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Cancer risk after *in utero* exposure to diethylstilbestrol

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Abstract

In utero exposure to diethylstilbestrol (DES) is associated with increased risk of clear cell adenocarcinoma (CCAC) of the vagina or cervix. It is not clear whether these risks remain increased at older ages, and if the risks of other cancer sites, including breast cancer, are increased. This nationwide cohort study included 12,249 DES-exposed women and 2,070 unexposed sisters. Hormone-related risk factors and medical history were assessed through questionnaires, and cancer incidence through linkages with nationwide registries. Comparison with general population rates showed no difference in overall cancer risk (SIR = 0.98, 95%CI 0.93–1.04) or breast cancer risk (SIR = 1.03, 95%CI 0.96–1.11) for DES-exposed women. The rate of vaginal cancer was strongly increased for DES-exposed women (SIR = 10.5, 95%CI 5.72–17.6) and was increased in all age categories, including age 60–69 years (SIR = 8.3, 95%CI 1.00-29.9). Risks of both CCAC (SIR = 49.1, 95%CI 21.2–96.8) and squamous cell carcinoma (SCC; SIR = 5.86, 95%CI 2.15–12.8) of the vagina were significantly elevated. When comparing DES-exposed women with DES-unexposed sisters, overall cancer risk and risk of breast cancer were similar (HR = 0.93, 95%CI 0.78–1.11 and HR = 0.97, 95%CI 0.76–1.23, respectively). Apart from the established increased risk of vaginal cancer, women exposed to DES *in utero* do not seem to be at increased risk of cancer, including breast cancer. The risk of vaginal cancer remains increased also for women in their fifties/sixties. Moreover, the increased risk of vaginal cancer was seen for both subtypes CCAC and SCC. Screening for vaginal cancer up to higher ages than currently recommended (< 60 years) should be considered.

Keywords Diethylstilbestrol · DES · Breast cancer · Cervical cancer · Cancer of the vagina · Clear cell adenocarcinoma · Squamous cell carcinoma · DES daughters · Screening

Introduction

Diethylstilbestrol (DES) is a synthetic estrogen that was prescribed to several millions of pregnant women worldwide from the 1940s to the early 1970s, with the aim of preventing miscarriages and other pregnancy complications [1, 2]. Starting in 1971, however, studies showed that *in utero* exposure to DES is associated with several severe adverse health effects, including reproductive tract abnormalities, increased risk of infertility and clear cell adenocarcinoma (CCAC) of the vagina and cervix [3–6]. The risks of CCAC of the vagina and cervix are strongly increased from the age of 15–20 years [6–8]. In 2010, we showed that women exposed to DES *in utero* had a 24-times increased risk of CCAC of the vagina or cervix compared to age- and sex-specific general population rates, and that also women aged forty years and older were at increased risk [9]. No excess risks were observed for other sites. In their most

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recent follow-up, the National Cancer Institute DES Follow-up Study observed a comparable, 27-times, increased risk of CCAC of the vagina or cervix compared to the general population [10]. In addition, they did observe a slightly increased breast cancer rate among DES-exposed women compared with the general population (SIR 1.17, 95%CI 1.01–1.36), but no increased risk of breast cancer when comparing DES-exposed and DES-unexposed women (hazard ratio [HR] 1.05, 95%CI 0.79–1.41). Median attained age of both cohorts, however, was still relatively young (44.0 [9] and 54.7 [10] years, respectively).

The aim of this study is to assess whether the risk of cancer in women exposed to DES *in utero* is increased at older ages, with a special focus on cancers of the vagina, cervix and breast.

Methods

Study population

The DES-net project is a nationwide, retrospective cohort study with prospective follow-up among DES daughters, i.e. women exposed to DES *in utero*, in the Netherlands. DES daughters were identified through the registry of the Netherlands DES Center. This center was established in 1992 for dealing with future DES-related health claims. All women exposed to DES *in utero* were advised to register, both individuals with and without health problems at time of registration. A system for financial compensation was achieved in March 2007 [11–13]. In the period 2000–2004, all registered DES daughters were sent a questionnaire about risk factors for hormone-related cancers and medical history. To establish an internal reference group, all DES daughters were asked to provide contact details of any sisters they had who had not been exposed to DES *in utero*. All reported, unexposed sisters were approached in 2004–2005, and received a questionnaire comparable to the DES daughters.

Non-responders to the questionnaire were sent a reminder after two months and a second reminder after six months. The final response rate to the questionnaire was 60% for the DES daughters, and 84% for the approached unexposed sisters (Supplementary Figs. 1 and 2). The study was approved by the Institutional Review Board of the Netherlands Cancer Institute and the Surveillance Committees of PALGA, the nationwide Dutch Pathology Registry, and the Netherlands Cancer Registry (NCR).

Assessment of cancer incidence and death

Vital status was obtained through linkage with the Dutch Office of Death Registry (CBG). Cancer incidence was assessed through linkage with PALGA and NCR, which have nationwide coverage since 1991 and 1989, respectively. The Surveillance Committees of PALGA and NCR granted us permission to link both responders and non-responders to the questionnaire under strict privacy procedures, excluding 204 DES-exposed women and 18 unexposed sisters who had actively refused. Linkage information was complete up to April 2023 for 12,249 DES-exposed women and 2,070 unexposed sisters (Supplementary Figs. 1 and 2). Diagnostic data provided by the NCR included disease stage, which consisted of International Federation of Gynecology and Obstetrics (FIGO) stage for lower genital tract carcinomas. PALGA provided histories of all lower genital tract screening results for women diagnosed with cancer of the vagina.

Classification of DES exposure

Great efforts were previously made to classify DES exposure [9]. For DES daughters, a copy of proof of their documented DES exposure was requested at time of questionnaire. If unavailable, the mother was asked to provide permission to have her medical record traced. Documented proof of DES exposure of the mother was available for only a minority of the women (12%). Mothers' medical records were hard to trace as archives of hospitals and general practitioners had been destroyed. Because surrogates for DES exposure were frequent among self-reported DES daughters - including medically verified DES-related reproductive tract abnormalities (such as adenosis, squamous cell metaplasia in the vagina or portio, cox comb, vaginal ridges, T-shaped uterus) (for 22%) and frequent gynecological screening before the age of the national screening program (additional 26%) - all women with self-reported DES exposure were included in the analyses, irrespective of whether DES exposure was medically verified. For a subgroup of participants ($n=115$) whose mother was treated in one out of four hospitals where all medical records were archived, we were able to verify self-reported DES exposure with medical records. For 76% of these women DES exposure was confirmed, in 3% a drug different from DES was recorded and in 21% no DES was mentioned in the hospital medical file. Prescription by general practitioners could not be excluded, however [9].

Statistical analyses

To assess the risk of cancer after *in utero* DES-exposure, two types of comparisons were performed: internal and external comparison. In the external comparison, the entire

analytical cohort ($n=12,249$ DES-exposed, $n=2,070$ unexposed women) was used to calculate Standardized Incidence Ratios (SIRs), comparing the observed cases with the expected number based on the general population age-, sex- and calendar period–specific incidence rates from the NCR for calendar years 1989–2022. The 95% confidence intervals (CI) were calculated assuming a Poisson distribution. For comparison of vaginal cancer stage, reference data from the NCR was used [14] taking into account the age of the cohort and years of diagnosis, i.e. restricting the NCR general population data to diagnoses before the age of 70 years in the period 1994–2022.

Internal comparison of cancer incidences was performed within the responders to the questionnaire to enable adjustment for potential confounders ($n=7,673$ DES-exposed, $n=1,757$ unexposed women). Cox proportional hazard models with attained age as the underlying time parameter were used, yielding Hazard Ratios (HR) and 95% CIs. Potential confounders considered as additional covariates were education (primary, secondary, college/university), Body Mass Index (BMI, <20, 20–24, 25–29, 30+kg/m^2), age at menarche (≤11, 12–14, 15+years), parity (n/y), and age at first full-term pregnancy (nulliparous, <25, 25–29, 30–34, 35+years).

All analyses were performed endpoint-specific, taking into account every first occurrence of the specific cancer of interest (irrespective of history of cancer diagnoses other than the specific cancer of interest), excluding from this analysis women diagnosed with the cancer of interest before start of time-at-risk. In the external comparison, time-at-risk started at the date of identification of the cohort, i.e. at registration at the DES Center for DES-exposed women and at the date of questionnaire completion for unexposed sisters (see Supplemental Table 1 for distribution of year of study entry). In the internal comparison, time-at-risk for both the DES-exposed women and unexposed sisters started at time of questionnaire completion. In all analyses, time at risk for all women ended at April 2023, the date of specific cancer diagnosis, or date of death, whichever came first.

Analyses were performed using STATA (version 15, StataCorp, College Station, TX).

Table 1 Characteristics of the full cohort by DES-exposure status

	DES-exposed women		DES-unexposed women	
Characteristic	No.	%	No.	%
Total	12,249	100.0%	2,070	100.0%
Year of birth				
1928–1958	2,927	23.9%	737	35.6%
1959–1963	3,026	24.7%	441	21.3%
1964–1968	3,198	26.1%	441	21.3%
1969–1987	3,098	25.3%	451	21.8%
History of cancer at start of follow-up[a]				
No	12,159	99.3%	2,032	98.2%
Yes	90	0.7%	38	1.8%
Vagina	16	0.1%	0	0.0%
CCAC	11	0.1%	0	0.0%
Cervical	33	0.3%	5	0.2%
CCAC	11	0.1%	1	0.0%
Breast cancer	18	0.1%	17	0.8%
Age at end of follow-up				
Median (IQR), years	59 (54–64)		61 (55–67)	
<50 years	617	5.0%	211	10.2%
50–54 years	3,059	25.0%	298	14.4%
55–59 years	3,174	25.9%	460	22.2%
60–64 years	2,933	23.9%	436	21.1%
65–69 years	1,819	14.9%	329	15.9%
70+years	647	5.3%	336	16.2%
Vital status at end of follow-up				
Alive	11,626	94.9%	1,971	95.2%
Deceased	623	5.1%	99	4.8%

Abbreviations: CCAC, clear cell adenocarcinoma; IQR, interquartile range

[a] Source of history of cancer from 1989 onwards is the Netherlands Cancer Registry. Cancer diagnoses before 1989 are only known for responders through the questionnaire. For DES-exposed women, follow-up starts at time of registry. For unexposed, follow-up starts at time of questionnaire. Because follow-up started at different points in time for the DES-exposed and unexposed women, DES-unexposed women were older at start of follow-up (29 years IQR 24–34 vs. 43 years IQR 37–49, for exposed vs. unexposed, respectively)

Results

The entire cohort consisted of 12,249 women exposed to DES *in utero* and 2,070 unexposed sisters. (Table 1) Year of birth ranged from 1928 to 1987. DES-unexposed women were more often born in earlier birth cohorts (35.6% versus 23.9% born in 1928–1958 for DES-unexposed versus exposed, respectively). Follow-up started at different points in time for the DES-exposed and unexposed (see Methods section), causing DES-unexposed women to be older at start of follow-up (29 years (IQR 24–34) versus 43 years (IQR 37–49) for exposed versus unexposed, respectively). Consequently, history of cancer diagnosis before start of follow-up differed between the two groups. None of the DES-unexposed women, however, had a history of cancer of the vagina, whereas 16 women in the exposed group did (of which 11 were CCAC). Median age at end of follow-up was 59 years for DES-exposed women (IQR 54–64 years) and 61 for unexposed women (IQR 55–67 years).

External comparison with age- and calendar-year-specific general population rates showed that overall cancer risk was neither increased in DES-exposed women (SIR 0.98, 95%CI 0.93–1.04) nor in unexposed women (SIR 1.02, 95%CI 0.89–1.16). (Table 2) For breast cancer, both the invasive breast cancer and ductal carcinoma in situ incidence rates in

Table 2 Comparison of cancer rates among DES-exposed and DES-unexposed women with age and calendar-year specific general population rates

		DES-exposed women				DES-unexposed women			
Type of cancer	**ICD-10**	**Obs**	**Exp[a]**	**SIR**	**95%CI**	**Obs**	**Exp[a]**	**SIR**	**95%CI**
Total cancer	C00-C80	1,378	1401.7	0.98	0.93–1.04	231	226	1.02	0.89–1.16
Breast	C50	727				135			
Invasive breast cancer		719	698.3	1.03	0.96–1.11	115	104.9	1.10	0.91–1.62
Ductal carcinoma in situ		104	89.6	1.16	0.95–1.41	16	14.6	1.10	0.63–1.79
Cervix uteri	C53	30	45.6	**0.66**	**0.44–0.94**	4	4.6	0.87	0.24–2.22
Clear cell adenocarcinoma		2[b]	0.37	5.48	0.66–19.8	0	0.0	-	-
Squamous cell carcinoma		15[b]	32.3	0.46	0.26–0.77	1	3.3	0.31	0.01–1.72
Adenocarcinoma		9[b]	6.8	1.32	0.61–2.51	3	0.70	4.32	0.89–12.6
Adenosquamous carcinoma		1[b]	1.41	0.71	0.02–3.95	0	0.1	-	-
Vagina	C52	14	1.3	**10.50**	**5.72–17.6**	0	0.2	-	-
Clear cell adenocarcinoma		8	0.2	49.13	21.2–96.8	0	0.0	-	-
Squamous cell carcinoma		6	1.0	5.86	2.15–12.8	0	0.2	-	-
Adenocarcinoma		0	0.2	-	-	0	0.0	-	-
Adenosquamous carcinoma		0	0.0	-	-	0	0.0	-	-
Vulva	C51	5	9.2	0.54	0.18–1.27	1	1.6	0.63	0.02–3.48
Corpus uteri	C54	38	45.8	0.83	0.59–1.14	7	9.5	0.74	0.30–1.52
Ovary and fallopian tube	C56	28	53.5	**0.52**	**0.35–0.76**	9	8.2	1.10	0.50–2.09
Pancreas	C25	21	21.3	0.99	0.61–1.51	6	4.6	1.31	0.48–2.85
Lung and bronchus	C34	111	151.4	**0.73**	**0.60–0.88**	14	29.6	**0.47**	**0.26–0.79**
Melanoma	C44, C51, C60, C632, C80	147	137.7	1.07	0.90–1.26	23	18.8	1.22	0.77–1.83
Colon and rectum	C18-C20	85	79.0	1.06	0.85–1.32	18	16.4	1.10	0.65–1.73
Anus and anal canal	C21	4	4.8	0.83	0.23–2.11	0	0.8	-	-
Thyroid glands	C73	16	21.9	0.73	0.42–1.19	3	2.8	1.08	0.22–3.14
Hodgin lymphoma	C81	5	7.0	0.71	0.23–1.67	1	0.6	1.55	0.04–8.65
Non-hodgin lymphoma	C85								
Brain	C71, C722, C723, C751, C753	18	17.7	1.02	0.60–1.61	2	2.5	0.82	0.10–2.95
Leukemia	C95								
Head and neck	C00-C14, C30-C32	14	28.5	0.49	0.27–0.83	4	5.1	0.79	0.22–2.02
Oesophagus	C15	5	11.1	0.45	0.15–1.05	0	2.4	-	-
Cardia stomach	C160	10	2.8	**3.60**	**1.72–6.61**	1	0.5	1.93	0.05–10.8
Stomach, other	C161-C169	9	9.7	0.93	0.42–1.76	0	1.7	-	-

Abbreviations: ICD-10, International Classification of Diseases, 10th edition; Obs, observed; Exp, expected; SIR, standardized incidence ratio; 95%CI, 95% confidence interval

[a] Expected numbers were calculated using age-, sex- and calendar period-specific cancer incidence rates for the Dutch population using incidence data from the Dutch Cancer Registry. Analyses were performed endpoint-specific, taking into account the first occurrence of the specific endpoint of interest, excluding from this analyses women diagnosed with the endpoint before start of follow-up

[b]Do not add up to total due to unknown/unspecified morphology for three cases of cervical cancer

our cohort were similar to the general population rates (SIR 1.03, 95%CI 0.96–1.11 and SIR 1.16, 95%CI 0.95–1.41, respectively). Also in age-stratified analyses, none of the age categories showed a statistically-significantly elevated breast cancer rate, with SIRs ranging from 0.94 to 1.06 for invasive breast cancer and from 0.62 to 1.76 for ductal carcinoma in situ. (Table 3)

For DES-exposed women, a strongly increased rate of cancer of the vagina was observed (SIR 10.5, 95%CI 5.72–17.6). Rates were increased in all age categories, including in the highest age category of 60–69 years (SIR 8.3, 95%CI 1.00-29.9). (Table 3) Oldest age at diagnosis of cancer of the vagina was 62 years. When focusing on specific subtypes of cancer of the vagina, increased rates were observed for both CCAC (SIR 49.1, 95%CI 21.2–96.8) and squamous cell carcinoma (SCC, SIR 5.86, 95%CI 2.15–12.8). While among women aged < 40 years all diagnoses of cancer of the vagina concerned CCAC, diagnoses among women aged ≥ 40 years often were SCC. (Supplemental Table 2) Age-specific analyses showed an increased risk of CCAC of the vagina among DES-exposed women aged both younger and older than 40 years, with SIRs of 97.7 (95%CI 31.7-228.1) based on five observations for age < 40 years and 26.9 (95%CI 5.54–78.5) based on three events for age ≥ 40 years. The risk of SCC vaginal cancer among DES-exposed women was increased only at ages ≥ 40 years (SIR 6.43, 95%CI 2.36-14.0, based on six events, no cases were observed < 40 years). Compared to the general population,

Table 3 Comparison of cancer rates among DES-exposed and DES-unexposed women with age and calendar-year specific general population rates, by attained age

	DES-exposed women				DES-unexposed women			
Type of cancer	Obs	Exp[a]	SIR	95%CI	Obs	Exp[a]	SIR	95%CI
Breast								
Invasive breast cancer								
< 40 years	*62*	*58.4*	*1.06*	*0.81–1.36*	*1*	*2.8*	*0.35*	*0.01–1.96*
40–49 years	*260*	*244.8*	*1.06*	*0.94–1.20*	*22*	*25.1*	*0.88*	*0.55–1.33*
50–59 years	*299*	*289.8*	*1.03*	*0.92–1.16*	*57*	*45.1*	*1.27*	*0.96–1.64*
60–69 years	*92*	*98.0*	*0.94*	*0.76–1.15*	*26*	*26.0*	*1.00*	*0.65–1.47*
Ductal carcinoma in situ								
< 40 years	*7*	*4.0*	*1.76*	*0.71–3.63*	*0*	*0.2*	-	
40–49 years	*25*	*21.2*	*1.18*	*0.76–1.74*	*3*	*2.3*	*1.28*	*0.26–3.74*
50–59 years	*63*	*48.9*	*1.29*	*0.99–1.50*	*8*	*7.5*	*1.07*	*0.46–2.10*
60–69 years	*9*	*14.5*	*0.62*	*0.28–1.17*	*5*	*3.8*	*0.79*	*0.16–2.32*
Cervix uteri								
< 40 years	*7*	*15.7*	***0.45***	***0.18–0.92***	*1*	*0.6*	*1.58*	*0.04–8.82*
40–49 years	*14*	*17.5*	*0.80*	*0.44–1.35*	*0*	*1.8*	-	
50–59 years	*6*	*10.0*	*0.60*	*0.22–1.61*	*2*	*1.5*	*1.31*	*0.16–4.74*
60–69 years	*3*	*2.3*	*1.33*	*0.27–3.89*	*1*	*0.6*	*1.81*	*0.05–10.1*
Vagina								
< 40 years	*5*	*0.2*	***31.54***	***10.2–73.6***	*0*	*0.0*	-	
40–49 years	*4*	*0.3*	***12.32***	***3.40–31.5***	*0*	*0.0*	-	
50–59 years	*3*	*0.6*	***5.09***	***1.05–14.9***	*0*	*0.1*	-	
60–69 years	*2*	*0.2*	***8.29***	***1.00-29.9***	*0*	*0.1*	-	
Ovary and fallopian tube								
< 40 years	*5*	*7.9*	*0.63*	*0.20–1.47*	*0*	*0.3*	-	
40–49 years	*8*	*16.3*	***0.49***	***0.21–0.97***	*3*	*1.5*	*1.95*	*0.40–5.71*
50–59 years	*8*	*19.5*	***0.41***	***0.18–0.81***	*3*	*3.2*	*0.95*	*0.20–2.78*
60–69 years	*6*	*8.9*	*0.68*	*0.25–1.47*	*3*	*2.4*	*1.23*	*0.25–3.58*
Lung and bronchus								
< 40 years	*5*	*3.6*	1.37	0.45–3.21	0	0.1	-	
40–49 years	*23*	*27.2*	0.84	0.54–1.27	0	2.7	-	
50–59 years	*49*	*66.8*	**0.73**	**0.54–0.97**	8	10.8	0.74	0.32–1.46
60–69 years	*29*	*49.3*	**0.59**	**0.39–0.85**	6	12.3	0.49	0.18–1.06
Melanoma								
< 40 years	*30*	*25.6*	*1.17*	*0.79–1.67*	*2*	*1.2*	*1.66*	*0.20–6.01*
40–49 years	*49*	*47.2*	*1.04*	*0.77–1.37*	*6*	*5.1*	*1.17*	*0.43–2.54*
50–59 years	*52*	*46.4*	*1.12*	*0.84–1.47*	*7*	*7.0*	*1.00*	*0.40–2.05*
60–69 years	*16*	*17.2*	*0.93*	*0.53–1.51*	*7*	*4.2*	*1.65*	*0.67–3.41*
Cardia stomach								
< 40 years	*0*	*0.1*	-		*0*	*0.0*	-	
40–49 years	*3*	*0.7*	*4.28*	*0.88–12.5*	*0*	*0.2*	-	
50–59 years	*3*	*1.2*	*2.61*	*0.54–7.6*	*1*	*0.2*	*5.46*	*0.14–30.4*
60–69 years	*4*	*0.8*	***5.16***	***1.4–13.2***	*0*	*0.1*	-	

Abbreviations: ICD-10, International Classification of Diseases, 10th edition; Obs, observed; Exp, expected; SIR, standardized incidence ratio; 95%CI, 95% confidence interval

[a] Expected numbers were calculated using age-, sex- and calendar period-specific cancer incidence rates for the Dutch population using incidence data from the Dutch Cancer Registry. Analyses were performed endpoint-specific, taking into account the first occurrence of the specific endpoint of interest, excluding from this analyses women diagnosed with the endpoint before start of follow-up

DES-exposed women had lower FIGO stages at diagnosis: stage I, II, and III were diagnosed in 70.6%, 11.8%, and 11.8%, respectively, of the vaginal carcinomas, compared to 40.7%, 27.3%, and 28.4% in the general population. Linkage to the nationwide Dutch Pathology Registry, enabling evaluation of screening adherence for women with a cancer diagnosis, showed that all women diagnosed with vaginal cancer had frequent cervical and vaginal Pap smears in the years before diagnosis.

No increased SIRs were observed for other gynecological cancer sites. On the contrary, lower rates of cervical cancer and cancer of the ovary and fallopian tube were observed among DES-exposed (SIR for cervical cancer 0.66, 95%CI 0.44–0.94; SIR for cancer of the ovary and fallopian tube 0.52, 95%CI 0.35–0.76). (Table 2) For cervical cancer, the lower rate was most apparent among women aged < 40 years (SIR 0.45, 95%CI 0.18–0.92). For ovary and fallopian tube, SIRs were lower for all age categories, but only statistically significant for the age groups 40–49 and 50–59 years (SIR 0.49, 95%CI 0.21–0.97 and SIR 0.41, 95%CI 0.18–0.81, respectively). (Table 3)

Lung cancer rates were also lower than in the general population, both for DES-exposed and for unexposed women (SIR 0.73, 95%CI 0.60–0.88 and SIR 0.47, 95%CI 0.26–0.79, respectively). An increased rate of cancer of the cardia was observed for DES-exposed women (SIR 3.6, 95%CI 1.72–6.61, with ten observed diagnoses). Rates for other tumor sites, including melanoma, were not statistically significantly different from the general population.

Among the responders to the questionnaire, multivariable analyses could be performed adjusting for established confounders for breast cancer, directly comparing DES-exposed and unexposed women. Characteristics of responders did not materially differ from non-responders (Supplemental Table 3). Median age at time of questionnaire slightly differed between DES-exposed and DES-unexposed (37 years and 43 years, respectively). (Table 4) Education, BMI and age at menarche were comparable between the two groups. DES-exposed women, however, more often were nulliparous, also when restricting the comparison to women aged ≥ 40 years at time of questionnaire, to diminish the possibility of childbirth after questionnaire completion, with 37% nulliparous among DES-exposed women compared with 19% for unexposed. Yet, median age at first full-term pregnancy was 28 years for both groups. Comparing DES-exposed women with DES-unexposed, risks of both any type of cancer and of breast cancer were similar, with HRs of 0.93 (95%CI 0.78–1.11) for all cancers combined and 0.97 (95%CI 0.76–1.23) for breast cancer. (Table 5) When restricting the analyses to an attained age of 50 years or less, however, slightly increased - yet statistically non-significant - HRs were observed for any breast cancer (including both invasive and ductal carcinoma in situ, HR 1.38, 95%CI 0.91–2.14) and for invasive breast cancer (HR 1.45, 95%CI 0.92–2.30). Risk of breast cancer at age ≥ 50 was not increased when comparing DES-exposed with DES-unexposed women (HR 0.79, 95%CI 0.58–1.08). Neither in the full nor in the age-restricted analyses did any of the potential confounders substantially affect the HRs associated with DES-exposure. (Supplemental Tables 4, 5 and 6)

Discussion

This large study with near-complete long-term follow-up data shows no increased risk of cancer overall and breast cancer in women exposed to DES *in utero*. However, a strongly increased risk of vaginal cancer was observed which remained increased among women in their fifties and sixties. Remarkably, the increased risk of vaginal cancer was observed both for morphologic subtype CCAC and SCC.

Concerns about a potentially increased risk of breast cancer after *in utero* DES exposure originate from the observed association between *in utero* exposure to higher estrogen levels and increased breast cancer risk [15], in addition to the increased risk of breast cancer found in women who received DES during pregnancy [16]. We found no evidence of an increased risk of breast cancer, neither when compared with general population rates nor with DES-unexposed sisters. In contrast, the National Cancer Institute DES Follow-up Study [10] observed a moderately increased risk of breast cancer (SIR 1.17, 95%CI 1.01–1.36), yet only when comparing DES-exposed women with the general population– not when comparing DES-exposed with DES-unexposed women (HR 1.05, 95%CI 0.79–1.41). We did not observe elevated breast cancer risks for women aged ≥ 50 years. We did see slightly higher, albeit statistically non-significant, HRs for breast cancer before the age of 50 years for women exposed to DES compared to unexposed-sisters. This difference, however, might be caused by our finding of a decreased breast cancer risk among DES-unexposed sisters compared with the general population (SIR for invasive breast cancer < 40 years 0.35 and for 40–49 years 0.88). Apart from nulliparity, no clear differences in established breast cancer risk factors were apparent between DES-exposed, DES-unexposed, and the general population, nor did correcting for these risk factors affect the HRs.

Our study is the first to report a persistent increase of vaginal cancer risk after the age of 50 years. The majority (~ 70%) of vaginal cancers was detected at stage I, implying a relatively good prognosis. Moreover, the stage distribution seems more favorable than in the general population, possibly as a result of screening. Two cases of vaginal cancer were detected at age ≥ 60 years, resulting in a strongly increased SIR for vaginal cancer also for the age group 60–69 years. The morphology for both cases concerned SCC, as was the case for the majority of vaginal cancers diagnosed above the age of 40 years. This increased risk of SCC of the vagina > 40 years after *in utero* exposure to DES is a novel finding, that may be explained by the larger cervical transformation zone observed in DES daughters, increasing the chances of the development of low- and high-grade intraepithelial lesions [17, 18]. Troisi et al. examined the rates of high-grade squamous intraepithelial lesions of

Table 4 Characteristics of the cohort with questionnaire data, by DES-exposure status

	DES-exposed women		DES-unexposed women	
Characteristic	No.	%	No.	%
Total	7,673	100.0%	1,757	100.0%
Age at questionnaire[a]				
Median (IQR), years	37 (32–52)		43 (37–49)	
<24 years	6	0.1%	13	0.7%
24–29 years	1,083	14.1%	88	5.0%
30–35 years	2,162	28.2%	205	11.7%
35–40 years	2,281	29.7%	423	24.1%
41–45 years	1,241	16.2%	295	16.8%
45+years	900	11.7%	733	41.7%
Highest education				
Primary school	2,007	26.6%	419	24.1%
Secondary school	2,639	35.0%	598	34.5%
College or university	2,886	38.3%	718	41.4%
Unknown	141	*n.a.*[b]	22	*n.a.*[b]
Body Mass Index, kg/m^2				
<20	626	8.3%	90	5.1%
20–24	4,388	58.4%	944	53.7%
25–29	1,854	24.7%	505	28.7%
30+	652	8.7%	180	10.2%
Missing	153	*n.a.*[b]	38	*n.a.*[b]
Age at menarche				
Median (IQR), years	13 (12–14)		13 (12–14)	
≤11 year	1,016	13.2%	189	10.8%
12–14 year	4,586	59.8%	1,129	64.3%
15+year	1,203	15.7%	272	15.5%
Unknown	868	*n.a.*[b]	167	*n.a.*[b]
Parity and number of children				
No	3,476	45.3%	430	24.5%
Yes	4,197	54.7%	1,327	75.5%
1 child	1,404	33.5%	205	15.4%
2 children	1,980	47.2%	705	53.1%
3 children	634	15.1%	303	22.8%
4+children	179	4.3%	114	8.6%
Women aged > 40 years only[c]				
No	947	37.2%	211	18.7%
Yes	1,599	62.8%	915	81.3%
1 child	392	24.5%	99	11.0%
2 children	777	48.6%	468	52.1%
3 children	321	20.1%	238	26.5%
4+children	109	6.8%	94	10.5%
Age at first full term pregnancy				
Median (IQR), years	28 (25–31)		28 (25–31)	
<25 year	769	10.0%	285	16.2%
25–29 year	2,009	26.2%	586	33.4%
30–34 year	1,285	16.7%	397	22.6%
35+year	272	3.5%	94	5.4%
Unknown	27	*n.a.*[b]	8	*n.a.*[b]
Not applicable	3,311	*n.a.*[b]	387	*n.a.*[b]

Abbreviations: n.a., not applicable; IQR, interquartile range

[a] Questionnaire was distributed at different moments in time for exposed and unexposed women. For DES-exposed women, questionnaires were sent out in the period 2000–2003. For unexposed, this was 2004–2005

[b] Percentages are calculated as proportions of all known cases, not taking into account cases that are unknown, missing or not applicable

[c] Age at time of questionnaire

Table 5 Within cohort comparison of cancer risk by DES-exposure status for women with questionnaire data

	Total cohort				Attained age							
					<50 years				≥50 years			
	n	N[a]	HR	95%CI	n	N[a]	HR	95%CI	n	N[a]	HR	95%CI
Total cancer												
Univariate model												
DES exposure												
No	191	1,666	1.00	Ref.	42	1,286	1.00	Ref.	149	1,462	1.00	Ref.
Yes	808	7,462	0.94	0.79–1.12	351	7,305	1.24	0.89–1.71	457	6,827	0.83	0.67–1.03
Multivariable model[b]												
DES exposure												
No	191	1,666	1.00	Ref.	42	1,286	1.00	Ref.	149	1,462	1.00	Ref.
Yes	808	7,462	0.93	0.78–1.11	351	7,305	1.25	0.90–1.74	457	6,827	0.81	0.65–1.01
Breast cancer												
Univariate model												
DES exposure												
No	96	1,686	1.00	Ref.	24	1,296	1.00	Ref.	72	1,494	1.00	Ref.
Yes	450	7,635	0.95	0.75–1.21	218	7,460	1.34	0.87–2.05	232	7,085	0.81	0.62–1.06
Multivariable model[b]												
DES exposure												
No	96	1,686	1.00	Ref.	24	1,296	1.00	Ref.	72	1,494	1.00	Ref.
Yes	450	7,635	0.97	0.76–1.23	218	7,460	1.38	0.91–2.14	232	7,085	0.79	0.58–1.08
Breast cancer, invasive only												
Univariate model												
DES exposure												
No	83	1,686	1.00	Ref.	21	1,269	1.00	Ref.	62	1,497	1.00	Ref.
Yes	397	7,640	0.99	0.76–1.28	199	7,465	1.40	0.89–2.21	198	7,109	0.83	0.62–1.11
Multivariable model[b]												
DES exposure												
No	83	1,686	1.00	Ref.	21	1,296	1.00	Ref.	62	1,497	1.00	Ref.
Yes	397	7,640	1.01	0.78–1.31	199	7,465	1.45	0.92–2.30	198	7,109	0.82	0.58–1.14

Abbreviations: n/N, number of events/number at risk; HR, hazard ratio; CI, confidence interval; Ref., reference category

[a]Number at risk differs per endpoint because the analyses were performed endpoint-specific, taking into account every first occurrence of the specific cancer of interest (irrespective of history of cancer diagnoses other than the specific cancer of interest), excluding from this analysis women diagnosed with the cancer of interest before start of time-at-risk

[b]Full multivariable model is shown in Supplementary tables 4, 5 and 6

the lower genital tract and did not find an increased risk for women aged ≥ 45 years [19]. However, they did not examine the risks of cancer at sub-sites within the lower genital tract.

The risk of SCC cervical cancer was significantly decreased among DES-exposed women. This is likely due to the high screening rate among Dutch women exposed to DES, leading to enhanced detection of precancerous lesions of the cervix [20]. Current Dutch guidelines advise DES-exposed women to have a Pap smear of the cervix and vagina every other year until at least the age of 60 years. For vaginal cancer, no evidence-based screening method currently exists for the general population. Nevertheless, Pap smears of the vagina have been recommended for women exposed to DES *in utero*. Also in our study, screening for vaginal cancer seems less effective in *preventing* invasive disease than for cervical cancer. Remarkably, all invasive vaginal cancers were diagnosed despite a history of frequent vaginal screening. However, in view of the favorable stage distribution of the vaginal cancers, it should be considered if a recommendation for screening up to higher ages for DES-exposed women is appropriate.

The reduced risk of cancer of the ovary and fallopian tube was not observed before; previously reported SIRs for cancer of the ovary were 1.20 (95%CI 0.71–1.90) [10] and 1.13 (95%CI 0.31–2.89) [9]. A possible explanation for this difference in findings is the aging of the cohort, as a clearly reduced risk of cancer of the ovary and fallopian tube was especially observed for the age category 50–59 years. No obvious explanations for this reduced risk are available; it might be speculated though that DES-exposed women are less inclined to use menopausal hormone treatment [21].

The observed increased rate of cardia cancer among DES-exposed women compared with the general population might well be a chance finding, as we considered many outcomes, and both observed and expected absolute numbers for cardia cancer were low (10 versus 2.8). An increased risk for this outcome has previously not been observed, nor was it hypothesized. The increased risk of melanoma that previously was seen mainly among women aged < 40 years [9], was not observed now in the updated cohort with also more person years among women aged < 40 years. An increased risk of pancreas cancer after *in utero* exposure to DES has been reported by others, based on small numbers [10], but was not observed in our cohort.

Women enrolled into our cohort might differ from the general population with respect to risk factors for cancer. Due to a higher percentage of subfertility among DES-exposed women, the proportion of nulliparous women was higher among DES-exposed women than among DES-unexposed women (37% versus 19% among women aged ≥ 40 years at time of questionnaire, respectively) and higher than in the general Dutch population (11.7 -17.8% among women born between 1935–1985 [22]). Correcting for parity in the internal comparison, however, did not affect our results. Other established risk factors, including age at menarche, BMI and age at first full-term pregnancy did not differ between DES-exposed, unexposed and the general population. Smoking status, however, was not collected for this cohort. Considering that a lower risk of lung cancer was observed for both DES-exposed women and DES-unexposed sisters compared with the general population, it is likely that smoking behavior differed from the general population.

All cancer diagnoses in this study were medically verified. Documentation of maternal DES exposure, unfortunately, could not be verified for a large part of the cohort. However, we have previously shown in a validation study, through stratification, and through sensitivity analyses, that the percentage of misclassification is relatively low, and is not likely to have influenced our results [9].

In conclusion, our long-term follow-up data overall show reassuring results; women exposed to DES *in utero* do not seem to be at increased risk of cancer, including breast cancer, other than the established increased risk of vaginal cancer. Importantly, the risk of vaginal cancer after *in utero* DES exposure remained increased also for women in their fifties and sixties. Moreover, the increased risk of vaginal cancer was seen for both subtypes, CCAC and SCC. Screening for vaginal cancer up to higher ages than the currently advised age of 60 years should be considered for DES-exposed women.

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Data availability The data underlying this article will be shared with non-commercial parties on reasonable request to the corresponding author (f.v.leeuwen@nki.nl). Only pseudonymized data will be transferred.

Declarations

Ethical approval This is an observational study. The study was approved by the Institutional Review Board of the Netherlands Cancer Institute and the Surveillance Committees of PALGA, the nationwide Dutch Pathology Registry, and the Netherlands Cancer Registry (NCR).

Competing interests None of the authors have conflicts of interest.

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