnRH-II receptors the activation of which could decrease apoptosis.

4. GnRH-a may up-regulate an intragoadadal antiapoptotic molecules such as sphingosine-1 phosphate (S-1-P).
5. GnRH-a may protect the undifferentiated germline stem cells, which ultimately generate de novo primordial follicles.

Criticism to Gn-RH agonist use is based on these considerations. Primordial follicles initiate follicle growth through an unknown mechanism, which is not gonadotropin dependent. There is some controversy regarding the existence of GnRH receptors on the human ovary, whereas GnRH receptors have clearly been detected in the rat ovary. The response may thus not be similar across species. If the sole mechanism of gonad protection with GnRH agonists were through the suppression of gonadotropins, especially FSH, then the treatment would not be expected to protect the primordial follicle population that represents the ovarian reserve.

Some prepubertal children receiving gonadotoxic chemotherapy may eventually have POF. As younger patients have a larger ovarian reserve, a decreased frequency of immediate amenorrhea does not mean that the gonads are unaffected by the chemotherapy, but simply that they have a sufficient number of oocytes not to demonstrate immediate ovarian failure.¹³

The hypogonadotropic state induced by GnRH agonists may have negative effects in breast cancer patients by arresting tumour cells in G0 phase and making them less responsive to chemotherapy.

At present, despite encouraging reports, the benefits and long-term effects of GnRHa co-treatment are unclear, and a consensus regarding the effectiveness of ovarian suppression is lacking. Therefore, GnRHa co-treatment for prevention of chemotherapy-induced gonadotoxicity should be offered to patients only with appropriate informed consent in an institutional review board approved investigational protocol.

Gonadotropin-releasing hormone agonist co-treatment with chemotherapy can be used:

1. as the only strategy, if no other option is available and the patient is informed about its limits
2. combined with other options.

Ovarian tissue cryopreservation

Ovarian tissue consisting of germ cells can be removed and stored before the gonadotoxic treatment. After patients are cured, this tissue might either be returned to patients *via* autotransplantation or matured *in vitro* to produce offspring by *in vitro* fertilisation. Ovarian tissue can be removed by the use of multiple biopsy samples from the ovary or by oophorectomy. The removal of ovarian cortical strips that can be done laparoscopically is better and it produces tissue that is rich in primordial follicles. Cortical strips and biopsies are ideal because the tissue survives cryopreservation and undergoes revascularisation on return, although most primordial follicles are lost.

The autologous transplantation of this tissue aims to restore natural fertility and also maintain sex-steroid production. The feasibility of this process has been shown in sheep and other mammals, with both the return of ovarian hormonal activity and the subsequent production of offspring. After such success in animals, the evidence of ovulation after orthotopic transplantation in a woman was reported.¹⁴ The reports by Oktay and colleagues¹⁵ and Donnez and co-workers¹⁶ are important in showing that the ovarian function could realistically be preserved after the sterilising treatment, although the continuing intermittent ovulation in the Donnez study raises questions as to whether pregnancy clearly resulted from the grafted tissue.

This technique of fertility preservation remains experimental and several issues

remain to be clarified, but perhaps the greatest concern is the potential to return malignant cells back to patients after they are cured. This factor is of particular importance in patients with haematological malignant disease. Oocyte maturation *in vitro*, followed by assisted reproduction, would eliminate this risk. Techniques to mature oocytes artificially, even from early stages of development, have yielded some success in mice. At present, little is known about the support needed for this process to take place in human tissue, and the clinical potential of this technique will need to be established.

Candidates to ovarian tissue cryopreservation are cancer patients who:

1. wish to be pregnant in the future or who don't exclude such possibility
2. have a realistic chance of long-term survival
3. still have at least a certain amount of follicles, possibly not damaged by previous treatments
4. accept, must be performed and don't have surgical contraindications to laparoscopy
5. have a low risk of primary tumour reimplantation or ovarian cancer
6. can't use ovarian hyperstimulation, because of neoplastic and/or thrombotic risk
7. need to start chemo/radiotherapy as soon as possible and who have not enough time to wait for *in vitro* fertilization (IVF) cycles
8. don't yet have a partner or have him but can't do IVF
9. are well informed about all the options and their risks
10. choose ovarian cryopreservation conscious that it is still experimental
11. have ethical concerns regarding ovulation induction and oocyte retrieval or other options.

Cryopreservation of unfertilized human oocytes

Fertility might be preserved by obtaining mature oocytes before the gonadotoxic treatment for IVF and subsequent embryo cryopreservation. This is the most effective method, but it is only applicable to sexually mature women, and needs a partner or donor sperm for fertilisation.

For women without a partner, cryopreservation of mature oocytes is an option, but subsequent pregnancy rates are substantially lowered because these cells sustain more damage during the freeze-thaw process than do embryos.¹⁷ These techniques are not suitable for most patients with cancer, because they need a period of ovarian stimulation that will delay treatment. The technique is also inappropriate for prepubertal patients, in whom all fertility preservation strategies remain experimental.

Cryopreservation of human oocytes can be performed if:

1. the laboratory is specifically highly competent on oocyte cryopreservation
2. a partner is not available
3. ovarian stimulation is possible
4. other options are discussed and discarded
5. the patient is properly conscious of the actual limited results of this technique.

Embryo cryopreservation

Embryo cryopreservation is still the most efficient method to preserve future fertility because of reasonable post-thaw survival, implantation, and delivery rates. Because the efficacy of IVF is dramatically reduced after even one round of chemotherapy, IVF should be performed before chemotherapy. Obviously, embryo freezing is predomi-

nantly suitable for women with a partner with whom they wish to procreate and it has legal limitations.

Embryo cryopreservation is an established technique that is available for fertility preservation if:

1. a small delay in the initiation of chemotherapy or radiotherapy is acceptable
2. a partner sperm is available (or a donor outside Italy)
3. ovarian hyperstimulation can be safely performed
4. this technique is chosen knowing its efficiency and the alternatives
5. there are non ethical or legal limitations.

Fertility-sparing surgery

Preservation of at least a part of an ovary and/or of the uterus can be done in certain neoplastic situations. Optimal cancer therapy should always supersede fertility preservation as a primary objective.

Ovarian neoplasms candidates for fertility-sparing surgery are ovarian tumours of low malignant potential, malignant ovarian germ cell tumours and ovarian sex cord-stromal tumours. Fertility-sparing surgery may be an option for invasive epithelial ovarian cancer which have early-stage disease, if the patient is well informed about risks, but this is highly controversial. Surgical procedures that would constitute fertility-sparing surgery for an ovarian malignancy include ovarian cystectomy, unilateral salpingo-oophorectomy, unilateral salpingo-oophorectomy plus hysterectomy, with the preservation of the contralateral ovary, and bilateral salpingo-oophorectomy, with the preservation of the uterus. Of course, after the latter two procedures, the assisted reproductive technology (ART) would be necessary to achieve a pregnancy.

The fertility sparing options for invasive cervical cancer are conization alone for stage IA1 or IA2 disease or radical trachelectomy for stage IA2 or IB1 disease. In addition, IVF techniques may be employed prior to definitive therapy if time delays are not significant.

The optimal candidate for medical treatment of endometrial cancer is a woman of childbearing age who has a stage IA, grade 1, adenocarcinoma. If such treatment is contemplated, it is recommended that a thorough hysteroscopy and curettage be performed to rule out a worse lesion prior to initiation.²

Candidates for fertility-sparing surgery or therapies are well informed patients with:

1. ovarian tumours of low malignant potential, malignant ovarian germ cell tumours, ovarian sex cord-stromal tumours and selected cases of epithelial malignant ovarian cancers stage Ia where one ovary could be saved.
2. stage IA1 or IA2 cervical cancer treated with conization alone or stage IA2 or IB1 where radical trachelectomy is performed
3. selected cases of stage IA, low-grade, endometrial cancer treated with progestins.

Transposition of the ovaries

Patients who receive pelvic irradiation might have their ovaries shielded or removed from the radiation field, a procedure known as oophoropexy which can be undertaken laparoscopically. Although the ovarian function can be preserved in 50% of cases, ischemia and radiation induced uterine and ovarian damage will reduce the chances of a successful pregnancy.

Transposition of the ovaries should be considered in case of:

1. planned pelvic or whole body irradiation
2. chemotherapy is not necessary
3. ovarian cancer involvement is unlikely
4. ovarian hypostimulation can be performed
5. can be combined with ovarian tissue cryopreservation.

Egg or uterus donation

Premature ovarian failure affects especially young female cancer patients who can only rely on egg donation. This technique has the highest effectiveness among fertility preservation options even for women candidates to other fertility preventive options: cumulative pregnancy rates are over 60%, if embryos are of good quality.

Uterus donation is still anecdotal and it is a possibility for women who did hysterectomy or pelvic radiotherapy. Strong ethical and legal concerns are the main limits.

Candidates to egg or uterus donation are women who:

1. are affected by premature ovarian failure
2. did hysterectomy or pelvic radiotherapy
3. have no ethical concerns nor legal limits to this.

Conclusions

Fertility preservation is often possible and should always be proposed and discussed to women undergoing treatment for cancer. Oncologists should be prepared on this subject or they must refer patients to proper reproductive specialists. To preserve the full range of options, fertility preservation approaches should be considered

as early as possible during the treatment planning. These methods have psychological, ethical and legal aspects that should be fully discussed before choosing the most appropriate for each case.¹⁸⁻²⁰ The fertility preservation techniques should be considered investigational and must be performed in centres with the necessary expertise.

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Zaščita plodnosti pri bolnicah z rakom

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Izhodišča. Biološko starševstvo je pomembno tudi za bolnike z rakom, zato vedno pogosteje že pred pričetkom onkološkega zdravljenja zaščitimo njihovo plodno sposobnost. Naloga onkologov je bolnike seznaniti z možnostjo, da bo onkološko zdravljenje lahko trajno okvarilo njihovo plodnost in kakšne so možnosti, da bi to jatrogeno okvaro zmanjšali ali preprečili. Metode zaščite plodnosti se zelo hitro razvijajo, čeprav o njih v strokovni literaturi ne poročajo velikokrat. Da bi onkologom pomagali pri svetovanju bolnicam z rakom, v članku navajamo indikacije, kontraindikacije, omejitve in kontraverznosti različnih zaščit plodnosti.

Zaključki. Ko onkolog obravnava bolnice z rakom, ki so v rodnem obdobju, naj bolnice seznanjeni z možnimi posledicami zdravljenja in z načini, da bi bolnica ohranila plodno sposobnost. Priporočamo, da bolnico čim prej napoti na posvet k strokovnjaku, ki ima izkušnje z zdravljenjem plodnosti. Če se bolnice odločijo za eno od metod zaščite plodnosti, predlagamo vključitve v študijske raziskave, ki bodo lahko pokazale učinkovitost posamičnih metod.

Različna lokalizacija cistatina C v nezrelih in zrelih dendritičnih celicah

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Izhodišča. Razgradnja antigenov s proteolitskimi encimi (proteazami) v endocitozni poti antigena predstavitevni celic (dendritičnih celic) ter njihova regulacija z inhibitorji proteaz predstavlja pomemben korak pri nastanku antigenskih peptidov.

Metode. Človeške dendritične celice so bile uporabljene kot celični model za študij proteaznega inhibitorja cistatina C. Pripravljene so bile tanke zamrznjene rezine nezrelih in zrelih dendritičnih celic ter označene s specifičnimi protitelesi za kvantitativno elektronsko mikroskopijo. Pod transmisijskim elektronskim mikroskopom so zrna koloidnega zlata, vezana na specifične sonde (protitelesa), pokazala natančno lokalizacijo označenega inhibitorja.

Rezultati. Ovrednotenje označenih celic s statističnim testom Hi-kvadrat je potrdilo razlike v vsebnosti cistatina C v različnih celičnih organelih.

Zaključki. Potrjena je bila statistično značilna razlika v znotrajcelični porazdelitvi cistatina C med populacijama nezrelih in zrelih dendritičnih celic.