VOLUME 30 · NUMBER 1 · JANUARY 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Genetic Disease in the Children of Danish Survivors of Childhood and Adolescent Cancer

Jeanette F. Winther, Jørgen H. Olsen, Huiyun Wu, Yu Shyr, John J. Mulvihill, Marilyn Stovall, Annelise Nielsen, Marianne Schmiegelow, and John D. Boice Jr

See accompanying editorial on page 3

Jeanette F. Winther, Jørgen H. Olsen, and Annelise Nielsen, Institute of Cancer Epidemiology, Danish Cancer Society; Marianne Schmiegelow, Clinic of Pediatrics, Copenhagen, Denmark; Jørgen H. Olsen, Huiyun Wu, Yu Shyr, and John D. Boice Jr, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN; John J. Mulvihill, University of Oklahoma, Oklahoma City, OK; Marilyn Stovall, The University of Texas MD Anderson Cancer Center, Houston, TX; and John D. Boice Jr, International Epidemiology Institute, Rockville, MD.

Submitted January 25, 2011; accepted July 22, 2011; published online ahead of print at www.jco.org on November 28, 2011.

Supported in part by Grant No. 1 RO1 CA 104666 from the National Institutes of Health through Vanderbilt University Medical Center and in part by the Danish Cancer Society.

None of the funding sources were involved in conducting this study, except for J.D.B., who is scientific director at International Epidemiology Institute as well as professor of medicine, Vanderbilt University (and principal investigator on National Institutes of Health grant). The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Jeanette F. Winther, MD, Institute of Cancer Epidemiology, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen; e-mail: jeanette@cancer.dk.

0732-183X/12/3001-27/$20.00

DOI: 10.1200/JCO.2011.35.0504

ABSTRACT

Purpose
Preconception radiation and chemotherapy have the potential to produce germ cell mutations leading to genetic disease in the next generation. Dose-response relationships were evaluated between cancer treatments and untoward pregnancy outcomes.

Patients and Methods
A case-cohort study was conducted involving 472 Danish survivors of childhood and adolescent cancer and their 1,037 pregnancies. Adverse outcomes included 159 congenital malformations, six chromosomal abnormalities, seven stillbirths, and nine neonatal deaths. Preconception radiation doses to the gonads, uterus, and pituitary gland and administered chemotherapy were quantified based on medical records and related to adverse outcomes using a generalized estimating equation model.

Results
No statistically significant associations were found between genetic disease in children and parental treatment with alkylating drugs or preconception radiation doses to the testes in male and ovaries in female cancer survivors. Specifically, the risk of genetic disease was similar among the children of irradiated survivors when compared with nonirradiated survivors (relative risk [RR], 1.02; 95% CI, 0.59 to 1.44; $P = .94$). A statistically significant association between abdominopelvic irradiation and malformations, stillbirths, and neonatal deaths was not seen in the children of female survivors overall ($P = .07$) or in the children of mothers receiving high uterine doses (mean, 13.5 Gy; max, 100 Gy; RR, 2.3; 95% CI, 0.95 to 5.56).

Conclusion
Mutagenic chemotherapy and radiotherapy doses to the gonads were not associated with genetic defects in children of cancer survivors. However, larger studies need to be conducted to further explore potential associations between high-dose pelvic irradiation and specific adverse pregnancy outcomes.

J Clin Oncol 30:27-33. © 2011 by American Society of Clinical Oncology

INTRODUCTION

With the advent of multimodality therapy, the overall 5-year survival rate from childhood cancer has improved considerably, from 25% in the 1950s to almost 80% today.[1-3] Survivors are now able to have children of their own, and numbers are sufficiently large to test whether preconception radiation or mutagenic chemotherapy can result in a detectable increase in heritable genetic effects.[4] Although these curative therapies have the potential to produce germ cell mutations leading to genetic disease in the next generation, there is still little understanding of the genetic consequences of such treatments. Radiation-induced heritable diseases have not been demonstrated in humans, and estimates of genetic risks for protection purposes are based on mouse experiments.[5] The most comprehensive study of the Japanese atomic bomb survivors and their children found little evidence for inherited defects attributable to parental radiation,[6-10] and studies of pregnancy outcomes[11,12] and the offspring of childhood cancer survivors[13-23] to date suggest that the risk of treatment-induced heritable genetic effects must be low. Few studies, however, have quantified chemotherapy or radiation dose to gonads or uterus, and none has been of sufficient size to detect a moderate increase in heritable risk in offspring.[24]

Cancer survivors are especially suitable for studies of heritable genetic effects, because they

have been exposed to a wide range of well-documented gonadal radiation doses as well as to genotoxic chemotherapeutic agents. The purpose of this study was to quantify the extent to which cancer therapy contributes to adverse genetic outcomes in children of childhood cancer survivors, including chromosomal abnormalities, congenital malformations, stillbirths, and neonatal deaths as possible indicators of genetic damage in the next generation. Computation of individually estimated gonadal doses for all radiation therapy delivered before the date of relevant conception makes it possible to interpret the epidemiologic results in light of dose-response evaluations.

PATIENTS AND METHODS

Danish Childhood Cancer Survivors and Their Offspring

From the Danish Cancer Registry, we identified 4,676 childhood cancer survivors diagnosed before age 20 years between 1943 and 1996 who survived until onset of fertility (age 15 years). Survivors had to be alive on or born after April 1, 1968, when the Central Population Register was established, and a unique personal identification number was assigned for all citizens that permit linkage among registers. A search in the Central Population Register and Medical Birth Register identified all liveborn offspring (after exclusion of 87 children born outside Denmark and 35 children born before or up to 9 months after their parent's cancer diagnosis) and stillbirths. Multiple births were excluded because of the known association with stillbirths and certain malformations. Among the 4,676 survivors of childhood cancer, 1,474 had 2,767 pregnancies resulting in a liveborn singleton and/or stillbirth, excluding spontaneous abortions. Patients included in the case-cohort study were sampled from this cohort of fertile survivors. No indications of nonbiologic children were found after linking the female survivors to the Danish population-based infertility cohort[25] or to the In Vitro Fertilisation Register.[26]

Genetic Disease

From population-based and nationwide Danish health registries, we obtained information on genetic disease defined as chromosomal abnormalities (Danish Cytogenetic Registry includes information on abnormal karyotypes since 1960 diagnosed pre- or postneonatally for offspring and parents, if tested), congenital malformations (National Hospital Register includes those recorded at birth and later in life since 1977; approach has been used previously[15]), stillbirths (defined as infant with gestational age $\geq$ 28 weeks showing no sign of life; Medical Birth Register since 1973), and neonatal deaths (death within first 28 days of life; Danish Cause-of-Death Register since 1970, including underlying and contributing causes of death). Cancer was not regarded as a genetic disease because of the small likelihood of having a genetic component (except for rare cancer syndromes with clear genetic etiology, which were excluded). However, for completeness and descriptive purposes, cancers diagnosed before age 20 years were identified from the Danish Cancer Registry (since 1943). Cause of death was evaluated to exclude nongenetic causes of neonatal deaths. Hereditary patient cases of chromosomal abnormalities (same abnormal karyotype in parent and offspring) and cancers (cancer in parent and offspring compatible with familial cancer syndromes)[27] were excluded.

Medical Record Abstraction

Medical records on survivors included in the case-cohort study were reviewed to abstract detailed information on radiotherapy and chemotherapy for the primary cancer of the parent and for recurrence or new primaries provided more than 9 months before the birth of the child or stillbirth. Because high-dose radiation and chemotherapy can cause infertility,[22,28,29] it was important to identify assisted pregnancies, sperm banking, and other procedures performed to preserve or enhance fertility.

All information on radiotherapy was submitted to MD Anderson Cancer Center (Houston, TX), including procedures to reduce gonadal dose, if documented. Testicular shielding and field blocking might reduce high gonadal doses to 10% or less of the unshielded dose. For individual patients, doses to the ovaries and uterus (women) and testes (men) were calculated based on information in the radiotherapy schemes. Also, radiation dose to the pituitary gland was assessed as a potential risk factor for stillbirths and neonatal deaths among female survivors, because it might have led to a permanent disruption of the hypothalamic-pituitary-gonadal axis.[22,28,29]

Radiation dose outside the treatment beam was measured in a water phantom. To calculate the organ dose of interest, the measured beam data were positioned on a three-dimensional mathematic phantom simulating the appropriate size for the patient of a given age.[30] Also, information on all chemotherapy agents was abstracted from the medical record.

Statistical Analysis

Cases in the case-cohort analyses were survivors who had children with any of the genetic conditions (malformations, chromosomal abnormalities, stillbirths, neonatal deaths). The subcohort was randomly selected from the fertility cohort of 1,474 cancer survivors. A stratified fractional sampling procedure was based on sex and number of pregnancies, in which we sampled 25% ($\alpha = 0.25$) of the survivors who had fewer than four pregnancies (96.3%) and 100% ($\alpha = 1.00$) of those with four or more pregnancies (3.7%) regardless of case status. This study design enabled us to estimate the relative risk (RR) of

Table 1. Characteristics of Survivors of Childhood and Adolescent Cancer*

Characteristic	Cohort†		Cases‡		Subcohort§	
	No.	%	No.	%	No.	%
Total	1,474	100	145	100	372	100
Sex						
Male	722	49	60	41	183	49
Female	752	51	85	59	189	51
Main diagnostic group‖						
Leukaemias¶	102	7	8	6	20	5
Lymphomas	236	16	22	15	71	19
CNS neoplasms	299	20	28	19	72	19
Sympathetic nervous system tumors	30	2	4	3	7	2
Retinoblastoma	72	5	10	7	24	6
Wilms and other renal tumors	51	3	6	4	7	2
Hepatic	2	< 1	0	0	1	< 1
Malignant bone tumors	72	5	6	4	21	6
Soft-tissue sarcomas	148	10	19	13	45	12
Germ cell, trophoblastic, and other gonadal neoplasms	136	9	9	6	25	7
Carcinomas and other malignant epithelial neoplasms	308	21	30	21	77	21
Other and unspecified malignant neoplasms	18	1	3	2	2	1

*Includes entire cohort of 1,474 fertile survivors who had at least one liveborn singleton and/or stillbirth, 145 survivors who had at least one child with presumed genetic disease (cases), and random sample of 372 survivors (subcohort) drawn from cohort.

†Includes 100 cases, 327 noncase subcohort members, 45 survivors who were both case and subcohort member, and 1,002 survivors not selected. Total of 487 fertile cancer survivors originally selected for case-cohort study. However, 15 survivors excluded after medical record abstraction indicated that child was not biologic child of survivor or was conceived through sperm banking before chemotherapy or that survivor had nonmalignant diagnosis treated with surgery only. Thus, final case-cohort study consisted of 472 survivors (ie, approximately one third of fertility cohort).

‡Includes 100 cases and 45 survivors who were both case and subcohort member.

§Includes 327 noncase subcohort members and 45 survivors who were both case and subcohort member.

‖According to Danish Cancer Registry.

¶Comparatively low percentage of children among survivors of leukemia, because survival among such patients diagnosed before 1970 was not as successful as today, and for those diagnosed after 1970, young age at diagnosis has precluded many to have survived to ages of fertility when they could become pregnant.

the outcome in a cohort study framework in a cost-efficient manner without having to abstract medical records or reconstruct radiation and chemotherapy doses for all survivors in the cohort.

The clustered data structure was handled by generalized estimating equations (GEEs). For example, a cancer survivor could have up to seven pregnancies, the outcome could be normal or abnormal, and a child could have more than one abnormal outcome (eg, child with malformation might die early). In the analyses, multiple outcomes in one child were counted only once to simplify the data complexity and in light of the fact that the majority of pregnancies had only one outcome (98.1%). Furthermore, we only included the first four pregnancies in survivors (98.4%) for model convergence.

The association between the radiation or chemotherapy exposure and the risk of having at least one of the defined genetic conditions was evaluated using statistical models based on GEEs. Because 25% of the baseline fertility cohort was selected into the subcohort for those survivors with fewer than four pregnancies after 28 weeks gestation (in contrast with 100% of all cases and all survivors with ≥ four pregnancies), the stratified fractional sampling scheme could have distorted the parameter estimation if not appropriately handled. However, the uncertainty associated with sampling from the cohort was addressed in the estimation of the RRs and CIs. Each study member was given the weight α^{-1} to reflect the appropriate sampling fraction. All cases and subcohort members with four or more pregnancies were given the weight of 1, because all such survivors were included, whereas those with fewer than four pregnancies were given the weight of 4 to reflect the one in four sampling. The sandwich SE was adjusted for additional uncertainty associated with estimating the offset resulting from the fractional sampling of the subcohort.[31] The log weight was incorporated as an offset to the model. Estimation of the RR was computed assuming a binomial distribution.[32-34] The model specified an exchangeable variance-covariance structure for the clustering of the adverse pregnancy outcomes on survivors. Continuous log transformed doses were used in the model fitting and *P* value computation. RRs were calculated by using the median of each dose category prespecified in the fitted model. Polynomial regression was incorporated with the GEE model for possible nonlinear association. The models were adjusted for the following covariates considered to be potential confounders: maternal age (as continuous variable), birth order (continuous), and chemotherapy (yes or no; for evaluations of radiotherapy) or radiation (yes or no; for evaluations of chemotherapy). Missing data were addressed by multiple imputation procedures, using the outcome and all covariates specified in the regression model.[35] Averaged value of 100 imputations was used to replace the missing value. The estimation was implemented once on the single averaged value–filled complete data set. This procedure should yield satisfactory estimation, given only 8% missing information for the primary exposure variable (ie, radiation dose).[36,37] Additional adjustments were made for sex in the analysis when radiation exposure was measured as yes or no. All *P* values were two tailed, and computations were conducted using R 2.10.1 (http://www.R-project.org).

RESULTS

The case-cohort study constituted 472 survivors (one third of fertility cohort) who had 1,037 pregnancies resulting in a livebirth or stillbirth after 28 weeks gestation. There were 145 survivors with at least one affected child or stillbirth (cases with 152 affected children and seven stillbirths in total) and 372 survivors randomly selected for the subcohort, including 45 cases (Table 1). For case-cohort members, the median testicular dose in irradiated men was .039 Gy (mean dose, .41 Gy; range, .00005 to 8 Gy). In irradiated women, the median minimum ovarian dose was .10 Gy (mean, 1.16 Gy; range, .00005 to 40 Gy); median uterine dose, .10 Gy (mean, 2.30 Gy; range, .00005 to 100 Gy); and median pituitary dose, 1.10 Gy (mean, 9.09 Gy; range, .00004 to 60 Gy).

A total of 181 presumed genetic diseases occurred in the children of the 145 case survivors, with most being 159 congenital malforma-

Table 2. Genetic Outcomes Among Children of Cancer Survivors by Parental and Offspring Characteristics (n = 181)*

Characteristics	Chromosomal Abnormalities	Congenital Malformations†	Stillbirths	Neonatal Deaths
Total No.	6	159	7	9
Sex of offspring				
Male	1	102	NA	7
Female	3	57	NA	2
Unknown	2‡	0	0	0
Sex of parent				
Male	3	65	2	3
Female	3	94	5	6
Parental cancer treatment				
Chemotherapy				
No	5	115	6	8
Yes	0	26	1	1
Unknown§	1	18	0	0
Radiotherapy				
No	3	89	6	2
Yes	3	70	1	7
Parental gonadal and uterine dose, Gy				
Female survivor				
Ovarian minimum dose (mean, 1.16; range, .00005 to 40)‖				
0 (nonirradiated)	2	55	4	0
> 0 to < .50	1	19	1	1
≥ .50	0	9	0	4
Unknown¶	0	11	0	1
Uterine dose (mean, 2.30; range, .00005 to 100)‖				
0 (nonirradiated)	2	55	4	0
> 0 to < .50	1	20	1	1
≥ .50	0	8	0	4
Unknown¶	0	11	0	1
Pituitary dose (mean, 9.09; range, .00004 to 60)‖				
0 (nonirradiated)	2	55	4	0
> 0 to < .50	0	8	0	2
≥ .50	1	17	1	2
Unknown¶	0	14	0	2
Male survivor				
Testicular dose (mean, .41; range, .00005 to 8)‖				
0 (nonirradiated)	1	34	2	2
> 0 to < .50	2	18	0	1
≥ .50	0	5	0	0
Unknown¶	0	8	0	0

Abbreviation: NA, not applicable.

*For children with more than one outcome, all outcomes are included.

†Grouped into 12 diagnostic main diagnostic groups: nervous system (n = 6); eye, ear, face, and neck (n = 18); heart and blood vessels (n = 24); respiratory organs (n = 3); lip and palate (n = 7); digestive system (n = 9); genitalia (n = 41); urinary organs (n = 3); extremities (n = 26); musculoskeletal system (n = 6); skin, hair, and nails (n = 6); multiple organ systems (n = 9; primarily chromosomal abnormalities involving multiple malformations such as Down and Turner syndromes); and other/unspecified (n = 1). Prevalence of malformations in current study differs from that in our previous study,[15] because cohort of survivors is slightly larger, all malformations counted as outcomes (not just children with malformations), and all malformations recorded throughout life counted (not just those recognized within first year of life).

‡Two cases of Down syndrome diagnosed prenatally.

§Information not available based on medical record abstraction.

‖For those irradiated.

¶Doses could not be estimated because of incomplete information in medical record.

Table 3. Risk of Genetic Disease Among Children of Cancer Survivors by Parental Treatment With Radiation and Chemotherapy

Treatment of Survivor Parent	Cases			Subcohort Members			Adjusted RR*	95% CI	*P*†
	Offspring		No. of Survivors	Offspring		No. of Survivors			
	No.	%		No.	%				
Radiotherapy‡									.94
Nonirradiated	87	56	80	570	58	209	1.00	Referent	
Irradiated	69	44	65	411	42	163	1.02	0.59 to 1.44	
Chemotherapy§									
None	114	87	103	671	85	250	1.00	Referent	.51
Alkylating drug	17	12	17	114	15	49	0.82	0.53 to 1.28	
No chemotherapy or radiation	66	79	59	380	76	137	1.00	Referent	.49
Alkylating drug‖	17	11	17	114	24	49	0.75	0.26 to 2.13	

Abbreviations: GEE, generalized estimating equation; RR, relative risk.

*Based on 1,020 pregnancies: 864 normal and 156 adverse outcomes.

†*P* values computed with overall Wald statistic from logistic regression adjusting for birth order, maternal age, survivor sex, and chemotherapy in radiation model and for radiation only in chemotherapy model. The sandwich standard error was adjusted for additional uncertainty associated with estimating the offset resulting from the fractional sampling of the subcohort. GEE model with exchangeable variance-covariance structure was used to account for the clustered data structure.

‡Among 472 survivors, 215 received radiotherapy (52 cases only, 150 noncase subcohort members, 13 both), and 257 did not receive radiotherapy (48 cases, 177 subcohort members, 32 both).

§Alkylating agents were BCNU (carmustine), busulfan, chlorambucil, cisplatinum, CCNU (lomustine), cyclophosphamide (cytoxan), nitrogen mustard, procarbazine, DTIC, and triethylenemelamine TEM. Platinum compounds are not alkylating drugs but were included because of their DNA-damaging capability. Two different analyses of effect of alkylating drugs were conducted: one using no chemotherapy as referent, the other no chemotherapy or radiotherapy as referent. Among 472 survivors, 87 received chemotherapy (19 cases only, 60 noncase subcohort members, eight both), 324 did not receive chemotherapy (74 cases, 221 subcohort members, 29 both), and 61 had no information available (seven cases, 46 subcohort members, eight both).

‖A total of 85 of 87 survivors who received chemotherapy had information on exposure to alkylators available: 61 received alkylators (12 cases, 44 subcohort members, five both), and 24 did not (seven cases, 14 subcohort members, three both).

tions (Table 2). Four children were diagnosed with Down syndrome (two mothers were spouses of male survivors, and their maternal ages were $>$ 40 years), and two children had Turner syndrome. Only three of six parents with children with chromosomal abnormalities were irradiated: one women (ovarian, uterine, and pituitary doses of .016, .017, and 16.90 Gy, respectively) and two men (testicular doses of .024 and .23 Gy, respectively). Only one female survivor among the seven survivors with a stillbirth was irradiated (ovarian, uterine, and pituitary doses of .056, .052, and 24 Gy, respectively). Nine neonatal deaths were reported in seven parents (six irradiated). Two parents had two children who died early: one nonirradiated man (cause of death of children: intracranial hemorrhage in preterm child; congenital toxoplasmosis, no information on term) and one irradiated female survivor (ovarian, uterine, pituitary doses of 1.40, 1.30, and .64 Gy, respectively; intracranial hemorrhage and respiratory distress as respective causes of death; both preterm and immature). One father with a low testicular dose (.004 Gy) had a preterm child who died as a result of immaturity. Four mothers, each with one affected child, had received a low uterine dose (.12 Gy; pituitary dose unknown; child died as result of asphyxia), high dose (uterine and pituitary doses of 1.30 and .05 Gy and 4.90 and .02 Gy, respectively; both children were preterm and died as result of immaturity), and unknown dose (ventricular septum heart defect).

To handle the complex outcome data, we counted multiple adverse outcomes in one child as just one (exclusion of 22 adverse outcomes, which involved 18 pregnancies, so total outcomes were reduced from 1,059 to 1,037). Thirteen survivors had five or more pregnancies. For model convergence, we only included their four first pregnancies (exclusion of three adverse and 14 normal outcomes). Accordingly, the number of relevant pregnancies was reduced from 1,037 (181 adverse and 878 normal outcomes) to 1,020 (156 adverse and 864 normal outcomes) in the analyses. The risk of genetic disease among offspring of survivors was similar among irradiated survivors when compared with nonirradiated survivors (RR, 1.02; 95% CI, 0.59 to 1.44), whereas the risk was nonsignificantly decreased among those having received alkylating agents, both when compared with those who did not receive chemotherapy (RR, 0.9; 95% CI, 0.5 to 1.3) and those without any potential mutagenic treatment (RR, 0.8; 95% CI, 0.3 to 2.1); however, this was based on small numbers (Table 3).

An association between uterine dose and congenital malformations, stillbirths, and neonatal deaths, taken together, was of borderline statistical significance ($P = .07$) based on a two-tailed test and continuous dose. The highest uterine doses were associated with a two-fold increased risk (RR, 2.30; 95% CI, 0.95 to 5.56; Table 4), but this association was not statistically significant. Ten mothers with uterine doses of .50 Gy or greater (mean, 13.52 Gy; range, .95 to 100 Gy) gave birth to 11 children, four of whom were preterm babies who died shortly after birth, and seven children had different congenital malformations. We found no association between ovarian exposure and the risk of having affected children (Table 4). Furthermore, no association with testicular radiation exposure was observed, with different malformations seen in four children of male survivors within the highest testicular doses (.67, 1.10, 1.20, and 2.10 Gy). Small numbers prevented an evaluation of pituitary dose in female survivors and having a stillbirth or neonatal death, but doses were generally low.

Nine nonhereditary cancers were identified among the offspring of six male (five irradiated: three with testicular doses $<$ .50 Gy and two with doses of 1.80 and 4.50 Gy, respectively) and three female survivors (one irradiated: ovarian and uterine doses of .018 and .022 Gy, respectively). No clear cancer patterns emerged, and no cases of leukemia were found.

Table 4. Risk of Genetic Disease Among Children of Cancer Survivors by Radiation Dose to Ovary, Uterus, or Testes Received by Parent

Organ Dose of Survivor Parent (Gy)	Cases			Subcohort Members			Adjusted RR*	95% CI	*P*†
	Offspring		No. of Survivors	Offspring		No. of Survivors			
	No.	%		No.	%				
Female survivor									
Ovarian minimum dose‡ (mean, 1.16; range, .00005 to 40§)									.96
0 (nonirradiated)	52	69	45	306	68	115	1.00	Referent	
> 0 to < .50	21	28	20	124	29	46	1.12	0.52 to 2.38	
≥ .50	2	3	2	12	3	5	1.04	0.17 to 6.25	
Uterine dose‖ (mean, 2.30; range, .00005 to 100§)									.07
0 (nonirradiated)	50	61	43	305	66	114	1.00	Referent	
> 0 to < .50	21	26	20	131	28	49	1.34	0.77 to 2.32	
≥ .50 (mean, 13.52; range, .95 to 100)	11	13	10	26	6	12	2.30	0.95 to 5.56	
Male survivor¶									
Testicular dose (mean, .41; range, .00005 to 8§)									.72
0 (nonirradiated)	35	64	35	263	61	93	1.00	Referent	
> 0 to < .50	16	29	16	139	32	60	0.84	0.48 to 1.49	
≥ .50	4	7	4	28	7	12	1.12	0.44 to 2.88	

Abbreviation: RR, relative risk.

*Association between radiation exposure and genetic disease examined by using radiation dose as continuous measurement (ie, *P* values calculated and conclusion based on continuous dose). Continuous doses [log scale] used in model fitting and *P* value computation. RRs calculated using median of each category.

†*P* value computed with overall Wald statistic from logistic regression adjusting for birth order, maternal age, and chemotherapy. Sandwich standard error adjusted for additional uncertainty associated with estimating offset resulting from fractional sampling of subcohort. Generalized estimating equation model with exchangeable variance-covariance structure used to account for clustered data structure.

‡Maximum and minimum ovary doses estimated for ovary (left or right) that received higher and lower dose. Minimum dose to either ovary used as treatment exposure in analyses, because it was assumed that less exposed ovary was more likely to be functioning one. Because of limited information available in abstracted medical records, ovarian minimum dose could not be estimated in 22 of 247 female survivors included in case-cohort study (also 22 in this subanalysis). Because of close proximity of uterus and ovaries, radiation doses to these organs were highly correlated. To reduce confounding effect of high-dose uterine exposure in evaluation of possible germline damage to ovarian cells, we calculated effect of ovarian irradiation only among women who had dose of < 1.00 Gy to uterus (excluding 19 survivors from this subanalysis). Using this restrictive strategy (as described previously[38]) still allowed us to examine doses to ovary over wide range as well as those constituting relatively high exposure.

§For those irradiated.

‖Total of 22 female survivors had no estimated uterine dose. Genetic disease in this analysis defined as congenital malformations, stillbirths, and neonatal deaths only. Not biologically plausible that chromosomal abnormalities would be related to uterine dose, so they were excluded.

¶Among 225 male survivors included in case-cohort study, 23 had no estimated testicular dose.

DISCUSSION

No associations were seen between the risk of genetic disease in children and parental treatment with alkylating drugs or preconception radiation doses to the testes in male and ovaries in female cancer survivors. Specifically, the risk of genetic disease among offspring of survivors was similar among irradiated survivors when compared with nonirradiated survivors (RR, 1.02; 95% CI, 0.59 to 1.44; ie, study was able to reject RRs as high as 1.44 with 95% confidence). A statistically significant association between abdomino-pelvic irradiation and malformations, stillbirths, and neonatal deaths was not seen in the children of female survivors receiving the highest uterine doses (RR, 2.3; 95% CI, 0.95 to 5.56).

In this study, we address low-dose effects that might have genetic implications as well as high-dose effects that might damage the uterus. Uterine development and function may be compromised after pelvic irradiation, whereas chemotherapy does not seem to have any significant lasting adverse effect on uterine function.[22,28,29,39] Pregnancy outcome may be adversely affected by reduced uterine elasticity and fibrosis related to abdominal irradiation and possible damage to the uterine vasculature, leading to fetal death, preterm birth, and fetal growth restriction. Observations regarding the impact of uterine irradiation on pregnancy outcome were first mentioned in the 1980s[40,41] and confirmed in subsequent publications,[11,38,42-50] including studies reporting dose-response findings based on radiation dose to the flank or uterus[38,44,48,49]; they were further supported by null findings in partners of male survivors. Because infant mortality is increased in preterm births, our finding of several cases of neonatal deaths in preterm immature babies of women who received the highest uterine doses was not unexpected. The reason for our low number of stillbirths compared with that in a recent study in the United States[48] is likely the result of differences in the definition of stillbirths in the two countries. In the United States, a fetal death arising before gestational week 20 is classified as a miscarriage and after week 20 as a stillbirth. Accordingly, stillbirths in weeks 20 to 27 in the United States would be classified as spontaneous abortions or miscarriages in Denmark. It is noteworthy that a slight excess risk for spontaneous abortion among women with cancer treated with high-dose pelvic radiotherapy was previously reported in Denmark.[11]

Most previous studies of the risk of congenital malformation in offspring of cancer survivors have not reported any increased risk, but these studies did not include accurate individually calculated gonadal or uterine doses.[13,15,23,45,49] However, conflicting results have recently been published on the risk of malformations in offspring of male survivors.[51-54] Congenital malformations, stillbirths, and neonatal deaths, taken together, might be associated with high-dose uterine

irradiation, conceivably related to the relatively high number of neonatal deaths (four of nine in total) in offspring of survivors in the highest uterine dose category. However, numbers were too small to provide stable estimates of risk for individual outcome categories.

The hypothesis of a possible leukemogenic risk after parental preconception exposure to ionizing radiation suggested by Gardner et al[55] in 1990 prompted several studies of radiation workers, which failed to confirm the initial report.[56] Such a preconception effect has not been reported in higher-dose studies of atomic bomb survivors[57] or in survivors of childhood cancer.[16,17] Not a single case of leukemia was observed in Danish survivor parents (722 were men; mean dose, .41 Gy; maximum, 8.00 Gy).

Our study involved a population-based cohort of Danish survivors of childhood cancer and all of their children. Our definition of genetic disease is consistent with that used in the atomic bomb survivor study.[6] The range of gonadal doses was broad and, for many survivors, high and just below the threshold for infertility. Because childhood cancer and many of the selected outcomes are rare, the study is limited by small numbers, despite inclusion of all survivors and outcomes in Denmark. In addition, the heterogeneity of the outcomes evaluated likely has diluted the analyses by inclusion of some outcomes with a small genetic component, although we attempted to remove those outcomes with known nongenetic etiologies. In conjunction with an international study of trans-generational effects of cancer treatment (www.gcct.org), we have increased the sample size by adding offspring of cancer survivors diagnosed in early adulthood (age $<$ 35 years). Nonetheless, our nationwide study provides no convincing evidence that radiotherapy or chemotherapy caused adverse pregnancy outcomes that conceivably could be related to inherited germline mutations, consistent with our recent studies of minisatellite mutation frequencies in cancer families.[20] Although the absence of clear evidence for a relationship between radiation and chemical treatments and genetic disease in children is reassuring, larger studies are still needed to further explore possible associations between high-dose pelvic and abdominal radiotherapy and specific adverse pregnancy outcomes.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** John J. Mulvihill, Radiation Effect Research Foundation Hiroshima/Nagasaki (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Jeanette F. Winther, Jørgen H. Olsen, John J. Mulvihill, John D. Boice Jr

Collection and assembly of data: Jeanette F. Winther, Marilyn Stovall, Annelise Nielsen, Marianne Schmiegelow

Data analysis and interpretation: Jeanette F. Winther, Jørgen H. Olsen, Huiyun Wu, Yu Shyr, John J. Mulvihill, Marilyn Stovall, John D. Boice Jr

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

1. de Nully Brown P, Olsen JH, Hertz H, et al: Trends in survival after childhood cancer in Denmark, 1943-87: A population-based study. Acta Paediatr 84:316-324, 1995
2. Gatta G, Zigon G, Capocaccia R, et al: Survival of European children and young adults with cancer diagnosed 1995-2002. Eur J Cancer 45:992-1005, 2009
3. Smith MA, Seibel NL, Altekruse SF, et al: Outcomes for children and adolescents with cancer: Challenges for the twenty-first century. J Clin Oncol 28:2625-2634, 2010
4. Boice JD Jr, Tawn EJ, Winther JF, et al: Genetic effects of radiotherapy for cancer. Health Phys 85:65-80, 2003
5. The 2007 recommendations of the International Commission on Radiological Protection: ICRP Publication 103. Ann ICRP 37:1-332, 2007
6. Schull WJ, Otake M, Neel JV: Genetic effects of the atomic bombs: A reappraisal. Science 213: 1220-1227, 1981
7. Otake M, Schull WJ, Neel JV: Congenital malformations, stillbirths, and early mortality among the children of atomic bomb survivors: A reanalysis. Radiat Res 122:1-11, 1990
8. Neel JV: Genetic studies at the Atomic Bomb Casualty Commission-Radiation Effects Research Foundation: 1946-1997. Proc Natl Acad Sci U S A 95:5432-5436, 1998
9. Izumi S, Suyama A, Koyama K: Radiation-related mortality among offspring of atomic bomb survivors: A half-century of follow-up. Int J Cancer 107:292-297, 2003
10. Kodeira M, Izumi S, Takahashi N, et al: No evidence of radiation effect on mutation rates at hypervariable minisatellite loci in the germ cells of atomic bomb survivors. Radiat Res 162:350-356, 2004
11. Winther JF, Boice JD Jr, Svendsen AL, et al: Spontaneous abortion in a Danish population-based cohort of childhood cancer survivors. J Clin Oncol 26:4340-4346, 2008
12. Winther JF, Boice JD Jr, Svendsen AL, et al: Induced abortions in Danish cancer survivors: A population-based cohort study. J Natl Cancer Inst 101:687-689, 2009
13. Byrne J, Rasmussen SA, Steinhorn SC, et al: Genetic disease in offspring of long-term survivors of childhood and adolescent cancer. Am J Hum Genet 62:45-52, 1998
14. Winther JF, Boice JD Jr, Mulvihill JJ, et al: Chromosomal abnormalities among offspring of childhood-cancer survivors in Denmark: A population-based study. Am J Hum Genet 74: 1282-1285, 2004
15. Winther JF, Boice JD Jr, Frederiksen K, et al: Radiotherapy for childhood cancer and risk for congenital malformations in offspring: A population-based cohort study. Clin Genet 75:50-56, 2009
16. Sankila R, Olsen JH, Anderson H, et al: Risk of cancer among offspring of childhood-cancer survivors: Association of the Nordic Cancer Registries and the Nordic Society of Paediatric Haematology and Oncology. N Engl J Med 338:1339-1344, 1998
17. Madanat-Harjuoja LM, Malila N, Lähteenmäki P, et al: Risk of cancer among children of cancer patients: A nationwide study in Finland. Int J Cancer 126:1196-1205, 2010
18. Winther JF, Boice JD Jr, Thomsen BL, et al: Sex ratio among offspring of childhood cancer survivors treated with radiotherapy. Br J Cancer 88: 382-387, 2003
19. Reulen RC, Zeegers MP, Lancashire ER, et al: Offspring sex ratio and gonadal irradiation in the British Childhood Cancer Survivor Study. Br J Cancer 96:1439-1441, 2007
20. Tawn EJ, Rees GS, Leith C, et al: Germline minisatellite mutations in survivors of childhood and young adult cancer treated with radiation. Int J Radiat Biol 87:330-340, 2011
21. Winther JF, Boice JD Jr, Christensen J, et al: Hospitalizations among children of survivors of childhood and adolescent cancer: A population-based cohort study. Int J Cancer 127:2879-2887, 2010
22. Edgar AB, Wallace WH: Pregnancy in women who had cancer in childhood. Eur J Cancer 43:1890-1894, 2007

23. Nagarajan R, Robison LL: Pregnancy outcomes in survivors of childhood cancer. J Natl Cancer Inst Monogr 34:72-76, 2005

24. Blatt J: Pregnancy outcome in long-term survivors of cancer (review). Med Pediatr Oncol 33:29-33, 1999

25. Brinton LA, Krüger Kjær S, Thomsen BL, et al: Childhood tumor risk after treatment with ovulation-stimilating drugs. Fertil Steril 81:1083-1091, 2004

26. Andersen AN, Westergaard HB, Olsen J: The Danish in vitro fertilisation (IVF) register. Dan Med Bull 46:357-360, 1999

27. Winther JF, Sankila R, Boice JD, et al: Cancer in siblings of children with cancer in the Nordic countries: A population-based cohort study. Lancet 358:711-717, 2001

28. Wo JY, Viswanathan AN: Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. Int J Radiat Oncol Biol Phys 73:1304-1312, 2009

29. Hudson MM: Reproductive outcomes for survivors of childhood cancer. Obstet Gynecol 116: 1171-1183, 2010

30. Stovall M, Donaldson SS, Weathers RE, et al: Genetic effects of radiotherapy for childhood cancer: Gonadal dose reconstruction. Int J Radiat Oncol Biol Phys 60:542-552, 2004

31. Barlow WE, Ichikawa L, Rosner D, et al: Analysis of case-cohort designs. J Clin Epidemiol 52:1165-1172, 1999

32. Rothman KJ, Greenland S: Modern Epidemiology (ed 2). Philadelphia, PA, Lippincott-Raven, 1998

33. McNutt LA, Wu C, Xue X, et al: Estimating the relative risk in cohort studies and clinical trials of common outcomes. Am J Epidemiol 157:940-943, 2003

34. Zou G: A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 159:702-706, 2004

35. Harrell FE: Regression Modeling Strategies With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York, NY, Springer, 2001

36. Shrive FM, Stuart H, Quan H, et al: Dealing with missing data in a multi-question depression scale: A comparison of imputation methods. BMC Med Res Methodol 6:57, 2006

37. Allison PD: Missing Data. Thousand Oaks, CA, Sage Publications, 2001

38. Signorello LB, Cohen SS, Bossetti C, et al: Female survivors of childhood cancer: Preterm birth and low birth weight among their children. J Natl Cancer Inst 98:1453-1461, 2006

39. Critchley HOD, Thomson AB, Wallace WHB: Ovarian and uterine function and reproductive potential, in Wallace H, Green D (eds): Late Effects of Childhood Cancer. London, United Kingdom, Arnold, 2004, pp 229-231

40. Green DM, Fine WE, Li FP: Offspring of patients treated for unilateral Wilms' tumor in childhood. Cancer 49:2285-2288, 1982

41. Li FP, Gimbrere K, Gelber RD, et al: Outcome of pregnancy in survivors of Wilms' tumor. JAMA 257:216-219, 1987

42. Chiarelli AM, Marrett LD, Darlington GA: Pregnancy outcomes in females after treatment for childhood cancer. Epidemiology 11:161-166, 2000

43. Green DM, Whitton JA, Stovall M, et al: Pregnancy outcome of female survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. Am J Obstet Gynecol 187:1070-1080, 2002

44. Green DM, Peabody EM, Nan B, et al: Pregnancy outcome after treatment for Wilms tumor: A report from the National Wilms Tumor Study Group. J Clin Oncol 20:2506-2513, 2002

45. Mueller BA, Chow EJ, Kamineni A, et al: Pregnancy outcomes in female childhood and adolescent cancer survivors: A linked cancer-birth registry analysis. Arch Pediatr Adolesc Med 163: 879-886, 2009

46. Reulen RC, Zeegers MP, Wallace WH, et al: Pregnancy outcomes among adult survivors of childhood cancer in the British Childhood Cancer Survivor Study. Cancer Epidemiol Biomarkers Prev 18:2239-2247, 2009

47. Madanat-Harjouja LM, Malila N, Lähteenmäki PM, et al: Preterm delivery among female survivors of childhood, adolescent and young adulthood cancer. Int J Cancer 127:1669-1679, 2010

48. Signorello LB, Mulvihill JJ, Green DM, et al: Stillbirth and neonatal death in relation to radiation exposure before conception: A retrospective cohort study. Lancet 376:624-630, 2010

49. Green DM, Lange JM, Peabody EM, et al: Pregnancy outcome after treatment for Wilms tumor: A report from the National Wilms Tumor long-term follow-up study. J Clin Oncol 28:2824-2830, 2010

50. Green DM, Whitton JA, Stovall M, et al: Pregnancy outcome of partners of male survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. J Clin Oncol 21:716-721, 2003

51. Magelssen H, Melve KK, Skjaerven R, et al: Parenthood probability and pregnancy outcome in patients with a cancer diagnosis during adolescence and young adulthood. Hum Reprod 23:178-186, 2008

52. Chow EJ, Kamineni A, Daling JR, et al: Reproductive outcomes in male childhood cancer survivors: A linked cancer-birth registry analysis. Arch Pediatr Adolesc Med 163:887-894, 2009

53. Ståhl O, Boyd HA, Giwercman A, et al: Risk of birth abnormalities in the offspring of men with a history of cancer: A cohort study using Danish and Swedish national registries. J Natl Cancer Inst 103: 398-406, 2011

54. Signorello LB, Friedman DL, Boice JD Jr: Congenital abnormalities: A legacy of cancer treatment? J Natl Cancer Inst 103:358-359, 2011

55. Gardner MJ, Snee MP, Hall AJ, et al: Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. BMJ 300:423-429, 1990

56. Draper G: Preconception exposures to potential germ-cell mutagens. Radiat Prot Dosimetry 132: 241-245, 2008

57. Yoshimoto Y, Neel JV, Schull WJ, et al: Malignant tumors during the first 2 decades of life in the offspring of atomic bomb survivors. Am J Hum Genet 46:1041-1052, 1990

