Long-term health effects after DES exposure in utero

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Chapter 3

Risk of cervical intra-epithelial neoplasia and invasive cancer of the cervix in DES daughters

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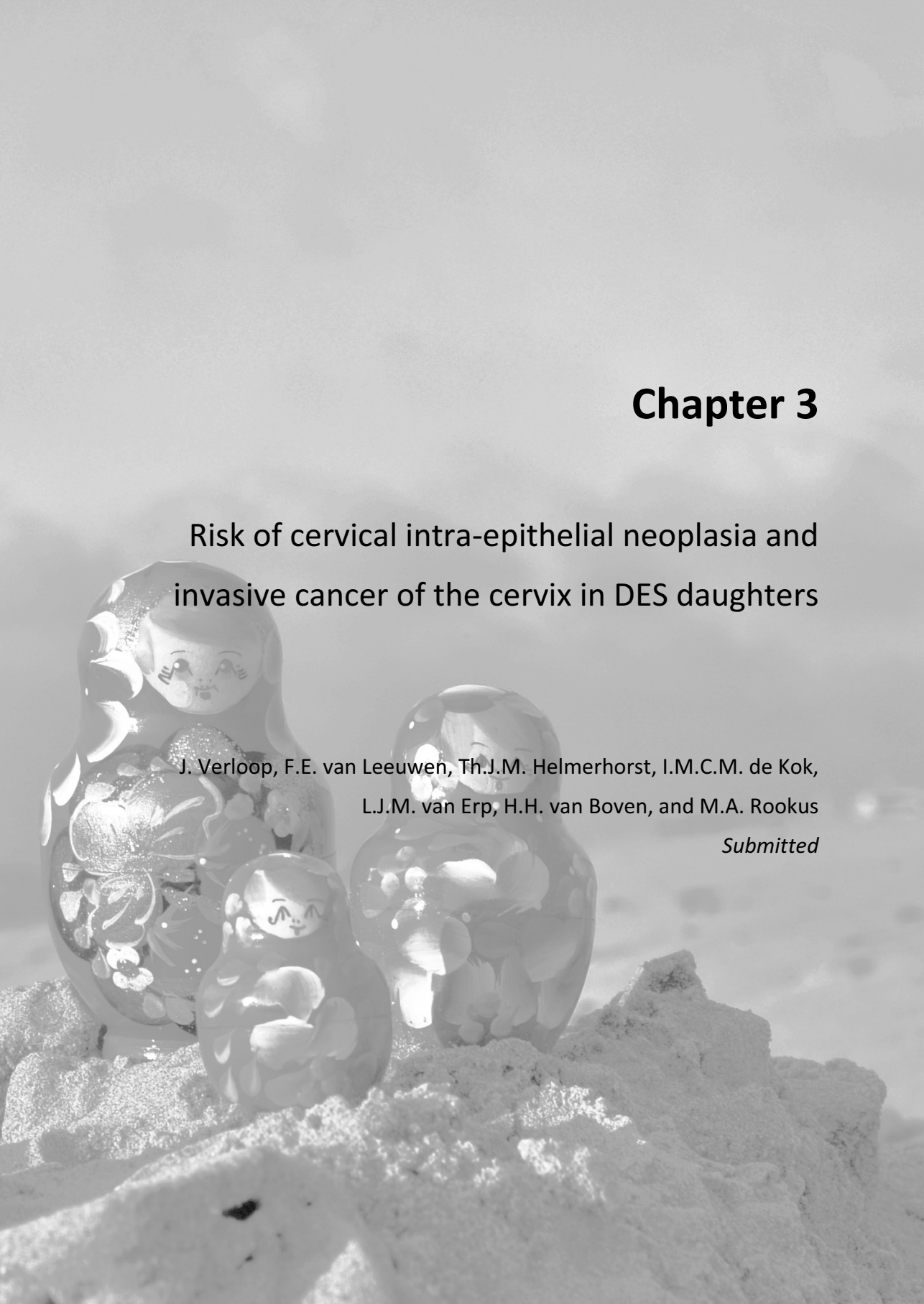

3.1 Abstract

Background

Women exposed to diethylstilbestrol *in utero* (DES) have an increased risk of clear cell adenocarcinoma of the vagina and cervix at young ages. Because of an enlarged transformation zone is has been speculated that the risk of cervical intra-epithelial neoplasia (CIN) and cervical cancer might be increased among DES daughters at older ages.

Methods

We studied the risk of CIN and cervical cancer in a cohort of 12,182 DES daughters. Women were prospectively followed from January 2000 till November 2008. Incidence was obtained through linkage with the Netherlands Nationwide Pathology database (PALGA). General population data were also derived from PALGA. Information concerning covariates was collected through a mailed questionnaire and reported DES-related malformations were verified by medical file.

Results

No increased risk was observed for CIN2+ (CIN grade 2, 3 or cancer) compared to the screened general population (Standardized incidence ratio (SIR) 0.96, 95% Confidence Interval (CI) 0.69-1.3). The incidence of CIN grade 1 was borderline statistically significantly increased (SIR=1.5, 95%CI=0.99-2.3). The incidence rate of CIN (all grades) was largely determined by the number of screening episodes. Among women with DES-related malformations the risk of CIN1 was somewhat higher (SIR=4.6, 95%CI=2.6-7.5), but risk of CIN2+ was not increased (SIR=0.87, 95%CI=0.38-1.7).

Conclusions

In this study an increased risk of CIN1 was observed, especially in women with DES-related malformations. The risk of CIN2+ (including cancer) was not increased suggesting that DES daughters in general do not have an increased risk of squamous cell cervical cancer.

3.2 Introduction

In the late 1940s to the early 1970s several millions of pregnant women worldwide received Diethylstilbestrol (DES) in order to prevent miscarriages and other pregnancy complications[1;2]. Following publications on an increased risk of clear cell adenocarcinoma of the vagina and cervix (CCA) among women exposed *in utero*[3;4] DES was banned from usage in pregnant women in the early seventies. Still, the study of late health effects in women exposed prenatally to the drug is relevant because DES daughters have only recently reached the ages at which chronic health conditions commonly occur. Next to CCA the now well-established adverse health effects include several reproductive tract abnormalities, such as the presence of glandular tissue (adenosis) and metaplastic squamous epithelium (which is considered as the physiological transition from glandular tissue to normal epithelial tissue) in the vagina and ectocervix (enlarged ectropion, wider transformation zone). It has been speculated that dysplastic changes in the vaginal and cervical epithelium might result in a higher risk of cancer, not only adenocarcinoma, but also squamous cell cancer and precancerous lesions[5-7]. A two-fold risk of cervical dysplasia was observed in the National Cancer Institute's DES Combined Cohort Follow-up Study (NCI DES Follow-up study). A recent analysis of the same cohort, with longer follow-up, confirmed this finding, with a nearly three-fold risk among DES daughters who had vaginal epithelial changes, with changes defined as adenosis and metaplastic squamous epithelium[7]. For invasive squamous cell cervical cancer findings were inconclusive[8]. In a recent report of our Dutch DES cohort a (non-statistically significant) decreased risk of invasive squamous cell cervical cancer was found[9]. The apparently opposite risks found for precancerous and invasive lesions might be due to screening bias.

In this report we examine the risk of cervical intra-epithelial lesions and cancer in a large cohort of Dutch DES daughters, with long-term and complete follow-up. A unique feature of this study is that all outcomes and reference data were obtained from the Netherlands nationwide pathology database (PALGA), which enabled us to account for screening behavior.

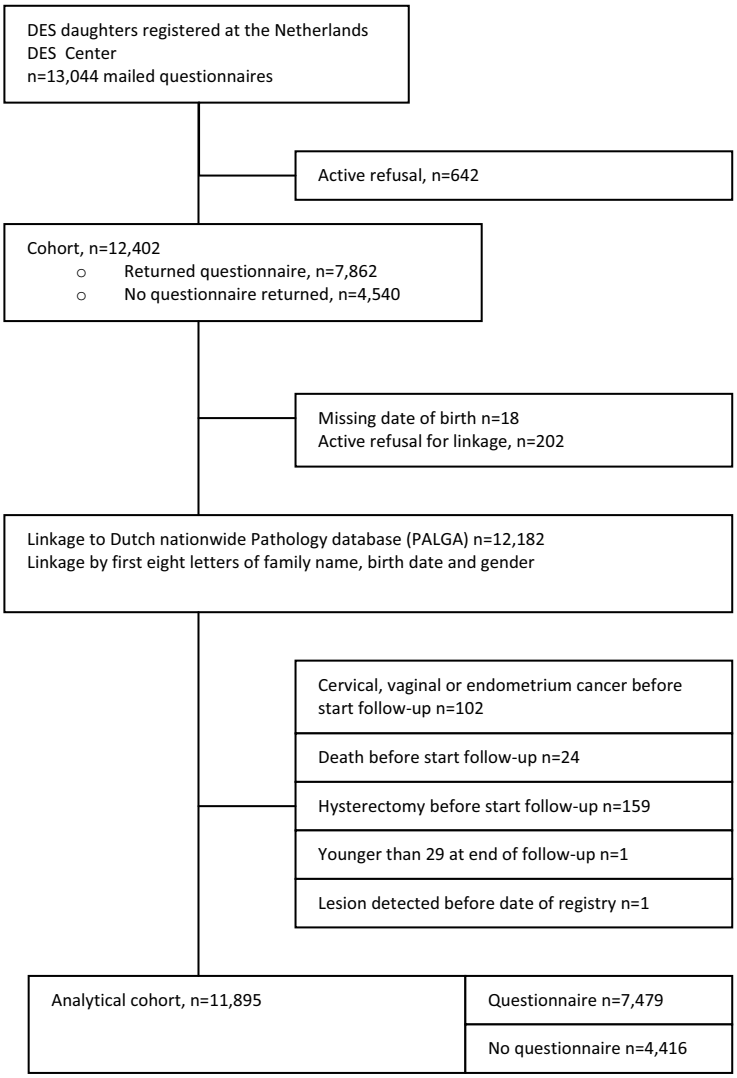

Figure 1 Follow-up information of DES daughters included in the study

3.3 Methods

Study population

The DES-net project is a nationwide retrospective cohort study with prospective follow-up among DES daughters in the Netherlands. Women were identified through the registry of the Netherlands DES Center that was established in 1992 in order to deal with future health claims. Documented DES exposure at time of registration was not required. In the period March 2000 – December 2004 all DES daughters with known addresses were sent a 16-page self-administered questionnaire about risk factors for hormone-related cancers and medical history (response 63%, Figure 1). In addition to the questionnaire, women granted permission to abstract data from their medical records by means of a written informed consent. Furthermore, women provided us, if available, with a copy of the medical file of their mothers in which DES exposure *in utero* was confirmed.

Data collection

Assessment of outcome and screening history

Information on cervical intra-epithelial neoplasia (CIN) and screening history was retrieved from PALGA[10]. PALGA is a nationwide database of excerpts of all histopathology and cytopathology reports made since 1989. The PALGA Surveillance Committee granted us permission to link all study subjects (both responders and non-responders to the questionnaire) with PALGA under strict privacy procedures. Furthermore, the study was approved by the Institutional Review Board of the Netherlands Cancer Institute. From PALGA we retrieved information on type of cervical examination (smear or histology by excision or biopsy), date of examination and outcome. All cervical intra-epithelial lesions and cancer were coded according to the systemized nomenclature of medicine (SNOMED). For each woman the first occurrence of the highest grade of cervical intra-epithelial neoplasia (CIN) was used in the analysis (see Supplement table 1). CIN1 was defined as mild dysplasia, CIN2 as moderate dysplasia and CIN3 as severe dysplasia or carcinoma in situ. Furthermore, for each woman the number of episodes, as a proxy for the number of screening rounds, was calculated. By definition, a screening episode started with a primary smear, if necessary followed by secondary smears in case of an abnormal smear or a smear of inadequate quality. An episode

ended when follow-up was complete according to the Dutch guidelines (i.e. three consecutive negative smears after a primary smear with high grade squamous cell intra-epithelial lesion (HSIL) smear, two consecutive negative smears after a primary smear with low grade squamous cell intra-epithelial lesion (LSIL), one consecutive adequate smear after a primary smear of inadequate quality within 6 months, or when no (adequate) follow-up smears were done within 4 years from the primary smear). Thus, by definition, post-diagnostic follow-up smears were attributed to the same episode as the diagnosed lesion.

Covariates

Questionnaire data on DES-related reproductive tract abnormalities (defined as adenosis, squamous cell metaplasia, transverse vaginal ridges, cockscomb, cervical collars, hoods, pseudo-polyps, hypoplastic cervix, uterine cavum malformations and tubal malformations) were verified by medical file. The term vaginal/cervical epithelial changes (VCEC) is used to refer to adenosis or squamous cell metaplasia of both the vagina and the ecto-cervix (enlarged ectropion). Other covariates included in this analysis (educational level, medical indication for maternal DES usage, age at first gynaecological DES examination, and number of smears and colposcopies during five years preceding the questionnaire) were based on self-report. Vital status was obtained by linkage with the Netherlands Office of Death Registry (CBG) and updated till October 2007.

Verification of DES exposure

Documented DES exposure was available for a minority of subjects. The chance of tracing the mothers' medical records was found to be increasingly difficult, also since DES has been prescribed on a large scale by general practitioners in the Netherlands. For a subgroup of participants (n=115) we verified self-reported DES exposure with medical records in four hospitals where all records had been kept[9]. For 76% of the women DES exposure was confirmed, in 3% a medicine different from DES was recorded and in 21% no DES was mentioned in the hospital medical file. For the latter group it was still possible that DES was prescribed by the general practitioner. Because the agreement between self-report and verified DES exposure was good, we included all women in the analyses, irrespective whether DES exposure was medically verified.

Statistical analyses

Follow-up started on January 1st 2000 or on the date of the 29th anniversary if a woman was younger than 29 years of age on January 1st 2000. We excluded women before age 29 because of the national general population screening program is restricted to women aged 30-60 years, and women are invited in the year they become 30 years. Follow-up ended on 30th November 2008, the date of first occurrence of intra-epithelial cervical neoplasia (CIN) or cervical cancer, death, date of uterus extirpation/cervix amputation, date of the 65th anniversary, whichever came first. After exclusion of ineligible women (n=287, see Figure 1), 11,895 women (100,287 person-years) were left for analyses.

Standardized Incidence Ratios (SIRs) were calculated by comparing the numbers of observed cases with CIN and invasive cervical cancer in our study with age-, sex- and calendar period–specific numbers from PALGA[11;12], which were originally derived from PALGA. The number of women at risk in the general population was obtained from Statistics Netherlands (www.cbs.nl) and adjusted for women without an uterus (from the Dutch Hospital Discharge database (LMR), based on 5-year age categories (30-64)). 95% confidence intervals (95%CI) were calculated assuming a Poisson distribution[13].

Separate analysis were done using screened women from the general population, estimated by applying 5-year coverage rates per five year age category from the year 2005[14] (see Supplement table 2). We also conducted analyses with and without exclusion of lesions detected during the first screening episode, in order to adjust for the occurrence of prevalent lesions, because it is generally known that first screens have higher detecting rates than following tests. For the latter analysis, calculations were restricted to DES-net participants who reported to have had a smear during the five years preceding the questionnaire. We also calculated SIRs for women stratified according to the number of episodes, the presence of DES-related malformations and attained age. Furthermore, we examined which type of examination (biopsy or cytology) and which outcome directly preceded the histological diagnosis of CIN.

We used the Kaplan-Meier method to compare the cumulative incidence of CIN lesions among subgroups of women. Furthermore, Cox regression analysis was performed to calculate hazard ratios (HR) in order to quantify the effect of different covariates on the risk of CIN within the exposed cohort, with adjustment for the other covariates. Both in the Kaplan-Meier and the Cox regression model, age was used as the time metric. All analyses were conducted using STATA release 11 SE.

Table 1 Characteristics of 11,895 DES daughters and the subgroup of cases diagnosed with cervical intra-epithelial neoplasia (all grades) and cancer during follow-up, 2000-2008

Characteristic	All women (n=11,895)	CIN (all grades) and cancer* (n=393)
	No. (%)	No. (%)
Year of birth		
≤1955	1,446 (12)	27 (7)
1956-1960	2,497 (21)	67 (17)
1961-1965	2,926 (25)	108 (27)
1966-1970	3,405 (29)	147 (37)
>1970	1,621 (14)	44 (11)
Age at end of follow up		
30-40 y	3.124 (28)	117 (30)
40-49 y	6,089 (51)	217 (55)
50+ y	2,682 (23)	59 (15)
Year of registration		
1992	11,010 (93)	358 (91)
1993-1999	601 (5)	13 (3)
2000-2005	284 (2)	22 (6)
Questionnaire completed		
Yes	7,479 (63)	264 (67)
No	4,416 (37)	129 (33)
Number of screening episodes during follow-up †		
0 episodes	2,074 (17)	0 (0)
1-2 episodes	4,780 (40)	129 (33)
3-5 episodes	3,612 (30)	189 (48)
>5 episodes	1,429 (12)	75 (18)
Number of cervical/vaginal smears		
Cervical smears		
0	2,091 (18)	3 (1)
1-2	4,401 (37)	28 (7)
3-5	3,496 (29)	112 (28)
>5	1,907 (16)	250 (64)
Vaginal smears		
0	8,867 (75)	218 (55)
1	1,148 (10)	43 (11)
2-5	1,511(13)	98 (25)
>5	369 (3)	34 (9)
Number of biopsies		
Cervical biopsies		
0	11,023 (93)	0 (0)
1	584 (5)	151 (38)
>1	288 (2)	242 (62)
Vaginal biopsies		
0	11,794 (99)	374 (95)
>=1	102 (1)	21 (5)
Treatment		
No treatment	11,025 (93)	192 (49)
Biopsy	517 (4)	8 (2)
Lis excision, conisation	264 (2)	179 (46)
Resection, extirpation	25 (0)	14 (4)

Table 1 (continued)

Women with questionnaire only	**All women** n=7,479	**CIN (all grades) and cancer** n=264
DES confirmation		
Medical file mother	871 (12)	36 (14)
DES-related abnormalities (VCEC,structural abnormalities)	1,630 (22)	87 (33)
History of frequent vaginal screening (before age 50) or frequent cervical screening before age 30)	1,954 (26)	83 (31)
No information available	3,024 (40)	58 (22)
Year of first gynaecological examination‡		
No DES-related gynaecological examination	1,396 (19)	36 (14)
<1992	4,639 (62)	179 (68)
1992-1999	891 (12)	33 (12)
>2000	23 (0)	2 (1)
Missing	530 (7)	14 (5)
Age at first gynaecological examination‡		
No DES-related gynaecological examination	1,396 (19)	36 (14)
<20	2,426 (32)	112 (42)
20-29	2,402 (32)	84 (32)
30+	725 (10)	18 (7)
Missing	530 (7)	14 (5)
Medical indication DES use mother		
Both threatened and habitual abortion	1,289 (17)	50 (19)
Threatened abortion only	1,879 (25)	58 (22)
Habitual abortion only	3,791 (51)	138 (52)
Other reason/unknown	520 (7)	18 (7)
Highest educational level		
Primary school	939 (13)	31 (12)
Secondary school	3,552 (47)	124 (47)
College or university	2,835 (38)	100 (38)
Unknown or missing	153 (2)	9 (3)
Number of Pap smears during 5 yrs preceding questionnaire		
No smears	431 (6)	4 (2)
1-2	2,646 (35)	67 (25)
>3	4,031 (54)	178 (67)
Unknown or missing	371 (5)	15 (6)
Number of colposcopies during 5 years preceding questionnaire	1,829 (24)	48 (18)
No colposcopies	2,236 (30)	91 (35)
1-2	1,169 (16)	76 (29)
>3	2,245 (30)	49 (18)
Unknown or missing		
Medically verified DES-related malformations§		
Not reported	5,322 (71)	155 (59)
Adenosis, squamous neoplasia (VCEC)	1,347 (18)	79 (30)
Structural anomalies	555 (7)	22 (8)
Not specified	205 (3)	7 (3)
Missing	50 (1)	1 (0)

*CIN: cervical intra-epithelial lesion † Screening episodes defined as primary smear and, if necessary, followed by secondary smears in case of an abnormal smear of a smear of inadequate quality ‡ gynaecological examination (any of these): palpation, colposcopy, cervical smears and/or vaginal smears § 813 women with both VCEC and structural abnormalities were categorized as VCEC. VCEC included cervical and vaginal adenosis and squamous cell metaplasia. Structural abnormalities included transverse vaginal ridges, cockscomb, cervical collars, hoods, pseudo-polyps, hypoplastic cervix, uterine cavum malformations and tubal malformations

Table 2 Standardized incidence ratios of cervical intraepithelial lesions and cancer among DES daughters, overall and by number of screening episodes, follow-up period 2000-2008

		CIN1*				CIN2*				CIN3*			
	PY†	Obs	Exp	SIR	95%CI	Obs	Exp	SIR	95%CI	Obs	Exp	SIR	95%CI
Overall	100,287	169	79	2.1	1.8-2.5	89	75.5	1.2	0.95-1.5	129	145.9	0.9	0.74-1.1
Screened women‡	82,787	169	85.5	2.0	1.7-2.3	89	82.6	1.1	0.87-1.3	129	160.9	0.8	0.67-0.95
1-2 episodes§	40,076	51	40.9	1.2	0.93-1.6	31	39.3	0.79	0.54-1.1	42	76.6	0.55	0.40-0.74
> 2 episodes	42,711	118	44.6	2.6	2.2-3.2	58	43.2	1.3	1.02-1.7	87	84.3	1	0.83-1.3
Screened women, first episode excluded ‖	50,991	118	41.6	2.8	2.3-3.4	49	37.4	1.3	0.97-1.73	75	65.8	1.1	0.90-1.4
1-2 episodes	20,420	25	16.4	1.5	0.99-2.3	16	14.6	1.1	0.63-1.8	23	25.5	0.9	0.57-1.4
> 2 episodes	30,570	93	25.2	3.7	3.0-4.5	33	22.8	1.5	0.99-2.0	52	40.3	1.3	0.96-1.7

		Cervical cancer*				≥CIN2*				≥CIN3*			
	PY†	Obs	Exp	SIR	95%CI	Obs	Exp	SIR	95%CI	Obs	Exp	SIR	95%CI
Overall	100,287	6	16.6	0.36	0.13-0.79	224	238	0.94	0.82-1.07	135	162.6	0.8	0.70-0.98
Screened women‡	82,787	6	17.7	0.34	0.13-0.74	224	261	0.86	0.75-0.98	135	178.6	0.76	0.63-0.90
1-2 episodes§	40,076	5	8.5	0.59	0.19-1.4	78	124	0.63	0.50-0.78	47	85.1	0.55	0.41-0.74
> 2 episodes	42,711	1	9.2	0.11	0.003-0.6	146	137	1.07	0.90-1.3	88	93.6	0.94	0.75-1.2
Screened women, first episode excluded ‖	50,991	3	7	0.43	0.09-1.3	127	110	1.2	0.96-1.4	78	72.8	1.1	0.85-1.3
1-2 episodes	20,420	2	2.8	0.72	0.09-2.6	41	42.8	0.96	0.69-1.3	25	28.3	0.9	0.57-1.3
> 2 episodes	30,570	1	4.2	0.24	0.006-1.3	86	67.3	1.3	1.0-1.6	53	44.5	1.2	0.89-1.6

* CIN: cervical intra-epithelial neoplasia. CIN1 (grade 1): Mild dyskaryosis CIN2 (grade 2): moderate dyskaryosis CIN3 (grade 3): severe dyskaryosis/ dysplasia or carcinoma in situ. CIN2+ and CIN3+ is including cancer. Cancer morphology: 2 squamous cell carcinoma, 1 clearcell adenocarcinoma, 2 adenocarcinoma 1 adenosquamous cell carcinoma

† PY=person years, Obs=observed, Exp=expected, SIR = Standardized incidence ratio, defined as observed number of cancers compared to the expected number of cancers in the general population with the same age; 95%CI = 95 percent confidence interval based on a Poisson distribution

‡ general population data adjusted for the coverage of screening (reference date: 31 december 2005)

§ Screening episodes defined as primary smear and, if necessary, followed by secondary smears in case of an abnormal smear of a smear of inadequate quality.

‖ general population data with exclusion of the first screening episode. Among study subjects was information about first episodes only available for women with questionnaire data.

3.4 Results

Characteristics of our study subjects are given in table 1. Among the 11,895 women in our analytic cohort, 393 women (3%) had a histologically confirmed CIN lesion or cervical cancer detected during follow-up. Fifteen women had two lesions (same or different grade) at different time points during follow-up (results not shown). The number of screening episodes was remarkably high among cases; 66% had more than two episodes during follow-up compared to 42% in the total DES-exposed cohort. In addition to this finding and also highly correlated to the number of episodes, women with CIN or cancer had undergone a higher number of smears and biopsies compared to the total cohort (more than 5 cervical smears: 64% and 16%, respectively). Seventy-four percent of the women with CIN or cancer reported to have had their first gynaecological examination before age 30 compared to 64% of the total cohort. Nearly 90% of all women, both women with CIN and other cohort members, had had a screening examination in the five years preceding the questionnaire, with the number of colposcopies being considerably higher among cases (64% versus 46% in the total group). Documented DES exposure was available for 871 women among whom were 36 cases. Cases more often had DES-related malformations compared to the entire cohort, of which VCEC occurred most frequently (32% and 20% for cases and entire cohort, respectively).

Table 2 describes the risk of CIN and cervical cancer among DES daughters compared to different reference data (female population or screened female population and in- and exclusion of lesions detected during the first episode, respectively). The screened reference population with prevalent lesions excluded was considered as the most valid comparison, since this reference population resembles the DES-net cohort most. For the remaining text, we will use the term ‘screened general population’ for the screened reference population with exclusion of the prevalent lesions. A borderline statistically significantly increased risk for CIN including cancer (CIN2+) among DES daughters was found compared to the screened general population (SIR=1.2, 95%CI=0.96-1.4). For CIN1 lesions a significantly increased risk was observed (SIR=2.8, 95%CI=2.3-3.4). On the other hand, the risk of cervical cancer seemed to be slightly decreased albeit not statistically significantly (SIR=0.43, 95%CI=0.09-1.3). Women with more than two screening episodes showed borderline statistically significantly increased risks for CIN1 and CIN2+ (SIR=3.7, 95%CI=3.0-4.5 and SIR=1.3, 95%CI=1.0-1.6, respectively). Within the subgroup of DES daughters with one to two episodes in 2000-2008 (suggestive of

Table 3 Standardized incidence ratios of cervical intraepithelial lesions and cervical cancer for DES daughters with questionnaire data, by DES-related malformations and number of screening episodes, follow-up period 2000-2008.

Screened women			**CIN1***				**CIN2***				**CIN3***			
First episode excl.	**PY†**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**
Overall	50,990	118	42	2.8	2.3-3.4	49	37	1.3	0.97-1.7	75	65.8	1.1	0.90-1.4
DES-related malformations‡													
No malformations	35,343	65	28.5	2.3	1.8-2.9	33	25.5	1.3	0.89-1.8	40	44.9	0.9	0.64-1.2
1-2 episodes	16,193	9	12.9	0.7	0.32-1.3	13	11.4	1.1	0.61-1.9	18	20	0.9	0.53-1.4
>2 episodes	19,149	56	51.6	3.6	2.7-4.6	20	14.1	1.4	0.87-2.2	22	24.9	0.88	0.55-1.3
Malformations	15,647	53	13.0	4.1	3.0-5.3	16	11.8	1.4	0.77-2.2	35	20.9	1.7	1.2-2.3
1-2 episodes	4,227	16	3.5	4.6	2.6-7.5	3	3.1	0.96	0.20-2.8	5	5.5	0.91	0.30-2.1
>2 episodes	11,421	37	9.6	3.9	2.7-5.3	13	8.7	1.5	0.80-2.6	30	15.4	1.9	1.3-2.8
VCEC	9,993	38	8.3	4.6	3.2-6.3	10	7.6	1.3	0.63-2.4	27	13.4	2.0	1.3-2.9
1-2 episodes	2,598	9	2.1	4.2	1.9-8.0	2	1.9	1	0.13-3.8	5	3.4	1.5	0.48-3.5
>2 episodes	7,395	29	6.2	4.7	3.1-6.7	8	5.7	1.4	0.61-2.8	22	10.1	2.2	1.4-3.3
Anatomical and unspecified malformations	5,655	15	4.7	3.2	1.8-5.3	6	4.2	1.4	0.52-3.1	8	7.5	1.1	0.46-2.1
1-2 episodes	1,629	7	1.3	5.2	2.1-10.8	1	1.2	0.8	0.02-4.6	0	2.1		
>2 episodes	4,026	8	3.4	2.4	1.0-4.7	5	3	1.6	0.5-3.8	8	5.4	1.5	0.6-2.9
Screened women			**Cervical cancer***				**CIN2+***				**CIN3+***			
First episode excl.	**PY†**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**
Overall	50,990	3	7	0.4	0.09-1.3	127	110	1.2	0.96-1.4	78	72.8	1.1	0.85-1.3
DES-related malformations‡													
No malformations	35,343	2	4.8	0.4	0.05-1.5	75	75.2	1	0.78-1.2	42	49.7	0.8	0.61-1.1
1-2 episodes	16,193	2	2.2	0.9	0.11-3.3	33	33.6	0.98	0.68-1.38	20	22.2	0.9	0.55-1.4
>2 episodes	19,149	0	2.6			42	41.6	1	0.73-1.4	22	27.8	0.8	0.50-1.2
Malformations	15,647	1	2.2	0.5	0.01-2.6	52	34.9	1.5	1.1-2.0	36	23.1	1.6	1.1-2.2
1-2 episodes	4,227	0	0.6			8	9.2	0.87	0.38-1.7	5	6.1	0.8	0.27-1.9
>2 episodes	11,421	1	1.6	0.63	0.02-3.5	44	25.7	1.7	1.2-2.3	31	17	1.8	1.2-2.6
VCEC	9,993	1	1.4	0.72	0.02-4.0	38	22.4	1.7	1.2-2.3	28	14.8	1.9	1.3-2.7
1-2 episodes	2,598	0	0.4			7	5.6	1.2	0.50-2.6	5	3.7	1.3	0.44-3.1
>2 episodes	7,395	1	1	0.97	0.03-5.4	31	16.8	1.8	1.3-2.6	23	11.1	2.1	1.3-3.1
Anatomical and unspecified malformations	5,655	0	0.8			14	12.5	1.1	0.61-1.9	8	8.3	0.97	0.42-1.9
1-2 episodes	1,629	0	0.2			1	3.6	0.28	0.01-1.6	0	2.3		
>2 episodes	4,026	0	0.6			13	9	1.5	0.77-2.5	8	5.9	1.4	0.58-2.7

* CIN: cervical intra-epithelial neoplasia. CIN1 (grade 1): Mild dyskaryosis CIN2 (grade 2): moderate dyskaryosis CIN3 (grade 3): severe dyskaryosis/dysplasia or carcinoma in situ. CIN2+ and CIN3+ is including cancer. Cancer morphology: 2 squamous cell carcinoma, 1 clearcell adenocarcinoma, 2 adenocarcinoma 1 adenosquamous cell carcinoma † PY=person years, Obs=observed, Exp=expected, SIR = Standardized incidence ratio, defined as observed number of cancers compared to the expected number of cancers in the general population with the same age; 95%CI = 95 percent confidence interval based on a Poisson distribution.‡ Definition (medically verified) DES-related malformations: Anatomical malformations: transverse vaginal ridges, cockscomb, cervical collars, hoods, pseudo-polyps, hypoplastic cervix, uterine cavum malformations and tubal malformations. VCEC: cervical and vaginal adenosis and squamous cell metaplasia. Malformations were self-reported in the questionnaire (at start of the follow-up) and validated by medical file.

following the general population cervical screening program) the risk increase of CIN1 was less pronounced (SIR=1.5, 95%CI=0.99-2.3) and the risk for CIN2+ (SIR=0.96, 95%CI=0.69-1.3) was not increased. Among women with DES-related malformations the risks of CIN1 and CIN2+ were both elevated compared to the screened general population, with the risk increases being more pronounced in the VCEC-group (SIR= 4.6, 95%CI=3.2-6.3 and SIR=1.7, 95%CI=1.2-2.3 for CIN1 and CIN2+, respectively) (Table 3). Increased risks were restricted to the group with more than two episodes.

The SIRs did not vary according to attained age, but numbers were small (Table 4). The risk of CIN1 seemed to be slightly higher among women younger than 40 than at older ages compared to the general population of similar age. To study whether the high number of histologically verified CIN1 lesions might be the result of more invasive diagnostic methods we examined which type of examination (biopsy or cytology) and which outcome preceded the histological diagnosis (Table 5). Half of the CIN1 lesions (53%) were preceded by a LSIL smear or a smear with atypical squamous cells of undetermined significance (ASCUS) which is quite comparable to general population data (appendix Table 1). A remarkable finding is the high proportion of biopsies without a preceding smear in CIN1 cases (36%), which might be indicative of biopsies directed by abnormal colposcopic findings or because of complaints (no reference data available).

Comparisons within the study group by the Kaplan-Meier method and multivariate Cox regression analysis revealed that the number of episodes was an important predictor for the chance of having a CIN1 lesion diagnosed (HR_{adj}=2.0, 95%CI=1.2-3.1) (Table 6), and illustrated by Figure 2a. Women with VCEC and non specified malformations showed a slightly not statistically significantly increased risk of CIN1 compared to women without DES-related malformations (HR_{adj}=1.38, 95%CI=0.90-2.12 and 1.24, 95%CI=0.50-3.13, respectively), see also Figure 2b. With respect to the risk of CIN2+, being intensively screened (>2 episodes in 2000-2008), was only weakly predictive of the chance of having a CIN2+ lesion diagnosed (HR_{adj}=1.22, 95%CI=0.82-1.81), also shown in Figure 2c. Borderline significantly increased HRs for CIN2+ were found for women with VCEC and structural abnormalities compared to women without DES-related malformations (HR_{adj}=1.47, 95%CI=0.96-2.27 and HR_{adj}=1.52, 95%CI=0.91-2.53, respectively), see Figure 2d. Furthermore, having had a DES examination preceding the questionnaire was associated with increased risk of CIN1 (HR_{adj}=1.71, 95%CI=0.71-4.11) and decreased the risk of CIN2+ (HR_{adj}=0.44, 95%CI=0.23-0.80).

Table 4 Risk of cervical dysplasia and cancer among screened DES daughters by attained age (exclusion of lesions diagnosed at first screening episode)

	Attained age <40§				Attained age 40+§				
All PY*	**24,345**				**26,645**				
Type of lesion†	**Obs***	**Exp**	**SIR**	**95%CI**	**Obs**	**Exp**	**SIR**	**95%CI**	**p difference‡**
CIN1	65	20.4	3.2	2.4-4.1	53	21.2	2.5	1.9-3.3	0.19
CIN2	27	20.1	1.3	0.88-2.0	22	17.2	1.3	0.80-1.9	0.86
CIN3	48	38	1.3	0.93-1.7	27	27.8	0.97	0.64-1.4	0.27
Cervical cancer	1	3	0.33	0.01-1.8	2	4	0.5	0.06-1.8	0.73
CIN2+	76	61.1	1.2	0.98-1.6	51	49	1	0.77-1.4	0.32
CIN3+	49	41	1.2	0.88-1.6	29	31.8	0.91	0.61-1.3	0.25

* PY=person years, Obs=observed, Exp=expected, SIR = Standardized incidence ratio, defined as observed number of cancers compared to the expected number of cancers in the general population

† CIN: cervical intra-epithelial neoplasia. CIN1 (grade 1): Mild dyskaryosis CIN2 (grade 2): moderate dyskaryosis CIN3 (grade 3): severe dyskaryosis/dysplasia or carcinoma in situ. CIN2+ and CIN3+ is including cancer. Cancer morphology: 2 squamous cell carcinoma, 1 clearcell adenocarcinoma, 2 adenocarcinoma 1 adenosquamous cell carcinoma

‡ p difference based on Poisson distribution

§ attained age= age at diagnosis of (first, maximum) CIN or age at end follow-up (30th November 2008)

Table 5 Preceding primary test results in relation to histologically confirmed lesions for 393 DES daughters with CIN lesions and (invasive) cervical cancer

	Histologically confirmed lesion*				
Result of primary test	**Cancer**	**CIN3**	**CIN2**	**CIN1**	**Total**
Smears†					
Cancer	0 (0%)	6 (5%)	0 (0%)	0 (0%)	6 (2%)
Carcinoma in situ	1 (17%)	11 (9%)	0 (0%)	2 (1%)	14 (4%)
Severe dyskaryosis (HSIL)	2 (33%)	34 (26%)	9 (10%)	4 (2%)	49 (12%)
Moderate dyskaryosis (HSIL)	0 (0%)	35 (27%)	23 (26%)	13 (8%)	71 (18%)
Mild dyskaryosis (LSIL)	0 (0%)	22 (17%)	27 (30%)	45 (27%)	94 (24%)
Borderline dyskaryosis/ASCUS	0 (0%)	16 (12%)	18 (20%)	44 (26%)	78 (20%)
Biopsies					
Cancer	3 (50%)	0 (0%)	0 (0%)	0 (0%)	3 (1%)
CIN3	0 (0%)	3 (2%)	0 (0%)	0 (0%)	3 (1%)
CIN2	0 (0%)	2 (2%)	11 (12%)	0 (0%)	13 (3%)
CIN1	0 (0%)	0 (0%)	1 (1%)	61 (36%)	62 (16%)
Total	6	129	89	169	393

* CIN: cervical intra-epithelial neoplasia. CIN1 (grade 1): Mild dyskaryosis CIN2 (grade 2): moderate dyskaryosis CIN3 (grade 3): severe dyskaryosis/dysplasia or carcinoma in situ. CIN2+ and CIN3+ is including cancer. Cancer morphology: 2 squamous cell carcinoma, 1 clearcell adenocarcinoma, 2 adenocarcinoma 1 adenosquamous cell carcinoma

† HSIL= High grade squamous intraepithelial lesion, LSIL= Lowgrade squameuze intra-epitheliale lesion, ASCUS= atypical squamous cells of undetermined significance

3.5 Discussion

In this study we comprehensively evaluated the risk of CIN and cervical cancer among DES daughters with almost complete follow-up and based on medically verified data. The overall risk of CIN2+ (CIN2/3 or invasive cancer) in DES daughters compared to the screened general population was not increased, whereas for low-grade CIN lesions (CIN1) a statistically significantly increased risk was observed. In women with DES-related malformations (both VCEC and other abnormalities, for whom DES exposure is quite certain) increased risks were observed of both CIN1 and CIN2+, with the increased risks being most pronounced in women with VCEC. However, when we restricted our analyses to DES daughters who had a screening frequency similar to the general population (1 to 2 screening episodes during 8.9 years), the risk of CIN2+ was no longer increased compared to the general screened population. We found a decreased risk of invasive cervical cancer, as observed in an earlier study[9], but based on 6 cases only. Among women who had been intensively screened (more than two screening episodes during 9 years), increased risks were found for all grades of CIN, except for invasive cancer.

To our knowledge, the risk of histologically confirmed CIN1 among DES daughters has never been studied before. In 1984, Robboy *et al* did examine the risk of *mild* dysplasia among DES daughters and unexposed women in the DESAD study, however the researchers also included mild lesions that were verified by cytology only[6]. When interpreting our results regarding CIN1, some issues need to be considered. First, the detection of (histologically confirmed) CIN1 depends on the preceding cytological outcome and subsequent referral of a woman for colposcopy. In absence of any biopsy no CIN will be detected. Consequently, the chance of detecting a CIN1 is highly influenced by differences in referral (diagnostic) criteria and may not be caused by a variation in background risk[15]. A screening program aiming at high specificity will have less referrals for colposcopy, whereas focus on high sensitivity will lead to many referrals for colposcopy to prevent missed cases[15]. With respect to DES daughters, it seems quite likely that physicians aim at detection of all lesions (high sensitivity). Indeed, we found that many CIN1 lesions in DES daughters were not preceded by cytological smear, but were detected by biopsy only (36% of all CIN1 lesions, table 5). It seems quite likely that these CIN1 lesions were detected because of (VCEC-related) complaints or because of regular

Table 6 Univariate and multivariate Hazard Ratios (HR) for different risk factors for CIN and cervical cancer

	CIN1*				CIN2 plus*			
Screened women, exclusion of the first episode	**Univariate**		**Multivariate‖**		**Univariate**		**Multivariate‖**	
	HR	**95%CI**	**HR**	**95%CI**	**HR**	**95%CI**	**HR**	**95%CI**
Episodes†								
1-2 episodes	1.00	reference	1.00	reference	1.00	reference	1.00	reference
>2 episodes	2.43	1.56-3.79	1.99	1.24-3.11	1.31	0.90-1.9	1.22	0.82-1.81
DES-related malformations‡								
No malformations	1.00	reference	1.00	reference	1.00	reference	1.00	reference
VEC	1.90	1.26-2.84	1.38	0.90-2.12	1.68	1.13-2.49	1.47	0.96-2.27
Structural abnormalities	1.22	0.69-2.16	1.05	0.59-1.86	1.49	0.90-2.46	1.52	0.91-2.53
Not specified	1.66	0.66-4.13	1.24	0.50-3.13	0.58	0.14-2.38	0.56	0.14-2.32
Education§								
Primary school	1.00	reference	1.00	reference	1.00	reference	1.00	reference
Secondary school	0.84	0.48-1.47	0.81	0.46-1.42	1.18	0.64-2.2	1.19	0.64-2.21
College or university	0.79	0.44-1.41	0.75	0.42-1.34	1.26	0.67-2.37	1.24	0.66-2.33
Unknown or missing	1.97	0.66-5.90	1.94	0.65-5.83	1.24	0.28-5.54	1.18	0.26-5.29
Age first gynaecological DES examination§								
No examination	1.00	reference	1.00	reference	1.00	reference	1.00	reference
<20	2.83	1.22-6.54	1.71	0.71-4.11	0.76	0.45-1.31	0.44	0.23-0.80
20-29	2.64	1.16-5.97	1.63	0.69-3.83	0.60	0.34-1.03	0.34	0.19-0.63
30+	2.02	0.78-5.40	1.29	0.47-3.51	0.45	0.18-1.13	0.26	0.10-0.69
Missing	1.39	0.44-4.42	0.98	0.30-3.18	0.56	0.24-1.33	0.36	0.15-0.88
Number of colposcopies (5 year preceding questionnaire)§								
No colposcopies	1.00	reference	1.00	reference	1.00	reference	1.00	reference
1-2	1.16	0.69-1.96	0.95	0.55-1.61	1.36	0.82-2.25	1.53	0.90-2.60
>3	2.09	1.23-3.54	1.46	0.84-2.54	1.92	1.13-3.27	2.13	1.19-3.81
Unknown or missing	0.65	0.35-1.20	0.64	0.35-1.19	0.71	0.39-1.30	0.74	0.40-1.36

* CIN: cervical intra-epithelial neoplasia. CIN1 (grade 1), CIN2+ : including CIN2, CIN3 and cervical cancer

† Screening episodes defined as primary smear and, if necessary, followed by secondary smears in case of an abnormal smear of a smear of inadequate quality

‡ Definition DES-related malformations: Anatomical malformations: transverse vaginal ridges, cockscomb, cervical collars, hoods, pseudo-polyps, hypoplastic cervix, uterine cavum malformations and tubal malformations. VCEC: cervical and vaginal adenosis and squamous cell metaplasia. Malformations were self-reported in the questionnaire (at start of the follow-up) and validated by medical file

§ Based on self-report

‖ Multivariate analysis: adjusted for all other risk factors

colposcopic examinations as part of the DES screening protocol (during the first years of surveillance, and thereafter only on indication, see Supplement, table 3) or potential misinterpretation of colposcopy images. Thus, the higher chance of biopsy after abnormal colposcopic findings might explain our result of an increased risk of histologically confirmed CIN1 among DES daughters. Second, a high frequency of screening will lead to overdiagnosis of CIN lesions that have a high chance of regression[16-18]. This is also manifested by the increased detection of CIN1 lesions among women with more than two screening episodes during follow-up. Third, the detection of CIN1 lesions in DES daughters might suffer from histological misclassification due to the common presence of VCEC and a wider transformation zone[19]. However, we found that the risk of CIN1 (compared to the screened general population) was also increased among women without DES-related malformations. In view of the arguments above, it seems rather unlikely that the observed risk increase for CIN1 reflects a true increase, unless the biopsy is considered as a treatment. Thus, the increased risk of CIN1 among DES daughters seems mainly attributable to intensive screening, not only cytological screening but also screening by regular colposcopic examinations as part of the DES screening protocol[20]. Nevertheless, our CIN1 findings are indicative of high compliance to the DES screening protocol.

Another important finding of our study is that we found no increased risk for CIN2+ in the entire cohort. In the subgroup of women with DES-related malformations, no increase was found in women who were screened 1-2 times (HR=0.87), while women with more than two episodes showed an increased risk (HR=1.7). As the general population is screened 1-2 times, the estimated HR of 1.7 is still biased by screening. Therefore, if women with DES-related malformations have an increased risk of CIN2+ it is likely to be small. The risk of CIN2+ in DES daughters has only been investigated in a single other cohort, the NCI DES follow-up study. Three papers on the risk of squamous cell neoplasia of the cervix in this cohort have been published, in 1984, 2001 and recently in 2011[5-7]. The latest paper extended the follow-up to approximately 26 years after study entry (1975) and included 208 DES-exposed women with high grade CIN. Different from our findings, the authors found a HR of 2.28 (95%CI=1.59-3.27) for high grade CIN lesions among 100% verified DES-exposed women, compared to non-exposed controls[7]. Quite similar to our study, an increased risk was shown for women with VCEC (HRs of 2.40 (95%CI=1.60-3.61) and 1.7 (95%CI=1.2-2.3) in the NCI-cohort study and our study, respectively). The ages at the end of follow-up were similar (48 and 44.2 years of age for

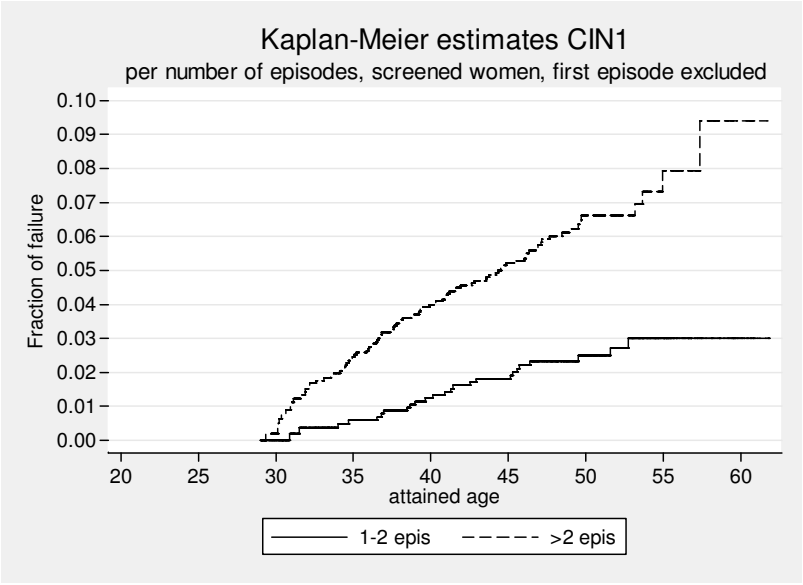

Figure 2a Kaplan Meier estimates for CIN1 lesions by number of episodes

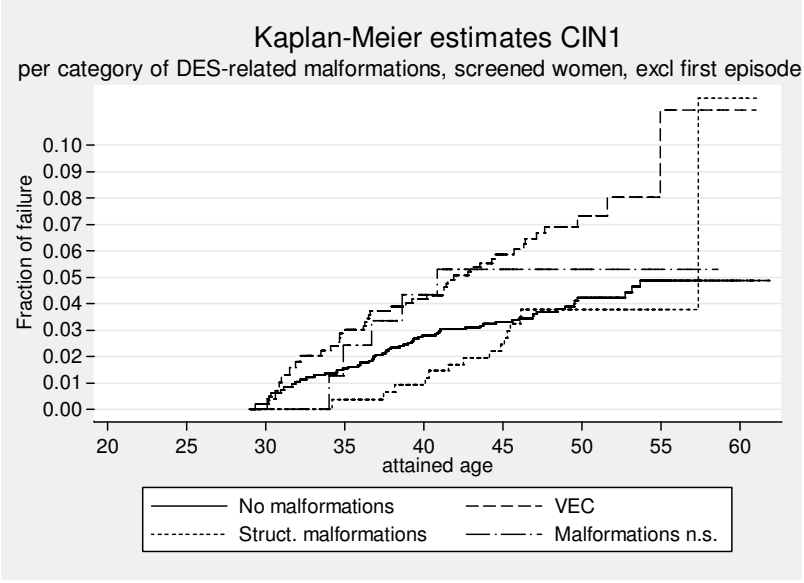

Figure 2b Kaplan Meier estimates for CIN1 lesions by DES-related malformation

the NCI cohort and our cohort, respectively), but age at start differed between the NCI-cohort (average age at entry approximately 23 years) and our cohort (minimum 29, average 35.9 ± 5.9 years of age). We had to exclude women younger than 29 from our analysis, because our national screening program (reference) starts at the year a woman turns 30 years. Thus, the higher HR of high grade lesions in the NCI DES study as compared with our estimate may result from a higher HR at younger ages (< 30 years). In addition, CIN treatment before age 30 may have somewhat lowered the subsequent risk of CIN lesions and cervical cancer in our study. As a consequence, the cumulative incidence cannot be compared between the two studies, and our estimate may be biased towards null. Another important difference between both studies is the reference group, which also might explain the different results. The NCI DES cohort used an internal non-exposed group as reference population whereas we used screened general population data. The use of general population data in our study enables adequate control for screening in the 1-2 episode group, but also has several drawbacks. First, the general population was inclusive DES daughters. However, since the prevalence of DES is estimated to be 3% at a maximum, this may have hardly affected our results. Second, a comparison with the general population suffers from the impossibility to adjust for confounding at an individual level. Therefore, our results might be subject to residual confounding by known risk factors for cervical cancer like infection with human papillomavirus (HPV), age at first intercourse, number of partners and smoking[18;21]. On the other hand, since data on health outcomes in the NCI-DES cohort were based on self-report, selective reporting of CIN might have occurred. While overreporting may be solved by verification, underreporting by the unexposed group may stay present, potentially biasing the results away from zero. The authors indicate that the observed increased risks might be due to the incomplete adjustment for screening[5]. Although we had detailed information on cervical screening, some residual bias by screening may still be present. First, as the general population was screened with 1-2 episodes, some residual screening bias remains in the group of DES daughters that had more than two episodes. Second, DES screening examinations may include not only cytology but also colposcopy in case of VCEC. We only had information about the number of colposcopies during the 5 years preceding the questionnaire, not during the follow-up period. Having undergone a colposcopy was a strong predictor for the detection of CIN2+ during the follow-up period (HR= 2.13, 95%CI=1.19-3.81), suggestive of continued participation in the DES screening program.

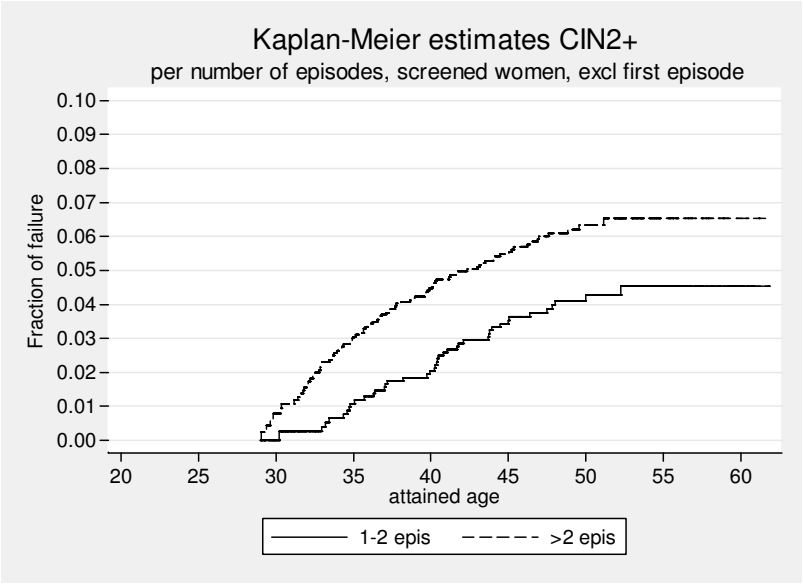

Figure 2c Kaplan Meier estimates for CIN2+ lesions by number of episodes

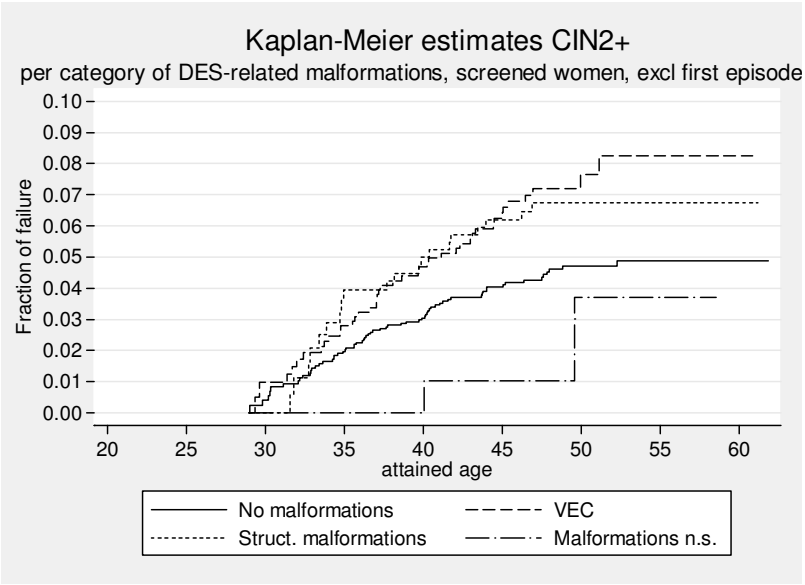

Figure 2d Kaplan Meier estimates for CIN2+ lesions by DES-related malformation
(Age=time metric in the model)

A limitation of our cohort is the relatively small number of women with verified DES exposure. However, according to our validation study[9] we estimate that at most 24% of our cohort members might be misclassified as DES-exposed. When we restricted our analysis to women with DES-related malformations, which are largely attributable to DES exposure (attributable risk of 90%[3;22;23]), such misclassification of exposure is expected to be minimal or absent. Women with medically confirmed DES-related malformations comprised 28% of the current analytic cohort, compared to approximately 45% (1864/3795) in the NCI-follow-up study. The true percentage of DES-related malformations in our cohort is even higher than 28% since we excluded self-reported malformations for which no medical files were available (8%). Clearly, we cannot rule out that our overall risks might be somewhat diluted by inclusion of non-exposed women.

Our study has several strengths. First, our data on outcome and screening were based on medical registry data that are much more complete and accurate than self-reported data. All outcomes were 100% medically verified and the number of cases (n=393) was high. Also, the loss to follow-up in our cohort was minimal. Additionally, we had complete information about cytological screening during the entire follow-up period and were therefore able to evaluate the impact of this important variable.

Biological mechanism

It is well established that HPV infection is the necessary cause of cervical cancer[24]. However, HPV infection alone may not be sufficient and other cofactors, like high parity, smoking and long-term oral contraceptive use, likely play a role in the transition from HPV infection to HSIL/invasive cancer[17]. It is uncertain whether HPV infection plays a role in DES-related tumors. With respect to cervical and vaginal clear cell adenocarcinoma no clear association with HPV infection has been found, but this was based on small studies[25]. It has been suggested[5;19] that the cervical tissue in women with VCEC might be more susceptible to carcinogenic factors like an HPV-infection, which might explain our increased risks of CIN for women with VCEC. Similarly, the susceptibility of women with an enlarged transformation zone might be increased. Another explanation might be that DES daughters have an altered immune system, which renders them more vulnerable to persistent HPV-infection[26;27].

In summary, in this study increased risk of CIN1 was observed, with the risk increase being most pronounced in women with DES-related malformations and largely due to the

intensive cytological and colposcopic screening in this group. If screening could adequately be taken into account, the risk of CIN2+ (including cancer) was not increased, suggesting that DES daughters do not have an increased risk of squamous cervical cancer compared to the general population. Among the more frequently screened DES daughters with VCEC or other DES-related malformations the effects of bias by screening and a potential small truly increased risk of CIN2+ could not be disentangled. These findings underscore the importance of being cautious with respect to invasive diagnostic procedures or treatment since overdiagnosis seems to occur in a group of women which is already strongly medicalized.

References

(1) Giusti RM, Iwamoto K, Hatch EE. Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med* 1995;122:778-788.

(2) SMITH OW. Diethylstilbestrol in the prevention and treatment of complications of pregnancy. *Am J Obstet Gynecol* 1948;56:821-834.

(3) Robboy SJ, Young RH, Welch WR et al. Atypical vaginal adenosis and cervical ectropion. Association with clear cell adenocarcinoma in diethylstilbestrol-exposed offspring. *Cancer* 1984;54:869-875.

(4) Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878-881.

(5) Hatch EE, Herbst AL, Hoover RN et al. Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States). *Cancer Causes Control* 2001;12:837-845.

(6) Robboy SJ, Noller KL, O'Brien P et al. Increased incidence of cervical and vaginal dysplasia in 3,980 diethylstilbestrol-exposed young women. experience of the national collaborative diethylstilbestrol adenosis project. *JAMA* 1984;252:2979-2983.

(7) Hoover RN, Hyer M, Pfeiffer RM et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. *N Engl J Med* 2011;365:1304-1314.

(8) Troisi R, Hatch EE, Titus-Ernstoff L et al. Cancer risk in women prenatally exposed to diethylstilbestrol. *Int J Cancer* 2007;121:356-360.

(9) Verloop J, van Leeuwen FE, Helmerhorst TJ, van Boven HH, Rookus MA. Cancer risk in DES daughters. *Cancer Causes Control* 2010;21:999-1007.

(10) Casparie M, Tiebosch AT, Burger G et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007;29:19-24.

(11) Rebolj M, van BM, Lynge E et al. Incidence of cervical cancer after several negative smear results by age 50: prospective observational study. *BMJ* 2009;338:b1354.

(12) Rebolj M, van BM, Berkers LM, Habbema D. Monitoring a national cancer prevention program: successful changes in cervical cancer screening in the Netherlands. *Int J Cancer* 2007;120:806-812.

(13) Breslow NE, Day NE. *Statistical methods in cancer research. Volume II - The design and analysis of cohort studies.* Oxford: Oxford University Press, 1987.

(14) Rebolj M. *Recent development in the Dutch Cervical Cancer Screening Programme* [Department of Public Health, Erasmus MC, Rotterdam, the Netherlands; 2008.

(15) Kotaniemi-Talonen L, Nieminen P, Hakama M et al. Significant variation in performance does not reflect the effectiveness of the cervical cancer screening programme in Finland. *Eur J Cancer* 2007;43:169-174.

(16) Moscicki AB, Shiboski S, Hills NK et al. Regression of low-grade squamous intra-epithelial lesions in young women. *Lancet* 2004;364:1678-1683.

(17) Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. *Gynecol Oncol* 2008;110:S4-S7.

(18) Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007;370:890-907.

(19) Bornstein J, Adam E, Adler-Storthz K, Kaufman RH. Development of cervical and vaginal squamous cell neoplasia as a late consequence of in utero exposure to diethylstilbestrol. *Obstet Gynecol Surv* 1988;43:15-21.

(20) Helmerhorst TJ, Wijnen JA, Direcks AP. [current guidelines for gynecological examination in des daughters]. [dutch]. *Ned Tijdschr Geneeskd* 1992;136:2065-2067.

(21) Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. *Int J Cancer* 2007;120:885-891.

(22) Antonioli DA, Burke L. Vaginal adenosis. Analysis of 325 biopsy specimens from 100 patients. *Am J Clin Pathol* 1975;64:625-638.

(23) Jefferies JA, Robboy SJ, O'Brien PC et al. Structural anomalies of the cervix and vagina in women enrolled in the Diethylstilbestrol Adenosis (DESAD) Project. *Am J Obstet Gynecol* 1984;148:59-66.

(24) Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. *J Natl Cancer Inst* 2011;103:368-383.

(25) Kocken M, Baalbergen A, Snijders PJ et al. High-risk human papillomavirus seems not involved in DES-related and of limited importance in nonDES related clear-cell carcinoma of the cervix. *Gynecol Oncol* 2011;122:297-302.

(26) Baird DD, Wilcox AJ, Herbst AL. Self-reported allergy, infection, and autoimmune diseases among men and women exposed in utero to diethylstilbestrol. *J Clin Epidemiol* 1996;49:263-266.

(27) Burke L, Segall-Blank M, Lorenzo C, Dynesius-Trentham R, Trentham D, Mortola JF. Altered immune response in adult women exposed to diethylstilbestrol in utero. *Am J Obstet Gynecol* 2001;185:78-81.

Supplement table 1 Definition of outcome in the DES-net project

CIN classification	Description	SNOMED
Invasive cancer	Invasive cancer	M83103 (clear-cell adenocarcinoma), M80703 (squamous cell carcinoma), M81403 (adenocarcinoma) M85603 (adeno/squamous cell carcinoma), M80103 (carcinoma unspecified)
CIN-3	Cervical intra-epithelial neoplasia, grade 3	M80102 in situ, M81402 adeno, M80502 papillary) M80702 (squamous cell)
CIN-3	Cervical intra-epithelial neoplasia, grade 3	M74##8, M74008, M69162 M69730
CIN-2	Cervical intra-epithelial neoplasia, grade 2	M74##7, M74007, M69720
CIN-1	Cervical intra-epithelial neoplasia, grade 1	M74##6,M74006, M69710 ,M69161,M74000, M69160, M69700

Supplement table 2 Percentage of women that had at least one cervical Pap smear 5 year preceding the reference date (5-year coverage rate), compared to the general population, in the period 2004-2008

	Reference date 31-12 2004				Reference date 31-12 2005				Reference date 31-12 2006				Reference date 31-12 2007			Reference date 31-12 2008		
age cate-gory	DES-net			General population	DES-net			General population	DES-net			General population	DES-net			DES-net		
	resp	non resp	all		resp	non resp	All		resp	non resp	all		resp	non resp	all	resp	non resp	all
<30	*10%*	*1%*	5%		0%	7%	6%		0%	17%	11%		0%	0%	0%			0%
30-34	*80%*	*62%*	72%	69%	79%	64%	73%	69%	80%	64%	72%	70%	77%	68%	72%	77%	70%	73%
35-39	*86%*	*69%*	79%	77%	84%	69%	79%	78%	84%	69%	78%	78%	84%	68%	78%	84%	66%	77%
40-44	*84%*	*69%*	79%	81%	83%	67%	77%	81%	84%	66%	77%	81%	84%	67%	78%	83%	68%	77%
45-49	*82%*	*63%*	76%	81%	83%	66%	77%	81%	83%	67%	77%	82%	82%	68%	77%	81%	66%	76%
50-54	*74%*	*60%*	70%	81%	73%	60%	69%	81%	75%	59%	70%	82%	76%	59%	70%	76%	66%	71%
55-59	*65%*	*48%*	58%	80%	67%	46%	58%	79%	68%	49%	61%	79%	68%	52%	63%	68%	59%	63%
60-64				75%				76%				76%	48%	50%	49%	61%	40%	52%
30-64	*82%*	*66%*	76%	78%	81%	66%	76%	78%	82%	66%	76%	78%	81%	66%	76%	80%	65%	75%

Population at risk: Adjustment for date of hysterectomy and death

Source: Rebolj M. Recent development in the Dutch Cervical Cancer Screening Programme. Thesis. Department of Public Health, Erasmus MC, Rotterdam, the Netherlands; 2008.

Supplement table 3 Guideline for surveillance of DES daughters, last revision in 1992

DES exposure	DES-related malformations*	Cytological examination†	Colposcopy	Gynecological examination‡
Certain	Present	Yearly	Yearly during first 5 year, thereafter only by indication	Yearly
Certain	Absent	Yearly during first 5 year followed by general population screening§	-	Yearly during first 5 year
Uncertain	Present	Yearly	Yearly during first 5 year, thereafter only by indication	Yearly
Uncertain	Absent	General population screening[3]	-	-

* DES-related malformations were defined as altered vaginal fornix, collar, transverse or longitudinal vaginal septum, cockscomb, hypoplastic cervix, pseudopolyp, collar, vaginal ridges, adenosis, extensive transformation zone

† Cytological examination: vaginal and cervical pap smears

‡ Gynecological examination: palpation of the vagina, uterus and adnexa

§ General population screening: 1992-1996: one cervical pap smear per three years (age group 35-53). From 1996 onwards: one cervical pap smear per five years (age group 30-60 years)