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Article type : 3 Original Article - Australia, Japan, SE Asia

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Title page

Title

Spontaneous pregnancies in female survivors of childhood allogeneic haemopoietic stem cell transplants for hematological malignancies

Short Running Title

Spontaneous pregnancies in childhood HSCT survivors

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Conflict of Interest Statement

No author has any conflict of interest in regard to any aspect of the current study.

Funding Statement

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CEN.14266

The current study was not supported by any funding source.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Summary

Objective: Spontaneous pregnancies and live births are rarely reported after hematopoietic stem cell transplant (HSCT). We report spontaneous pregnancy outcomes of sexually active female survivors of childhood allogeneic HSCT, to provide more data for future counselling.

Design, Patients and Measurements: Retrospective review of all female survivors of childhood hematological malignancies who had allogeneic HSCT at the Royal Children Hospital between 1985 and 2011. Data was retrieved from medical records, updates from treating hematologist or endocrinologist, and was cross-referenced with self-reported questionnaires. Female survivors who were sexually inactive were excluded from analysis.

Results: Six of 37 (16.2%) female survivors reported spontaneous pregnancies resulting in 8 live-births. Among 22 women who received total body irradiation (n=21) +/- cranial irradiation or isolated cranial irradiation (n=1), and high dose cyclophosphamide, three reported pregnancy resulting in live-births (14%), whilst three of 15 women who received chemotherapy alone had pregnancy with live-births (20%).

Conclusions: Our current finding, albeit a small sample size, reinforces the importance of counselling female survivors of HSCT about the possibility of spontaneous pregnancy occurring despite documented ovarian failure and for need of contraception to avoid unplanned pregnancy.

Keywords

Spontaneous pregnancy, live-births, allogeneic HSCT, childhood

Word Count

1985

Table

Two tables

Body of text

Introduction:

Despite the high cure rates of children with haematologic malignancies achieved by contemporary chemotherapeutic regimens, patients with high risk features at diagnosis including those who respond poorly to initial therapy or suffering a relapse of the disease, frequently progress to hemopoietic stem cell transplant (HSCT) as a chance of potential cure. Ovarian dysfunction is almost universal after HSCT for malignant disease. Reported pregnancies resulting in live-births amongst large patient cohorts surviving after HSCT for leukaemias are rare, rates below 2% when denominators for specific diagnostic subgroups are given. For non-malignant disease, principally severe aplastic anemia (SAA), post-transplant recovery of ovarian function and number of pregnancies and live-births are far greater despite significantly lower numbers of patients transplanted for SAA (1-3,8). Importantly, gonadotoxic effects of alkylating agents, particularly busulfan, total body irradiation (TBI) and irradiation of hypothalamic-pituitary (HP) axis, ovary and uterus (5-7) are both dose and age dependent, with 7% - 13.5% live-births being reported in those with underlying hematological malignancies (8,9). Our study aimed to describe spontaneous pregnancy outcome of sexually active female survivors of childhood allogeneic HSCT, to provide more objective data for counselling.

Materials and Methods:

We reviewed all patients surviving greater than five years after allogeneic HSCT for hematological malignancies performed from age 6 months to 18 years, between 1985 and 2011 at the Royal Children's Hospital (RCH), Melbourne, aiming to identify women with natural conception resulting in live-birth(s) post HSCT. This is a nested cohort of a much larger cohort of 230 female and male survivors who had undergone childhood HSCT, of whom 156 (55 females and 101 males) were transplanted for hematological malignancies and one had stage 3 neuroblastoma. We captured all individuals who underwent HSCT through a complete and updated database at the RCH oncology department and with a cross referenced database commenced in 1981, kept and updated by an author (KT). The first years were specifically excluded as survival was limited and oncology protocols were radically changed during that period , so 1985 was taken as the first date, with final data collected for all those who survived at least 5 years after transplant, that is, until the end of 2018. To maximize case ascertainment of pregnancies, information from medical records, current treating hematologists and endocrinologists was cross-referenced with anonymous questionnaires collected from an ongoing bone marrow transplant survivorship study encompassing the

same cohort. Girls lost to follow-up or dying before age 15 years or those never sexually active were excluded. Survivors ≥15 years of age at data collection in 2018 were eligible for study. Potentially sexually active female survivors were stratified according to pre-transplant conditioning regimen into total body irradiation (TBI) and non-TBI groups.

Results:

All children who underwent HSCT at RCH were included in this study, of whom 230 remained alive 5 years post-HSCT and 227 were alive, among whom 81 were female (79 alive, at the time of writing). Fifty-five female survivors were transplanted for haematological malignancy, of whom 43 had active follow-up. A total of 18 of 55 were excluded due to loss to follow up before age 15 (N=3), death from second malignancy (N=2), being <15 years (N=5), never sexually active (N=8). Of 37 who were sexually active, 68% (25/37) responded to a self-reported anonymous questionnaire.

Of the 37 female survivors, 34 was transplanted for leukemias and 3 transplanted for myelodysplasia, utilizing hemopoietic stem cells from bone marrow, cord blood or peripheral blood stem cells, from either related or unrelated donors. Twenty one of 37 had fractionated TBI, of whom 5 had cranial irradiation range 10-18Gy). One had 18 Gy cranial irradiation without TBI. For the non-TBI group, 13 had busulfan (16-20mg/kg) and cyclophosphamide (120-200mg/kg) based conditioning regimens, 2 had melphalan (160mg/kg) and Fludarabine (150mg/m^2). Table 1 summarizes clinical data for current age, year of HSCT, pubertal and ovarian function status of the 37 sexually active female survivors. Of those, 25 had spontaneous pubertal onset, 11 prior to and 14 after HSCT. Spontaneous onset of menarche occurred in 11 of 37 (limited by missing data). Premature ovarian failure post-HSCT occurred in 28 and one had elevated gonadotrophins but normal menstrual cycles. Normal menstrual cyclicity was retained in 7 of 37 at the time of writing, including the one with elevated gonadotrophins. Of the 7 with normal ovarian function, one had hypogonadotropic hypogonadism.

Of 37 female survivors, six (16.2%) had seven naturally conceived pregnancies resulting in 8 live-births at time of report, with one twin pregnancy and no premature births. Details of underlying diagnosis of hematological malignancies, cumulative chemotherapy dosage, TBI and gonadal function impairment post-transplant are detailed in Table 2. Of 21 women who had received TBI (12Gy, six fractions over three days), three (14%) conceived naturally, with four live-births. One of 37 women also had prior 18Gy cranial irradiation (CRT). Of 15 women who received non-TBI regimens with busulfan 16mg/kg and cyclophosphamide

120mg/kg (n=8) or 200mg/kg (n=4), or melphalan 160mg/m^2 and fludarabine (n=2), three (20%) reported naturally conceived pregnancies resulting in live births. Median time from HSCT to first pregnancy for the 6 with spontaneous pregnancy was 13.7 years (range 7.1 - 18.5 years).

Three of our six spontaneous pregnancies with live-births had no specific pre-HSCT risk factors for infertility, one had Imatinib for chronic myeloid leukemia, one had hydroxyurea for acute myeloid leukemia, and one had no chemotherapy prior to HSCT conditioning. Two women with acute lymphocytic leukemia (ALL) had CRT prior to transplant and had high infertility risks with ovarian failure soon after HSCT, requiring long term hormonal replacement therapy (HRT). Two survivors were treated before menarche and both had spontaneous menarche but premature ovarian failure necessitating HRT.

At time of conception, three remained on HRT, two had stopped the HRT treatment, one had unplanned pregnancy after stopping oral contraceptive pills (OCP). Of the three who had TBI, Patient 1 had ceased HRT and was amenorrhoeic for several (unrecorded number) months prior to conception with elevated FSH of 51 IU/L and AMI <1pmol/l, Patient 2 had ceased her HRT and remained amenorrhoeic before pregnancy, Patients 3,4,5 had taken HRT for14 years, 7.8 years and 16.2 years respectively before their first pregnancy. Patient 3 had secondary amenorrhoea post HSCT and remained on HRT from 16 years of age till pregnancy. For those who had chemotherapy alone, Patient 4 had documented low AMH prior to conception. Patient 5 had a trial cessation of HRT. She then became very symptomatic of estrogen deficiency with elevated FSH 42IU/L, necessitating resumptions of HRT, which remained ongoing at time of conception. Patient 6 refused to take OCP. She had low AMH and estradiol documented biochemically around 2.5 years prior to the unplanned pregnancy that was complicated by heart failure, requiring cardiac pacing and metoprolol.

Discussion:

Among sexually active female survivors, who had childhood HSCT for hematological malignancies and a history of ovarian dysfunction, 16.2% conceived naturally leading to 8 live-birth(s). We did not collect data regarding spontaneous or medical miscarriage. Overall natural conception rate may thus be higher. Actual live-birth incidence will only become evident with longer follow up of this young cohort.

Fertility post-transplant is dependent on residual ovarian reserve, an intact hypothalamic pituitary axis and a uterus capable of allowing implantation and sustaining pregnancy. The

follicular pool present at birth naturally depletes, to 50% by age 15, 12% by age 30 and 3% by age 40 (10). Exposure to gonadotoxins more rapidly depletes ovarian reserve, resulting in an earlier onset of ovarian failure with significantly increased risk of infertility for cumulative cyclophosphamide doses > 9g/m^2 (11,12). A childhood acute lymphocytic leukemia (ALL) survivor study looking specifically at fertility demonstrated no significant association between alkylating agent (cyclophosphamide) exposure and pregnancy rate (13),reflecting a relatively high ovarian reserve in children, with similar reports of normalization of ovarian function after HSCT for severe childhood aplastic anemia (8). However, of our cohort who conceived, all were older at time of HSCT and all received higher dosage of gonadotoxic therapy compared to that report. By contrast, in a cohort who predominately received CRT, lower first pregnancy rate in ALL survivors aged 18-21 and >21 was observed, compared to sibling controls (RR 0.60. For those received CRT (18-24Gy) after menarche, no first pregnancies occurred after age 30, with postulated impaired pulsatile mid-cycle luteinizing hormone peaks (13) and reflecting lower ovarian reserve.

Survivors of HSCT performed for haematologic malignancy face additional risks for infertility due to extensive prior chemotherapy with or without CRT, compared with HSCT for non-malignant conditions, who had no gonadotoxic insult prior to HSCT conditioning. Cumulative gonadotoxin exposure worsens ovarian reserve (1,8), busulfan consistently associated with ovarian dysfunction and low or no pregnancy rates, compared to TBI (1,2,8). TBI may impact fertility by direct gonadal damage or via effect on the HP axis. In doses commonly used in pre-transplant conditioning regimens (12-14.4 Gy), TBI has not been associated with gonadotrophin deficiency but after previous CRT, total HP axis exposure may exceed 30Gy, with increasing, time-related risk of gonadotrophin deficiency (7).

Dose related TBI impact upon ovarian function varies with increasing age, from an estimated 20.3Gy at birth, 18.4Gy at 10 years to 16.5Gy at 20 years. Regarding prediction of immediate or permanent ovarian failure, by assuming radiosensitivity of oocyte to be <2Gy (6), for women over the age of 40 years, radiation of 6Gy or more was reported to cause ovarian failure (14). Prepubertal children having HSCT with or without TBI may experience primary ovarian failure prior to puberty, have pubertal arrest or achieve menarche with later premature ovarian failure. Late recovery of ovarian function post-TBI was recently reported in 29% of children and adolescents (8), although we believe that such recovery is likely to be relatively short term with inevitable premature menopause well recognized (15).

Fertility is further compromised by early uterine irradiation, impacting upon uterine size, vascularity, distensibility (7), contributing to high spontaneous miscarriage rate and preterm

births (1,9). Although the youngest of our cohort at time of TBI was almost 14 years, no infants born to these women were born prematurely.

Low anti-Mullerian hormone (AMH) has been reported in 26/28 survivors of either autologous or allogeneic HSCT during childhood for a range of malignant and non-malignant conditions (16). Although low AMH predicts ovarian reserve, pregnancy is not infrequently reported in women with low AMH who have not been exposed to gonadotoxins (17-18), as seen in 2 of our cohort where AMH levels were available.

Our live-birth outcomes of naturally occurring pregnancy are similar to others. In a cohort under age 21 at HSCT (9), 13.5% had spontaneous pregnancies with live-births (assuming successful outcomes of late gestation in 2 survivors), compared with 16.2% in our cohort.

Our study finding is limited by difficulty in pregnancy ascertainment. As some survivors were lost to long-term follow-up, it is possible that not all pregnancies were captured. We considered that an anonymous questionnaire administered to adult women, with no perceived risk by honest response, should capture most live birth outcomes.

Despite a small sample size, our current finding reinforces the importance of counselling survivors about the possibility of pregnancy despite documented ovarian failure and the importance of contraception to avoid unplanned pregnancy. Whether there may have been a change in pattern of discussion, counselling and prescribing of OCP versus HRT in older members of the cohort that resulted in unplanned spontaneous pregnancy remains unclear, compounded by well recognized problems of loss to follow up or acceptance of a poorer QoL by patients. We draw attention to the paramount importance to cater to contraceptive needs of HSCT survivors. All medical staff involved with care of sexually active female HSCT survivors need to be aware pregnancy may occur despite ovarian failure, even of long duration. In selected subgroups of female survivors who received high dose anthracycline or chest irradiation, early referral for cardiac surveillance should be emphasized in pre-conception assessment and for those with pregnancy. Multi-disciplinary planning for optimal mode of delivery will enhance safety of both mother and baby.

References:

1) Sanders J, Hawley J, Levy W et al. Pregnancies following high-dose cyclophosphamide with or without high- dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87(7):3045-3052.
2) Loren A, Chow E, Jacobsohn D et al. Pregnancy after Hematopoietic Cell Transplantation: A Report from the Late Effects Working Committee of the Center for International Blood

and Marrow Transplant Research (CIBMTR). *Biology of Blood and Marrow Transplantation*. 2011;17(2):157-166.

3) Salooja N, Szydlo R, Socie G et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. The Lancet. 2001;358(9278):271-276.
4) Carter A, Robison L, Francisco L et al. Prevalence of conception and pregnancy outcomes after hematopoietic cell transplantation: report from the bone marrow transplant survivor study. Bone Marrow Transplant. 2006;37(11):1023-1029.
5) Meirow D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7(6):535-543.
6) Wo J, Viswanathan A. Impact of Radiotherapy on Fertility, Pregnancy, and Neonatal Outcomes in Female Cancer Patients. International Journal of Radiation Oncology*Biology*Physics. 2009;73(5):1304-1312.
7) Ogilvy-Stuart A, Shalet S. Effect of Radiation on the Human Reproductive System. Environ Health Perspect. 1993;101:109.
8) Vatanen A, Wilhelmsson M, Borgström B et al. Ovarian function after allogeneic hematopoietic stem cell transplantation in childhood and adolescence. Eur J Endocrinol. 2014;170(2):211-218.
9) Wilhelmsson M, Glosli H, Ifversen M et al. Long-term health outcomes in survivors of childhood AML treated with allogeneic HSCT: a NOPHO–AML Study. Bone Marrow Transplant. 2018;54(5):726-736.
10) Wallace W, Kelsey T. Human Ovarian Reserve from Conception to the Menopause. PLoS ONE. 2010;5(1):e8772.
11) Green D, Kawashima T, Stovall M et al. Fertility of Female Survivors of Childhood Cancer: A Report From the Childhood Cancer Survivor Study. Journal of Clinical Oncology. 2009;27(16):2677-2685.
12) Barton S, Najita J, Ginsburg E et al. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. The Lancet Oncology. 2013;14(9):873-881.
13) Byrne J, Fears T, Mills J et al. Fertility in women treated with cranial radiotherapy for childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2004;42(7):589-597.
14) Ash P. The influence of radiation on fertility in man. Br J Radiol. 1980;53(628):271-278.
15) Sklar CA, Mertens AC, Mitby P, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. J Natl Cancer Inst. 2006;98(13):890-896.

16) Wędrychowicz A, Wojtyś J, Starzyk J. Anti-Muellerian hormone (AMH) as only possible marker in the assessment of ovarian function and reserve after hematopoietic stem cell transplantation (HSCT) in prepubertal girls, young females with composed hypogonadism and females receiving hormonal replacement therapy. Bone Marrow Transplant. 2016;52(2):313-316.
17) Iwase A, Osuka S, Goto M et al. Clinical application of serum anti-Müllerian hormone as an ovarian reserve marker: A review of recent studies. Journal of Obstetrics and Gynaecology Research. 2018;44(6):998-1006.
18) Korsholm A, Petersen K, Bentzen J, Hilsted L, Andersen A, Hvidman H. Investigation of anti-Müllerian hormone concentrations in relation to natural conception rate and time to pregnancy. Reprod Biomed Online. 2018;36(5):568-575.

Legends

Table 1. Table 1. Baseline characteristics of the cohort

	Whole cohort (n=37)	Survivors with pregnancy - Spontaneous (n= 7) - ART (n= 3)	Survivors without pregnancy (n=27)
Median Age in years, current	25.5 (16.3 – 45.6)	30.1 (21.6 – 41.6)	24.4 (16.3 – 45.6)

Table 2. Female allogeneic HSCT survivors with live-birth(s) following natural pregnancy

Table

Table 1.

Year of HSCT			
- Year 1985 – 1999	14	7	7
- Year 2000 – 2011	23	3	20
Ovarian function			
- Ovarian failure after HSCT	28	8	20
- Raised gonadotrophins only	1	0	1
- Normal ovarian function	7[#]	2	5
- Missing data	1	0	1
Spontaneous pubertal onset			
- Before HSCT	11	4	7
- After HSCT	14	4	10
- Need induction	10	2	8
- Missing data	2	0	2
Spontaneous menarche			
- Yes	11	4	7
- No	10	2	8
- Missing data	16	4	12

* One of the 7 females survivors had normal ovarian function and spontaneous pregnancy, therefore she was not counted towards the group with ovarian dysfunction and spontaneous pregnancy.

[#] One of the 7 female survivors with normal ovarian function had hypogonadotropic hypogonadism.

Abbreviations: HSCT – hemopoietic stem cell transplantation, ART – Assisted reproduction technique

Table 2.

Group 1 –HSCT survivors with TBI conditioning (3 of 22 – 17 ALL, 1 NHL, 3 AML, 1 Ph+CML)

Patient	Age at transplant (years)	Diagnosis & disease status at HSCT	Cumulative dose of CPM (g/m^2)	Total irradiation dose	Other conditioning agents	Menarche before HSCT	Gonadal function post-HSCT	Age at pregnancy (years)	No. of live-birth(s)
1	15.6	CML(Ph+) in CP1 Post HSCT (Pre-pregnancy) lymphoid relapse	6.1 Received pre-HSCT CPM Conditioning dose: 120mg/kg	TBI 12 Gy	No	Yes	Premature ovarian failure at 23years age. - FSH 51.2 IU/L HRT from age 23years, self-stopped, then pregnancy.	28.5	2 (Twins)
2[#]	13.9	ALL CR3	6.2 Received pre-HSCT CPM Conditioning	TBI 12 Gy CRT 18 Gy	No	No	Premature ovarian failure. Menorrhagia during HSCT, then amenorrhea. - FSH 39.2 IU/L,	22.5	1

			dose: 120mg/kg				- LH 27.7 IU/L HRT from age 14.5 years, then taken off prior to pregnancy.		
3	15.5	ALL CR 3	9 Received pre-HSCT CPM No conditioning CPM	TBI 12 Gy CRT boost 12Gy	Etoposide 60mg/kg	Yes	Premature ovarian failure Persistent amenorrhea post HSCT - FSH 170 IU/L - LH 62 IU/L - E2 <20 pmol/l HRT from age 16 years, ongoing at conception.	~30	1

Group 2 –HSCT survivors with chemotherapy only conditioning (3 of 15 – 5 ALL, 5 AML, 3 MDS, 1 Ph+CML, 1 JMML)

Patient	Age at transplant	Diagnosis of malignancy	Total cumulative dose of CPM (gram/m^2)	Other Alkylating agent	Non-alkylating conditioning agents	Menarche before HSCT	Gonadal function post-HSCT	Age at pregnancy (years)	No. of live-birth(s)
4	5.7	CML (Ph +) Clonal evolution	5.1 Conditioning dose:120mg/kg No pre-HSCT CPM	Busulfan 16mg/kg	No	No	Primary amenorrhea at the age 15 years. Hormonal profile before HRT: - FSH 13.2 IU/L - LH 19.6 IU/L - E2 68 pmol/l Still on HRT at conception.	22.8	1
5	11.7	MDS (Monosomy 7)	3.5 Conditioning dose:120mg/kg No pre-HSCT CPM	Busulfan 16mg/kg	Etoposide 30mg/kg	Yes	Premature ovarian failure. Secondary amenorrhea at age 14 years: - FSH 88.3 IU/L - LH 67.3 IU/L - E2 19 pmol/l HRT from age 14 years, ongoing HRT at conception.	30.2	1
6*	11.3	AML, then relapsed as biphenotypic leukemia	2 Pre-HSCT CPM only No conditioning CPM	Melphalan 160mg/m^2	Fludarabine 150mg/m^2	Yes	Vasomotor symptoms & menstrual irregularity at age 15 years with reduced ovarian reserve: - E2 45 pmol/l - AMH 2.3 pmol/l Refused OCP.	18.4	2

CML – Chronic myeloid leukemia, CP1- chronic phase 1, ALL – Acute lymphoblastic leukemia, AML – Acute myeloid leukemia, Ph+ – Philadelphia chromosome positive, MDS – myelodysplastic syndrome, NHL – Non-Hodgkin lymphoma, JMML – Juvenile myelomonocytic leukemia, CPM –

Cyclophosphamide, TBI – Total body irradiation, CRT – Cranial irradiation, FSH – Follicular stimulating hormone, LH – Luteinizing hormone, AMH – Anti-Mullerian hormone, OCP – Oral contraceptive pills.

* Severe anthracycline and sepsis induced cardiomyopathy, live-births delivered by Caesarian section.

[#] Patient had chronic graft-versus-host disease, who had ceased immunosuppressant many years prior to pregnancy.