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Imminent ovarian failure in childhood cancer survivors

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ABSTRACT

The aim of this study was to investigate reproductive history and the prevalence of imminent ovarian failure (IOF) in female childhood cancer survivors. Reproductive history and ovarian function were evaluated by questionnaires ($n = 124$) and by measurement of follicle stimulating hormone (FSH) and oestradiol (E_2) levels ($n = 93$). IOF was defined as FSH > 10 IU/l or $E_2 > 0.28$ nmol/l on day 3 of the menstrual cycle, or FSH > 12.4 IU/l on day 7 of the pill-free interval. IOF was demonstrated in 22.6% of the participants and correlated with age at diagnosis ($P < 0.005$) and age at study ($P = 0.036$). IOF correlated inversely with methotrexate ($P = 0.046$). The incidence of miscarriages (22.7%) and recurrent miscarriages (7.3%) was increased. The male/female (M/F) ratio of the offspring was decreased. In conclusion, female childhood cancer survivors are at risk for IOF. If pregnant, the risk of (recurrent) miscarriages is increased. The M/F ratio in the offspring is decreased.

1. Introduction

Along with the increasing number of survivors of childhood cancer, there is growing concern about the late toxicity of cancer treatment in childhood, including the adverse effects on the female reproductive system. Although most female childhood cancer survivors have a normal menstrual cycle, prior studies found a diminished ovarian reserve and an increased risk of early menopause in these individuals.[1–3] In healthy women, ovarian reserve decreases spontaneously in life and as soon as the number of primordial follicles falls below 1100, menopause occurs.[4] From about 10 years before menopause, the reduced ovarian follicle reserve leads to sub-fertility without an alteration in menstrual pattern. This phase in the physiological decline of fertility in normal women is characterised by imminent ovarian failure (IOF). As in the general population menopause occurs physiologically from the age of 43 years, IOF may be considered extremely rare before the age of 33. Chemotherapy potentially damages primordial follicles in the ovaries and can cause early menopause.[5,6] In most chemotherapy-treated girls the ovarian reserve is apparently still large enough to enter puberty and to retain a normal menstrual cycle for several years. However, because of a diminished ovarian reserve they may be at risk of developing IOF at an early age, even before finishing family planning. Prior studies of hormonal assessments in childhood cancer survivors revealed that 10–50% of the patients had reduced ovarian function. However, most studies were limited to survivors of Hodgkin's disease or acute lymphoblastic leukaemia (ALL), and included small numbers of subjects and girls less than 18 years of age.[7–10] Byrne and colleagues have suggested that age at treatment above 12 years is a risk factor for developing early menopause and that girls treated around the time of menarche were especially at risk for fertility deficits.[2,11]

IOF can be detected by assessment of basal follicle stimulating hormone (FSH) and oestradiol (E_2) in the early follicular phase. In studies of in vitro fertilisation, high levels of

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basal FSH and/or E_2 are associated with a reduced number of oocytes collected and with low pregnancy rates.[12–15] Little is known about basal FSH concentrations in healthy women with respect to age. In newly registered sub-fertility patients ⩽40 years without oligomenorrhoea Van Montfrans and colleagues found elevated basal FSH (>10.0 IU/l) in 2.5% of subjects.[16]

The purpose of the current study was to estimate the prevalence of IOF by assessment of basal FSH and E_2, to evaluate risk factors for the development of IOF, and to evaluate pregnancy outcome in female childhood cancer survivors aged 18 years and older.

2. Patients and methods

2.1. Subjects

Females aged 18–45 years who had survived childhood cancer or Langerhans cell histiocytosis (LCH), had received chemotherapy with or without radiotherapy between 1968 and 1998 at the Paediatric Oncology Department of the University Medical Centre Groningen, the Netherlands, and who were at least 2 years off-treatment, were invited to participate in the study. Exclusion criteria were severe mental retardation or hypothalamic/pituitary irradiation of more than 25 Gy (because of potential pituitary insufficiency). The study consisted of a questionnaire about menstrual and reproductive history and assessment of basal FSH and E_2.

Of the 157 patients who met the inclusion criteria, 23 refused to participate or were lost to follow-up. Ten patients had permanent ovarian failure (POF) requiring hormonal supplementation treatment; all of them had received pelvic irradiation or total body irradiation. The remaining 124 completed the questionnaire. Patient characteristics are shown in Table 1. Patients were eligible for hormonal assessment if they experienced menstrual cycles. Twenty patients had no menstrual cycles for various reasons (pregnancy 10, depot-contraceptives 5, haemodialysis 1, hysterectomy 1, prolactinoma 1, normogonadotrophic amenorrhoea 1 and one 44-year-old patient was found to have been postmenopausal for 2 years). Thus hormonal assessment was performed in 104 patients. The results were complete in 93 patients and were incomplete or unsuccessful in 11 patients. Fourteen of these 93 patients had received infra-diaphragmatic irradiation; in four of them the irradiation field covered the ovaries.

Table 1 – Patient characteristics

	Questionnaire $n = 124$	Hormonal assessment $n = 104$
No. menstrual cycle (*n*)	20[a]	
Incomplete results (*n*)		11
Age at diagnosis (years)[b]	7.0 (0–19)	5.0 (0–19)
Age at study (years)[b]	27.0 (18–45)	26.0 (18–43)
Follow-up (years)[b]	20.0 (5–34)	20.0 (5–34)
XRT below diaphragm (*n*)	18	14
Ovaries involved (*n*)	4	4
Diagnoses (*n*)		
ALL	53	47
ANNL	3	3
Hodgkin's disease	14	11
NHL	4	2
Wilms'tumor	12	9
Neuroblastoma	3	3
Rhabdomyosarcoma	7	7
Osteosarcoma	10	5
Ewing's sarcoma	4	4
Germ-cell tumour	4	4
Nasopharynx carcinoma	1	1
Leiomyosarcoma	1	1
LCH	8	7

XRT, radiotherapy; ALL, acute lymphoblastic leukaemia; ANNL, acute non-lymphoblastic leukaemia; NHL, non-Hodgkin's lymphoma; LCH, Langerhans cell histiocytosis.
a Pregnancy $n = 10$, depot-contraceptives $n = 5$, haemodialysis $n = 1$, amenorrhoea $n = 4$ (see text).
b Median (range).

2.2. Questionnaire

Patients completed a questionnaire about menarche, menstrual cycle, reproductive history, and use of oral contraceptives (OC).

2.3. Hormonal assessment

Ovarian function was evaluated in each subject by measuring serum levels of FSH and E_2 in one single blood sample. In order to minimise the burden for potential participants, OC was not interrupted. The blood samples were taken at day 3 in a spontaneous menstrual cycle, and at day 7 of the pill-free interval in OC users. Serum was separated from all blood samples within 2 h after collection and was stored at −20 °C until centralised analysis. Serum FSH levels were measured by a time resolved fluoro-immunoassay (FIA-kits, delivered by Perkin–Elmer Life Sciences and used on the Delfia: Wallac Oy, Turkü, Finland). Intra- and inter-assay coefficients of variation were ⩽2.0% and ⩽3.5%. Serum levels of E_2 were measured by radio-immunoassay (RIA-kits, developed in the University Medical Centre, Groningen, The Netherlands). Intra- and inter-assay coefficients of variation were ⩽8.0% and ⩽13.0%.

Criteria for IOF in patients not using OC were FSH > 10.0 IU/l or E_2 > 0.28 nmol/l on day 3 of the menstrual cycle. Our cut-off point of 10 IU/l FSH is calibrated to World Health Organization Standard 78/549 and widely used in Europe and equals a level of 17 IU/l if the assay is calibrated against the second international reference preparation (IRP), which is widely used in the United States of America (USA).[12] The cut-off point of 0.28 nmol/l E_2 is derived from a prior study that found no ongoing pregnancies after IVF in women with basal E_2 > 0.274 nmol/l (75 pg/ml).[15] In OC users IOF was defined as FSH > 12.4 IU/l on day 7 of the pill-free interval. This cut-off point was derived from the study of Van Heusden and Fauser, who found FSH 12.4 IU/l to be the upper limit of the range in FSH on day 7 of the pill-free interval in healthy OC using female volunteers. As in that study E2 levels in the

pill-free interval showed a wide range, we decided not to use E2 for assessment of IOF in OC users.[17]

3. Statistical analysis

All data were processed with the Statistical Program for Social Sciences (SPSS Inc., Chicago, IL, USA). The significance of differences between groups were estimated using Mann–Whitney U test, χ^2 test, binomial test and Spearman's test. The association of IOF and age at diagnosis, age at study, length of follow-up, cytostatic drugs, or infra-diaphragmatic irradiation was evaluated by univariate and multivariate analysis. The following drugs were used as categorical predictors: methotrexate, vincristine, anthracyclines, alkylating agents, 6-mercaptopurine (6MP) and/or 6-thioguanine (6TG), actinomycin-D, mitoxantrone and cytarabine. Variables with probability score <0.1 were used in the logistic regression model. A logistic regression model was also used for the identification of cytostatic drugs that were associated with a decreased sex ratio. A probability score of $P < 0.05$ was considered significant. The confidence interval (CI) was 95%.

The study was approved by the institutional medical research ethics committee. Written informed consent was obtained from all patients.

4. Results

4.1. Prevalence of IOF (Table 2)

IOF was demonstrated in 21/93 patients (22.6%): 12/21 had increased serum FSH, 10/21 had increased serum E_2 including one patient without FSH data. One patient had both high serum FSH and E_2. The prevalence of IOF in subjects under 33 years was 14/75 (18.7%). The prevalence of IOF based on FSH > 10 IU/l in patients ⩽40 years, not using OC was 4/33 (12.1%), which was significantly more than the 2.5% incidence in sub fertile patients ⩽40 years found by Van Montfrans and colleagues ($P < 0.01$).[16]

In patients with OC ($n = 58$, median age at study 24.0 years, range 18–40 years) the prevalence of IOF was 12% versus 40% ($P < 0.01$) in patients without OC ($n = 35$, median age at study 28.0 years, range19–43 years). Patients with OC were significantly younger at time of study than patients without OC ($P = 0.01$).

Table 3 – Association between age at diagnosis, age at study and length of follow-up and imminent ovarian failure (IOF)

	No. IOF $n = 72$	IOF $n = 21$	P-value
Age at diagnosis (years, median (range))	5 (0–17)	11 (2–19)	0.005
Age at study (years, median (range))	25 (18–40)	30 (19–43)	0.036
Follow-up (years, median (range))	20 (5–34)	20 (5–29)	0.2

4.2. IOF and age or pubertal status (Table 3)

Patients with IOF had higher age at diagnosis ($P < 0.005$) and higher age at the time of study ($P < 0.036$) than patients without IOF. Age at diagnosis correlated with age at study ($P < 0.01$, correlation 0.360). The prevalence of IOF in patients treated after menarche was 9/20 (45.0%) compared with 12/73 (16.4%) in patients treated before menarche ($P < 0.01$).

IOF was not associated with length of follow-up.

4.3. IOF and treatment (Table 4)

If evaluated by univariate analysis we found an inverse association between IOF and treatment with 6MP or 6TG ($P = 0.043$) and methotrexate ($P = 0.036$). However, within the logistic regression model, IOF was only inversely associated with methotrexate ($P = 0.046$). We found no association of IOF and infra-diaphragmatic irradiation. In 4/14 patients with infra-diaphragmatic irradiation the ovaries were involved; one of these four patients had IOF. The median age at the time of study of these patients was 20.5 (range 19–32) years, which is little younger than the entire hormonal assessment group.

4.4. IOF and reproductive potential

Patients with IOF reported more often decreased reproductive function than patients without IOF: 8/21 versus 2/72 ($P < 0.01$), e.g., late conceiving, three or more miscarriages and infertility.

Table 2 – Prevalence of imminent ovarian failure (IOF) in 93 patients with hormonal assessments

	Total	IOF			P
	n	n	%	CI	
All patients	93	21	22.6	14.1–31.1	Unknown versus normal
Patients <33 years	75	14	18.7	9.9–27.5	Unknown versus normal[b]
Patients without OC[a]	35	14	40	13–67	
Patients with OC	58	7	12	8–12.5	<0.01 versus without OC
Patients ⩽40 years without OC	33	4	12.1	0.1–23.3	<0.01[c]

Criteria of IOF: see text. CI, confidence interval; OC, oral contraceptives.
a Age at study versus patients with OC $P = 0.01$, see text.
b Incidence of IOF in healthy women <33 years considered uncommon.
c Versus sub-fertile population ⩽40 years, without OC, of which 2.5% has a FSH > 10IU/l.[17]

Table 4 – Association between treatment and imminent ovarian failure (IOF)

Treatment	*n*	IOF (%)	*P*-value between received/not received[a]
Total	93	21 (22.6)	
Chemotherapy			
6MP/6TG	49	7 (14.3)	0.043
Methotrexate	58	9 (15.5)	0.036[c]
Anthracyclines	45	14 (31.1)	0.057
Mitoxantrone	4	1 (25.0)	0.906
Actinomycin-D	23	6 (26.1)	0.643
Cytarabine	27	4 (14.8)	0.252
Vincristine	79	17 (21.5)	0.561
MOPP	7	3 (42.9)	0.182
Cyclophosphamide	43	11 (25.6)	0.521
Any AA	49	13 (26.5)	0.336
Radiotherapy			
Cranial	30	7 (23.3)	0.905
Inphra-diaphragmatic (not involving ovaries)	10	4 (40.0)	0.159[b]

6MP/6TG, 6-mercaptopurine and/or 6-thioguanine; AA, alkylating agents; MOPP, combination of mechlorethamine, vincristine, procarbazine and prednisone.
a Univariate analysis: χ^2 test.
b Infra-diaphragmatic irradiation not involving ovaries versus no infra-diaphragmatic irradiation.
c Logistic regression $P = 0.046$.

4.5. *Pregnancy outcome*

Seventy-five pregnancies in 41 patients resulted in 58 healthy children (one pair of twins), one stillbirth and 17 miscarriages (22.7%, CI 14–34). Forty-one first pregnancies resulted in 32 healthy children, one stillbirth and eight miscarriages (19.5%; CI 9–35). The frequency of miscarriages in our study group is higher than the assumed 10–15% in the general population. Three patients demonstrated recurrent miscarriage (7.3%, CI 2–20), which is much more frequent than in the general population (0.5–1.0%). We found no association between miscarriages and infra-diaphragmatic irradiation not including the ovaries or any of the specific cytostatic drugs.

Table 5 – Association between treatment and sex ratio

Treatment	*n*	M:F	*P* versus normal population	*P* received versus not-received [a]
Total offspring	58	1:1.42	<0.05	
6MP/6TG	19	1:3.75	<0.01	0.028[b]
Methotrexate	33	1:1.75	<0.05	0.37
Anthracyclines	32	1:1.28	0.1	0.68
Mitoxanthrone	6	1:1	0.31	0.65
Actinomycin D	20	1:1	0.13	0.04
Cytarabine	8	1:7.0	<0.05	0.74
Vincristine	48	1:1.4	<0.05	0.92
MOPP	6	1:1	0.31	0.65
Cyclophosphamide	36	1:1	0.13	0.09
Any AA	42	1:1	0.12	0.03

6MP/6TG, 6-mercaptopurine and/or 6-thioguanine; AA, alkylating agents; MOPP, combination of mechlorethamine, vincristine, procarbazine and prednisone.
a Univariate analysis: χ^2 test.
b Logistic regression significant $P = 0.03$.

4.6. *Male/female (M/F) ratio (Table 5)*

The M/F ratio in the 58 newborns was 24/34 (1.00:1.42), which is significantly decreased compared with the general population (1.05:1.00; $P < 0.05$). This was due mainly to the M/F ratio in the offspring of 6MP/6TG treated patients, which was 4/15 (1.00:3.75), compared with 20/19 (1.00:0.95) in the offspring of patients not treated with 6MP/6TG ($P < 0.05$).

5. Discussion

IOF appears to be a frequent complication in females treated for childhood cancer. Our results are supported by those of Sklar and colleagues, who found a relative risk of premature menopause of 14.5 in young adult survivors of paediatric cancer.[18]

Although the prevalence of IOF in the normal population is not known exactly, it is considered rare under the age of 33 years; however, in our study population, which was restricted to menstruating women, 18.7% of those under 33 years had IOF. Furthermore, the women under 40 years without OC had significantly more often IOF, based on high FSH, than a comparable sub-fertile population.[16] It should be emphasised that 11 patients out of the total cohort of 157 already had permanent ovarian failure, and therefore were not eligible for hormonal assessments. In our study group the prevalence of IOF increases with age. This is in accordance with the normal physiological decline of the ovarian function with ageing.

It is difficult to identify single drugs that reduce the ovarian function, because all patients received combination chemotherapy. Unexpectedly, alkylating agents were not associated with IOF, although alkylating agents are generally known to be gonadotoxic. In the present study the use of methotrexate was associated with a lower risk of IOF, compared with the entire study group. Methotrexate is frequently used in treatment of ALL. Several authors have described preservation of normal gonadal function in adult female childhood ALL survivors.[19–21] Furthermore, ALL is over-represented in the youngest age group, as the incidence peak of ALL is between 2 and 6 years of age.

We found no association between infra-diaphragmatic irradiation and IOF. Even if the ovaries were not included in the irradiation field, they most likely have been exposed to scatter irradiation. Wallace and colleagues have estimated the lethal dose 50 (LD_{50}) of the human oocyte to be as low as <2 Gy.[22] However, due to the small group size any association may not have been detected. Only one out of four patients with ovarian irradiation had IOF. She had received total body irradiation prior to a bone marrow transplantation at age 14 years; the other three girls had stage III Wilms' tumour and were much younger at diagnosis (1–3 years).

In our study, treatment around or after menarche was associated with a higher prevalence of IOF than treatment before menarche (45% versus 16.4%). This is in accordance with a recent study of Byrne and colleagues, who found that girls treated around the time of menarche were especially at risk for fertility deficits.[22] Our results may have been confounded

by the fact that at the time of the study those patients who were treated around or after menarche were a few years older, although not significantly, than patients who were still premenarchal at treatment. More data are needed to determine whether pubertal status at treatment is a risk factor for the development of IOF.

We found that patients without OC at the time of the study had IOF significantly more often than those using OC (40% versus 12%; $P < 0.01$). An explanation might be that the women without OC were older than those using OC, and with ageing the risk of IOF increases. On the other hand, there may have been false negative results as a consequence of the definition of IOF we used in patients using OCs. In the study of van Heusden and Fauser OCs with 30 μg ethinylestradiol (EE), as used by nearly all of our OC patients, showed a rebound phenomenon of FSH at the end of the pill-free interval in which FSH reaches 12.4 IU/l as the upper limit of the range.[17] As in natural cycles, an FSH level of 10 IU/l is accepted as a marker for IOF, the question arises whether an FSH level of 12.4 U/l in OC users is discriminating enough. Thus underestimation of the prevalence of IOF in OC using patients may have happened. More research is warranted to estimate the value of hormone levels in the pill-free interval in the assessment of ovarian reserve in OC users.

As expected, patients with IOF reported sub-fertility more often than those without IOF. However, assessment of reproductive potential in these patients is incomplete, as at the time of the study not all of them were ready to start a family.

The incidence of (recurrent) miscarriages in our study population was considerably higher than in the normal population. Reports on the risk of miscarriage in childhood cancer survivors are conflicting; some authors found an increased risk, whereas others found a normal incidence of miscarriages compared with controls or with the general population.[23–25]

The sex ratio of the offspring of the entire study group was reversed, especially in the offspring of patients treated with 6MP/6TG. To our knowledge the relation of purine-antagonists and reversed sex ratio has not been reported before. Green and colleagues found reversed sex ratio in offspring of male childhood cancer survivors.[26] Although in that study it was suggested that the modification of sex ratio could be due to impaired testosterone levels in the male survivors, no relationship between sex ratio and specific alkylating agents was demonstrated. In our study, evaluating female survivors, the deficit of boys among the offspring could suggest a defect in the X-chromosome. However, Bajnoczky and colleagues did not found chromosomal instability in offspring of childhood cancer survivors.[27] It could be worthwhile to record gender of foetuses of childhood cancer survivors with miscarriages.

5.1. *Limitations*

Unfortunately we had no control group. Reference values were selected from the recent literature; however hormonal assessment of healthy females with and without OC could have provided more reliable age-dependent reference values. Perhaps it would have been better if we had more than one measurement for each patient. Hormone levels may fluctuate between cycles. However, in IVF studies, raised basal FSH levels measured in a single cycle were associated with a decline in oocyte numbers collectable after stimulation.[28] If one single raised FSH level represents a reduction in follicle number, the number of patients fulfilling the criteria for IOF in our study could even be more if more than one measurement for each patient had been performed. Moreover, ovarian reserve can be assessed more precisely by serum anti-müllerian hormone,[29] which at the time of the study was not available in our laboratory, or by assessment of the number of antral follicles by ovarian sonography. Larsen and colleagues found that childhood cancer survivors with spontaneous menstrual cycle had smaller ovarian volume and a lower number of antral follicles per ovary than controls.[10] Wallace and Kelsey showed that ovarian reserve and reproductive age in healthy pre-menopausal women can be determined from the measurement of ovarian volume by transvaginal sonography.[30] However, this applies only to women who are not using hormonal contraception. As more than half of the patients in our study used oral contraceptives, we decided not to do transvaginal ovarian sonography; moreover, it is experienced as unpleasant by many young women who still are virginal.

5.2. *Practical implications*

The prevalence of fertility problems in our study group does not seem to be different from that of the normal population. However, the high prevalence of IOF suggests a narrowed fertility window. If female childhood cancer survivors are not aware of the increased risk of early IOF they may delay family planning until their thirties, like their healthy peers in The Netherlands. Assessment of ovarian reserve can therefore be essential for optimal patient counselling.

Conflict of interest statement

None declared.

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