Fertility preservation in pre-pubertal girls with cancer: the role of ovarian tissue cryopreservation

W. Hamish B. Wallace[1*] MD, Thomas W. Kelsey[2] PhD, Richard A. Anderson[3] MD

1. Department of Haematology/ Oncology, Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, UK. Hamish.Wallace@nhs.net *Corresponding author.

2. School of Computer Science, University of St Andrews, St Andrews, UK

3. MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh.

Running Title: **Fertility preservation for girls with cancer.**

Capsule:

The focus of this review is to discuss the indications for ovarian tissue cryopreservation in young patients with cancer, and future potential uses for this tissue.

Narrative Abstract:

With increasing numbers of survivors of cancer in young people future fertility and ovarian function are important considerations that should be discussed before treatment commences. Some young people, by nature of the treatment they will receive, are at high risk of premature ovarian insufficiency and infertility. For them, ovarian tissue cryopreservation (OTC) is one approach to fertility preservation that remains both invasive and for young patients experimental. There are important ethical and consent issues that need to be explored and accepted before OTC can be considered established in children with cancer. In this review we have discussed a framework for patient selection which has been shown to be effective in identifying those patients at high risk of premature ovarian insufficiency (POI) and who can be offered OTC safely.

Key Words:

Ovarian tissue cryopreservation; Pediatric Cancer; Fertility preservation

Introduction

The number of survivors of childhood cancers has increased over the last four decades. In the USA, the five year actuarial survival rate of the age group 0-14 years for the period 1950-1954 was only 20% (1) whereas for the period 2008-2012, the crude survival rate (incidence less mortality per 100,000 children) was 87% (2). Across the EU, the crude death rate from cancer for the 0-14 age group has fallen from 3.9 per 100,000 in 1997 to 2.1 per 100,000 in 2012 (3). The corollary to these improvements is that the general population contains a progressively larger proportion of survivors of childhood cancer (4), many of whom are at risk of multi-faceted chronic morbidity as a result of their successful treatment (5). Although many survivors of childhood cancer go on to have children, the potential loss of fertility is a frequent concern of both patients, their parents and medical carers (6). Some children, by nature of the treatment they will receive, are at high risk of infertility. For example, a large-scale study by the Childhood Cancer Survivor Study (CCSS) found that, compared to their siblings, the relative risk (RR) for survivors of ever being pregnant is 0.56 after 5-10 Gy to a field including the ovaries, with the RR falling to 0.18 after more than 10 Gy to a field including the ovaries (7). Recent data from the CCSS also

suggest that the prevalence of subfertility, even where ovarian function is retained, is increased (8). The focus of this review is to discuss the indications for ovarian tissue cryopreservation in young patients with cancer, and future potential uses for this tissue.

At diagnosis, all patients deserve an informed consultation about their fertility prognosis (9, 10). For some, their prognosis is uncertain, but for the majority of patients it is clear whether – for the individual – the risk of compromising fertility with first line treatment is low, medium or high (11).

At the time of diagnosis, the young patient is often unwell, and the family is facing an extraordinarily difficult time. The patient is undergoing many complex investigations and procedures, and the treating pediatric team may be able to offer entry into complex research studies that require informed consent from the patient and the family. These investigations, procedures and discussions take time, and are challenging for the patient and their family. However, we believe that informed discussion about the fertility prognosis – while potentially an additional burden – can be a positive experience, even if a fertility

preservation procedure is not indicated (because the assessed risk to future fertility is low) or indeed is not possible.

It is, regrettably, clear that these discussions do not always take place (12, 13). Assessment of UK practice relating to information provision about the effects of cancer treatment on fertility and options for fertility preservation showed that discussions were less common with girls than with boys, with young age of the girl being the most common reason cited for not having such a discussion (10). The American Society of Clinical Oncology has produced two evidence-based recommendations for fertility preservation for patients with childhood cancer (14, 15). A recent study of compliance with these recommendations reported that none of 136 patients older than 13 years at their last visit were counselled on fertility preservation (16).

Background

The human ovary is active during childhood (17). Follicles are recruited towards maturation at all ages, differentiate to preantral and antral follicles, but – for pre-pubertal ages – in the absence of sufficient gonadotropic support undergo atresia before they reach pre-ovulatory sizes (18). We have devised an age-related model of the non-growing follicle (NGF) population in healthy females (Figure 1), and additionally calculated the age-related rates of recruitment of NGFs towards maturation (Figure 2) (19). Interestingly, the rate of recruitment peaks at around 14 – 15 years of age, irrespective of whether there is a high, medium or low ovarian reserve. In theory, the pre-pubertal ovary is an ideal candidate for ovarian tissue cryopreservation (OTC), with plentiful follicles available for cryopreservation and future re-implantation although there is evidence that the childhood ovary contains a significant proportion of morphologically abnormal follicles, that are lost in adolescence (20). No perfect index of ovarian reserve exists, but the best available indirect biomarker is anti-Müllerian hormone (AMH) (Figure 3)(21) which is of established value in adult women (22). However, its utility as an indirect marker of ovarian reserve in the pre-

pubertal child remains uncertain. There is some evidence that AMH in children newly diagnosed with cancer is lower than healthy age-matched children (23), with comparable data in adult women (24). The role for AMH in the assessment of ovarian reserve in young patients with cancer remains a subject of on-going research.

Treatment with cytotoxic drugs inhibits follicular growth (18, 25, 26), but other mechanisms for the reduction of ovarian reserve have been suggested. Cyclophosphamide is known to increase the rate at which follicles are recruited towards maturation, leading to what has been termed 'burnout' of the NGF population (27). A recent *in vitro* study in a mouse model indicates that different chemotherapy drugs are likely to be cytotoxic to different cells in the ovary, with cisplatin and doxyrubicin affecting primarily oocytes and granulosa cells respectively (28). Irradiation to field that includes the ovaries in childhood causes permanent damage by destroying the small follicles (30, 31). It has been suggested that abdominal irradiation impairs follicle development as well as destroying small follicles (32). By modelling NGF decline with increasing age, we have shown that the LD_{50} of the human oocyte is less than 2 Gray (30) . This may be helpful in predicting the age at which

premature ovarian insufficiency (POI) develops if the dose to the ovary furthest away from the radiation field can be calculated (31). While cytotoxic treatment may accelerate depletion of NGF pool, leading to impaired fertility and POI, analysis of a prospective cohort of young patients with cancer has shown that serum AMH can indicate POI even in very young girls, before conventional markers e.g. FSH are elevated (33).

Radiation to a field that includes the pelvis (including total body irradiation) has been shown to impair uterine growth and blood flow. Those patients who have achieved a pregnancy but who have been exposed to radiation to the uterus have a very high incidence of late miscarriage, fetal growth restriction and premature birth (34-38).

Potential Indications for Ovarian Tissue Cryopreservation.

Ovarian tissue cryopreservation is one relevant approach to fertility preservation for the pre-pubertal girl facing treatment-induced loss of fertility, although ovarian shielding from radiotherapy and potentially oophoropexy may also be appropriate considerations (39). OTC is an invasive and still experimental procedure for young patients, requiring laparoscopic surgery. Our practice is to take multiple ovarian cortical strips from one ovary if possible, rather than a whole ovary as advocated by some authorities (40). The importance of patient selection, considering the clinical need, patient and parental consent, and surgical risk in what are necessarily unwell children cannot be overstated. We have previously described a framework for consideration of the issues that are relevant, divided into issues that are intrinsic to the patient (her age and general health, capacity for consent, and ovarian reserve), and extrinsic (notably the risk to her fertility of the treatment proposed) (9). The validity of the 'Edinburgh criteria' for OTC has recently been assessed (Table 1) (40). We have offered this procedure, performed laparoscopically, to girls with newly diagnosed cancer who met these criteria since 1996. Only 34 (8%) of the 410 patients treated in a

regional children's cancer centre met the Edinburgh selection criteria and were offered ovarian tissue cryopreservation before starting cancer treatment. 13 patients declined the procedure and 21 consented, and the procedure was completed successfully in 20 patients of whom 14 were available for assessment of ovarian function. The median age at the time of follow-up for the 20 patients was only 16·9 years (IQR 15·5–21·8), however, of the 14 assessable patients who had successfully undergone OTC, six had developed POI at a median age of 13·4 years (IQR 12·5–14·6). Assessment of ovarian function was possible for 141 of the 376 patients who were not offered cryopreservation; only one of these patients had developed POI. The cumulative probability of developing POI after treatment was completed was significantly higher for patients who met the criteria for ovarian tissue cryopreservation than for those who did not (15-year probability 35% [95% CI 10–53] *vs* 1% [0–2]; p<0·0001; hazard ratio 56·8 [95% CI 6·2–521·6] at 10 years) (Figure 4). While these criteria are proposed as a starting point for further research and refinement, they do appear to be a clinically useful guide to aid patient selection. Longer-term follow up will be necessary to assess later onset POI and the prevalence of subfertility of this cohort.

Potential Indications for re-implantation of ovarian tissue.

The key indication for re-implantation of ovarian tissue is for restoration of fertility. While the freeze/thaw followed by the re-implantation process causes attrition of about three quarters of the NGF population (41), over sixty live births have been reported after transplantation of cryopreserved ovarian tissue (42). The majority of women show restoration of ovarian activity by four months after transplantation, with live birth in about 25% (42). All these lives births derived from ovarian tissue had been taken from adult women. In a recent report (43) the authors have described a live birth in a woman with sickle-cell anaemia treated with a myeloblative conditioning regimen as part of a stem cell transplantation, after autograft of cryopreserved ovarian tissue taken at the age of 14 years, confirming the validity of this approach in adolescent as well as adult women.

While restoration of fertility is the main indication for re-implantation of ovarian tissue to patients with POI, the ovary is also an endocrine organ and restoration of hormonal function may also be a valid indication. Re-implantation of ovarian tissue for pubertal induction has also been

described (44, 45), with in both cases pubertal development and onset of menses. Induction of puberty with exogenous steroid hormones either orally or trans-dermally is well established in pediatric endocrine practice, with the main guiding principal being slow increases in estrogen exposure and delayed progesterone administration. The re-implantation of ovarian tissue in a hypergonadotrophic environment will result in ovulatory cycles with exposure to adult steroid concentrations much more rapidly than occurs physiologically, and waste of a finite number of germ cells (46). Additionally, in the cancer patient, there remains a possibility of recrudescence of the original cancer, particularly in hematological malignancies (47, 48).

Ethical considerations

OTC in the young patient with cancer offers ethical challenges for the patient, their family, and the treating team. The young patients themselves are usually unable to give informed consent for an experimental procedure. Therefore, in the case of OTC for young patients, informed consent is obtained from the parent or guardian. For an experimental procedure to be ethical in a child it must considered to be in the child's best interests. At the time of presentation, the patient is often unwell and may be at increased risk of bleeding and/or infection from a laparoscopic procedure. However, if the chances of a cure are good, and if future fertility is likely to be compromised, it may be considered to be in the best interests of the child to have their ovarian tissue cryopreserved for future use. If the ovarian tissue is not taken before treatment commences, the opportunity to preserve fertility may have been lost and will certainly be compromised. If OTC were to be reclassified as established rather than experimental (15, 49), then the procedure may then be considered to be in the best interests of the child, and therefore consent from parent or guardian to be ethically justifiable (50-52). In some European countries, but not in the United

Kingdom or United States of America, OTC is already considered an established procedure. Return of the tissue happens at a later date when the child is mature and able to give valid informed consent (9). In summary, obtaining ovarian tissue for cryopreservation remains experimental and in our Institution is carried out under the auspices of an ethically approved clinical trial.

Conclusions

OTC in children remains experimental, and is an invasive procedure requiring a general anesthetic, which may carry additional risks in the new young patient with cancer. There are important ethical and consent issues that need to be explored and accepted before OTC can be considered established in children with cancer. The majority of young patients with cancer will not have their fertility significantly compromised by their planned treatment and so patient selection is essential. We have proposed a framework for patient selection which has been shown to be effective in identifying those patients at high risk of POI and who can be offered OTC safely.

References

1. Ries LAG EM, Kosary CL, et al. SEER cancer statistics review, 1975–2001. In. Bethseda, MD: National Cancer Institute, 2004.
2. . United States Cancer Statistics: 1999–2012 Incidence and Mortality Web-based Report. In. Atlanta: U.S. Cancer Statistics Working Group, 2015.
3. . Crude death rate (per 100,000 inhabitants), all childhood cancers. In: Heidi Data Tool (ECHI - European Core Health Indicators), 2015.
4. Skinner R, Wallace WH, Levitt GA, Group UKCsCSGLE. Long-term follow-up of people who have survived cancer during childhood. Lancet Oncol 2006;7:489-98.
5. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT *et al.* Chronic health conditions in adult survivors of childhood cancer. N Engl J Med 2006;355:1572-82.
6. Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer. A pilot survey of survivors' attitudes and experiences. Cancer 1999;86:697-709.
7. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC *et al.* Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol 2009;27:2677-85.
8. Barton SE, Najita JS, Ginsburg ES, Leisenring WM, Stovall M, Weathers RE *et al.* Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. Lancet Oncol 2013;14:873-81.
9. Wallace WH, Critchley HO, Anderson RA. Optimizing reproductive outcome in children and young people with cancer. J Clin Oncol 2012;30:3-5.
10. Anderson RA, Weddell A, Spoudeas HA, Douglas C, Shalet SM, Levitt G *et al.* Do doctors discuss fertility issues before they treat young patients with cancer? Hum Reprod 2008;23:2246-51.
11. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 2005;6:209-18.
12. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol 2002;20:1890-7.
13. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. J Clin Oncol 2002;20:1880-9.
14. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K *et al.* American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917-31.
15. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH *et al.* Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500-10.
16. Salih SM, Elsarrag SZ, Prange E, Contreras K, Osman RG, Eikoff JC *et al.* Evidence to incorporate inclusive reproductive health measures in guidelines for childhood and adolescent cancer survivors. J Pediatr Adolesc Gynecol 2015;28:95-101.

17. Peters H, Himelstein-Braw R, Faber M. The normal development of the ovary in childhood. Acta Endocrinol (Copenh) 1976;82:617-30.
18. Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. Clin Endocrinol Metab 1978;7:469-85.
19. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. PLoS One 2010;5:e8772.
20. Anderson RA, McLaughlin M, Wallace WH, Albertini DF, Telfer EE. The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence. Hum Reprod 2014;29:97-106.
21. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH. A validated model of serum anti-mullerian hormone from conception to menopause. PLoS One 2011;6:e22024.
22. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R *et al.* The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370-85.
23. van Dorp W, van den Heuvel-Eibrink MM, de Vries AC, Pluijm SM, Visser JA, Pieters R *et al.* Decreased serum anti-Mullerian hormone levels in girls with newly diagnosed cancer. Hum Reprod 2014;29:337-42.
24. Lawrenz B, Fehm T, von Wolff M, Soekler M, Huebner S, Henes J *et al.* Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma--evaluation by using antimullerian hormone and retrieved oocytes. Fertil Steril 2012;98:141-4.
25. Himelstein-Braw R, Peters H, Faber M. Morphological study of the ovaries of leukaemic children. Br J Cancer 1978;38:82-7.
26. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Update 2012;18:525-35.
27. Roness H, Gavish Z, Cohen Y, Meirow D. Ovarian follicle burnout: a universal phenomenon? Cell Cycle 2013;12:3245-6.
28. Morgan S, Lopes F, Gourley C, Anderson RA, Spears N. Cisplatin and doxorubicin induce distinct mechanisms of ovarian follicle loss; imatinib provides selective protection only against cisplatin. PLoS One 2013;8:e70117.
29. Yuksel A, Bildik G, Senbabaoglu F, Akin N, Arvas M, Unal F *et al.* The magnitude of gonadotoxicity of chemotherapy drugs on ovarian follicles and granulosa cells varies depending upon the category of the drugs and the type of granulosa cells. Hum Reprod 2015;30:2926-35.
30. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod 2003;18:117-21.
31. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys 2005;62:738-44.
32. Himelstein-Braw R, Peters H, Faber M. Influence of irradiation and chemotherapy on the ovaries of children with abdominal tumours. Br J Cancer 1977;36:269-75.
33. Brougham MF, Crofton PM, Johnson EJ, Evans N, Anderson RA, Wallace WH. Anti-Mullerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. J Clin Endocrinol Metab 2012;97:2059-67.

34. Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ, Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. Br J Obstet Gynaecol 1999;106:1265-72.
35. Critchley HO, Wallace WH, Shalet SM, Mamtora H, Higginson J, Anderson DC. Abdominal irradiation in childhood; the potential for pregnancy. Br J Obstet Gynaecol 1992;99:392-4.
36. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ *et al.* Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood 1996;87:3045-52.
37. Teh WT, Stern C, Chander S, Hickey M. The impact of uterine radiation on subsequent fertility and pregnancy outcomes. Biomed Res Int 2014;2014:482968.
38. Signorello LB, Mulvihill JJ, Green DM, Munro HM, Stovall M, Weathers RE *et al.* Stillbirth and neonatal death in relation to radiation exposure before conception: a retrospective cohort study. Lancet 2010;376:624-30.
39. Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol 2015;3:556-67.
40. Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol 2014;15:1129-36.
41. Baird DT, Campbell B, de Souza C, Telfer E. Long-term ovarian function in sheep after ovariectomy and autotransplantation of cryopreserved cortical strips. Eur J Obstet Gynecol Reprod Biol 2004;113 Suppl 1:S55-9.
42. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. J Assist Reprod Genet 2015;32:1167-70.
43. Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C *et al.* Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod 2015;30:2107-9.
44. Ernst E, Kjaersgaard M, Birkebaek NH, Clausen N, Andersen CY. Case report: stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. Eur J Cancer 2013;49:911-4.
45. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet 2012;379:588.
46. Anderson RA, Hindmarsh PC, Wallace WH. Induction of puberty by autograft of cryopreserved ovarian tissue in a patient previously treated for Ewing sarcoma. Eur J Cancer 2013;49:2960-1.
47. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril 2013;99:1514-22.
48. Sorensen SD, Greve T, Wielenga VT, Wallace WH, Andersen CY. Safety considerations for transplanting cryopreserved ovarian tissue to restore fertility

in female patients who have recovered from Ewing's sarcoma. Future Oncol 2014;10:277-83.
49. Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril 2015;104:1097-8.
50. Di Pietro ML, Virdis A, Gonzalez-Melado FJ, De Luca D. Cryopreservation of ovarian tissue in pediatrics: what is the child's best interest? J Matern Fetal Neonatal Med 2012;25:2145-8.
51. Grundy R, Larcher V, Gosden RG, Hewitt M, Leiper A, Spoudeas HA *et al.* Fertility preservation for children treated for cancer (2): ethics of consent for gamete storage and experimentation. Arch Dis Child 2001;84:360-2.
52. Hershberger PE, Finnegan L, Pierce PF, Scoccia B. The decision-making process of young adult women with cancer who considered fertility cryopreservation. J Obstet Gynecol Neonatal Nurs 2013;42:59-69.

Figures and Tables

Figure 1. Normative model for NGF population. The best model for the establishment of the NGF population after conception, and the subsequent decline until age at menopause. The figure shows the dataset (n = 325), the model, the 95% prediction limits of the model, and the 95% confidence interval for the model. The horizontal axis denotes age in months up to birth at age zero, and age in years from birth to 51 years. Reprinted with permission (19).

Figure 2. Rates of NGF recruitment towards maturation. Each sub-figure describes the absolute number of NGFs recruited per month, for ages from birth to 55 years, based on population decline predicted by the ADC model. The red curve denotes recruitment for individuals whose decline is in line with the average age at menopause; maximum recruitment of 880 follicles per month occurs at 14 years 2 months. The green curve denotes recruitment for individuals whose decline is in line with early age at menopause (the lower 95% prediction limit of the model); maximum recruitment of 104 follicles per month occurs at 14 years 2 months. The yellow curve denotes recruitment in line with late age at menopause (the upper 95% prediction limit of the model); maximum recruitment of 7,520 follicles per month occurs at 14 years 2 months. Reprinted with permission (19).

Figure 3. The normal range for serum AMH in girls and women. The red line is the log-unadjusted validated AMH model using IBC assay values. The blue and green lines are the 68% and 95% prediction limits for the model (plus and minus one and two standard deviations respectively). Reprinted with permission (21).

Figure 4. Cumulative probabilities of not having premature ovarian insufficiency. Reprinted with permission (40).

Table 1. The Edinburgh OTC selection criteria. Reprinted with permission (40).

Age younger than 35 years
No previous chemotherapy or radiotherapy if aged 15 years or older at diagnosis, but mild, non-gonadotoxic chemotherapy acceptable if younger than 15 years
A realistic chance of surviving for 5 years
A high risk of premature ovarian insufficiency (>50%)
Informed consent (from parents and, where possible, patient)
Negative serology results for HIV, syphilis, and hepatitis B
Not pregnant and no existing children