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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**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 haemopoietic 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 3 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/m²). 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/m² 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 amenorrhoecic 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 amenorrhoecic before pregnancy, Patients 3,4,5 had taken HRT for 14
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
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
203 births (1,9). Although the youngest of our cohort at time of TBI was almost 14 years, no
204 infants born to these women were born prematurely.
205 Low anti-Mullerian hormone (AMH) has been reported in 26/28 survivors of either
206 autologous or allogeneic HSCT during childhood for a range of malignant and non-malignant
207 conditions (16). Although low AMH predicts ovarian reserve, pregnancy is not infrequently
208 reported in women with low AMH who have not been exposed to gonadotoxins (17-18), as
209 seen in 2 of our cohort where AMH levels were available.
210 Our live-birth outcomes of naturally occurring pregnancy are similar to others. In a cohort
211 under age 21 at HSCT (9), 13.5% had spontaneous pregnancies with live-births (assuming
212 successful outcomes of late gestation in 2 survivors), compared with 16.2% in our cohort.
213 Our study finding is limited by difficulty in pregnancy ascertainment. As some survivors
214 were lost to long-term follow-up, it is possible that not all pregnancies were captured.
215 We considered that an anonymous questionnaire administered to adult women, with no
216 perceived risk by honest response, should capture most live birth outcomes.
217 Despite a small sample size, our current finding reinforces the importance of counselling
218 survivors about the possibility of pregnancy despite documented ovarian failure and the
219 importance of contraception to avoid unplanned pregnancy. Whether there may have been a
220 change in pattern of discussion, counselling and prescribing of OCP versus HRT in older
221 members of the cohort that resulted in unplanned spontaneous pregnancy remains unclear,
222 compounded by well recognized problems of loss to follow up or acceptance of a poorer QoL
223 by patients. We draw attention to the paramount importance to cater to contraceptive needs
224 of HSCT survivors. All medical staff involved with care of sexually active female HSCT
225 survivors need to be aware pregnancy may occur despite ovarian failure, even of long
226 duration. In selected subgroups of female survivors who received high dose anthracycline or
227 chest irradiation, early referral for cardiac surveillance should be emphasized in pre-
228 conception assessment and for those with pregnancy. Multi-disciplinary planning for optimal
229 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
233 with or without high-dose busulfan or total-body irradiation and bone marrow
235 2) Loren A, Chow E, Jacobsohn D et al. Pregnancy after Hematopoietic Cell Transplantation:
236 A Report from the Late Effects Working Committee of the Center for International Blood

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Table 1. Baseline characteristics of the cohort

<table>
<thead>
<tr>
<th>Cohort Description</th>
<th>Median Age in years, current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cohort (n=37)</td>
<td>26.5 (16.3 – 45.6)</td>
</tr>
<tr>
<td>Survivors with pregnancy</td>
<td></td>
</tr>
<tr>
<td>- Spontaneous (n=7)</td>
<td>30.1 (21.6 – 41.6)</td>
</tr>
<tr>
<td>- ART (n=3)</td>
<td></td>
</tr>
<tr>
<td>Survivors without pregnancy (n=27)</td>
<td>24.4 (16.3 – 45.6)</td>
</tr>
</tbody>
</table>
### Table 2.

**Year of HSCT**
- Year 1985 – 1999: 14, 7, 7

**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 female 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

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at transplant (years)</th>
<th>Diagnosis &amp; disease status at HSCT</th>
<th>Cumulative dose of CPM (g/m²)</th>
<th>Total irradiation dose</th>
<th>Other conditioning agents</th>
<th>Menarche before HSCT</th>
<th>Gonadal function post-HSCT</th>
<th>Age at pregnancy (years)</th>
<th>No. of live-birth(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.6</td>
<td>CML(Ph+) in CP1 Post HSCT (Pre-pregnancy lymphoid relapse)</td>
<td>6.1</td>
<td>TBI 12 Gy</td>
<td>No</td>
<td>Yes</td>
<td>Premature ovarian failure at 23 years age. FSH 51.2 IU/L, HRT from age 23 years, self-stopped, then pregnancy.</td>
<td>28.5</td>
<td>2 (Twins)</td>
</tr>
<tr>
<td>2*</td>
<td>13.9</td>
<td>ALL CR3</td>
<td>6.2</td>
<td>TBI 12 Gy CRT 18 Gy</td>
<td>No</td>
<td>No</td>
<td>Premature ovarian failure. Menorrhagia during HSCT, then amenorrhea. FSH 39.2 IU/L,</td>
<td>22.5</td>
<td>1</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at transplant</th>
<th>Diagnosis of malignancy</th>
<th>Total cumulative dose of CPM (gram/m²)</th>
<th>Other Alkylating agent</th>
<th>Non-alkylating conditioning agents</th>
<th>Menarche before HSCT</th>
<th>Gonadal function post-HSCT</th>
<th>Age at pregnancy (years)</th>
<th>No. of live-birth(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15.5</td>
<td>ALL CR 3</td>
<td>9</td>
<td>TBI 12 Gy CRT boost 12Gy</td>
<td>Etoposide 60mg/kg</td>
<td>Yes</td>
<td>Premature ovarian failure Persistent amenorrhea at age 14 years, then taken off prior to pregnancy.</td>
<td>~30</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>5.7</td>
<td>CML (Ph +)</td>
<td>5.1</td>
<td>Busulfan 16mg/kg</td>
<td>No</td>
<td>No</td>
<td>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.</td>
<td>22.8</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>11.7</td>
<td>MDS (Monosomy 7)</td>
<td>3.5</td>
<td>Busulfan 16mg/kg</td>
<td>Etoposide 30mg/kg</td>
<td>Yes</td>
<td>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.</td>
<td>30.2</td>
<td>1</td>
</tr>
<tr>
<td>6*</td>
<td>11.3</td>
<td>AML, then relapsed as biphenotypic leukemia</td>
<td>2</td>
<td>Melphalan 160mg/m²</td>
<td>Fludarabine 150mg/m²</td>
<td>Yes</td>
<td>Vasomotor symptoms &amp; menstrual irregularity at age 15 years with reduced ovarian reserve: - E2 45 pmol/l - AMH 2.3 pmol/l Refused OCP.</td>
<td>18.4</td>
<td>2</td>
</tr>
</tbody>
</table>

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