Long-term health effects after DES exposure in utero

Verloop, J.

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Chapter 4

Effectiveness of screening in DES daughters

J. Verloop, F.E. van Leeuwen, Th.J.M. Helmerhorst and M.A. Rookus

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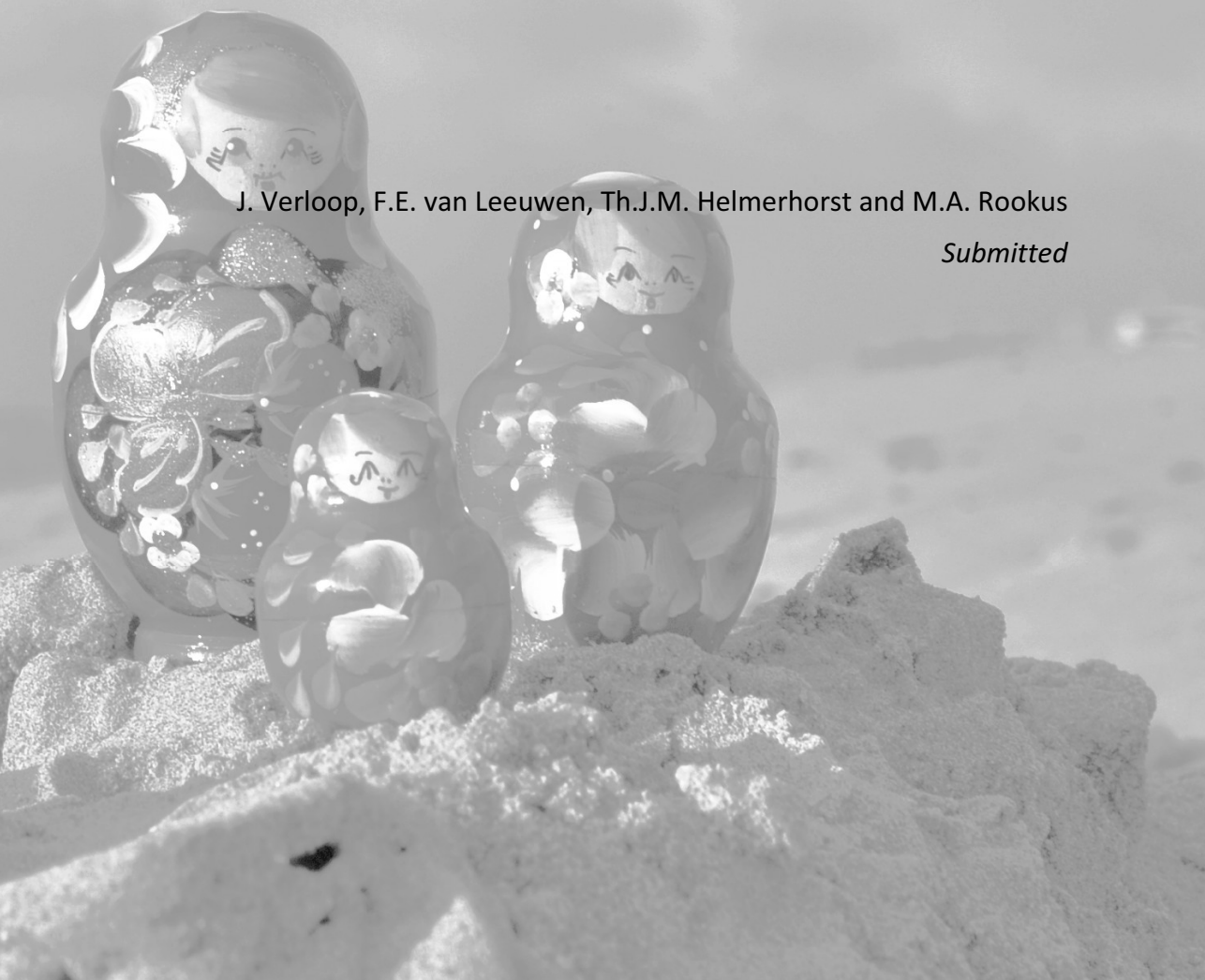

4.1 Abstract

Purpose

Because of increased risk of clear cell adenocarcinoma of the vagina and cervix, women exposed to diethylstilbestrol (DES) *in utero* have been advised to undergo frequent gynecological examinations, in order to early detect and treat (pre)cancerous lesions. We evaluated the effectiveness of cytological screening in DES daughters.

Patients and methods

We conducted a nested case-control study of 39 DES daughters (aged 30-54 years) with vaginal or cervical cancer and 186 DES-exposed controls, matched on age and date of birth. Information on screening history was collected through linkage with the Dutch pathology database (PALGA).

Results

Women with a Pap smear within 5.5 to 0.5 years preceding (pseudo)diagnosis had no reduced risk of vaginal and cervical cancer (OR=1.48, 95%CI=0.56-3.9) compared to non-screened women. The stage distribution for cervical cancer was slightly more favorable in screened DES daughters with cancer compared to the general population although not significantly (stage I in 87% and 77%, respectively, p=0.15). Women with moderate or severe dyskaryotic smears during screening had a strongly increased risk of cervical/vaginal cancer compared to women with a normal smear (OR=29.3, 95%CI=7.5-115). Women with DES-related malformations more often had abnormal smears detected during screening than other DES daughters (52% and 39% respectively).

Conclusion

Cytological screening is not effective in preventing cervical or vaginal cancer (neither squamous cell nor CCA) in DES daughters, although stage at diagnosis might be more favorable. As women with DES-related malformations more often present with abnormal smears during screening, careful monitoring of DES daughters with DES malformations seems to be warranted.

4.2 Introduction

Although the effectiveness of cervical cancer screening in the general population has never been addressed in randomized controlled trials, numerous observational studies have shown that cytological cervical screening is effective in detecting precancerous lesions, followed by a reduction of the incidence and mortality of invasive cervical cancer[1;2]. Because DES daughters have a strongly increased risk of clear cell adenocarcinoma (CCA) of the vagina and cervix and also may have a higher risk of squamous cell cervical cancer, millions of DES daughters worldwide have undergone regular gynecological examinations in order to early detect and treat (pre)cancerous lesions of the vagina and cervix[3]. Whereas many studies have reported on the health outcomes after screening of DES daughters, with screening mainly focused on the detection of adenosis[4;5], only few studies evaluated the effectiveness of screening on cancer risk in DES daughters; these studies only focused on CCA[6-8].

In 1975, the Dutch Gynecological Working Group developed guidelines for the surveillance of DES daughters[9-11]. This Dutch guideline (latest revision in 1992) recommends annual cervical and vaginal smears starting at age 14 (or menarche), colposcopic examinations during the first five year of screening, with individual recommendations for women with and without DES-related malformations and for different levels of (un)certainty about DES exposure (Supplement, table 1). The Dutch guidelines for DES-exposed women agree with the US guidelines[4;12], with omission of the individualized recommendations regarding the presence of malformations and uncertainty about exposure. In the 1980s, a national Dutch screening program for cervical cancer was established, initially inviting all women aged 35-55 every 3 years. After a reorganization of the program in 1996, the invitational age range was broadened to 30-60 years and the time interval between two consecutive screening tests extended to five years[13]. The Dutch DES screening differs from the national population screening with respect to additional cytological screening of the vagina[9-11], earlier age at start of the screening, higher frequency and colposcopic examinations during the first years of surveillance.

Our ongoing cohort study among DES daughters and the availability of the Dutch nationwide pathology database (PALGA) created a unique possibility to evaluate the effectiveness of the Dutch screening program for DES daughters[14]. Our evaluation focused on the cytological part of the guideline.

In a nested case-control study we addressed the following questions: 1) Is screening among DES daughters effective in preventing invasive vaginal and cervix cancers; 2) Are screened cases diagnosed at earlier stages; 3) Is screening at older ages (>40) still necessary; 4) is the follow-up of abnormal findings adequate; and 5) should screening according to the Dutch DES guideline be restricted to DES daughters with DES-related malformations? To our knowledge, this is the first report on effectiveness of cytological screening on cervical and vaginal cancer risk in DES daughters with all morphologies included (CCA, squamous cell cancer (SCC) and other morphologies).

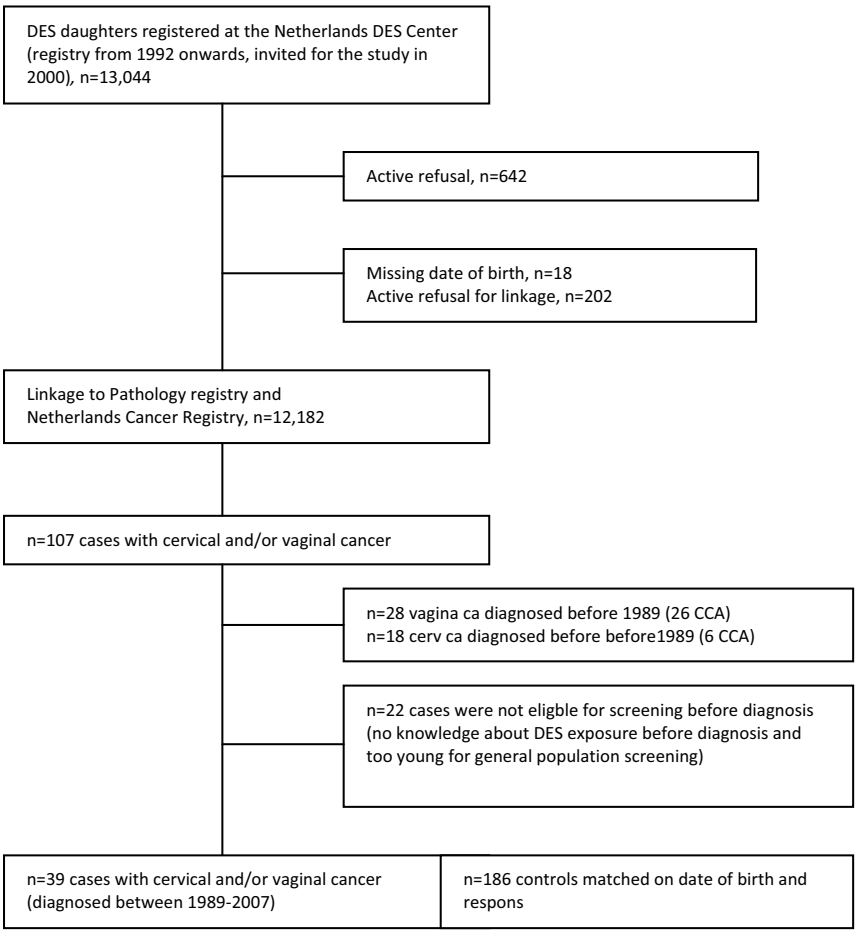

Figure 1 Follow-up information on DES daughters included in the study

4.3 Methods

Study population

The DES-net project is a nationwide retrospective cohort study with prospective follow-up of 12,182 DES daughters in the Netherlands. DES daughters were identified through the registry of the Netherlands DES Center. The study rationale and design have been described previously[14]. In short, the cohort of the Netherlands DES Center was established in 1992 in order to deal with future health claims. From March 2000 till December 2004 all DES daughters registered at the DES Center received an invitation for the study. Women were asked permission to abstract data from their medical records and to link their data with several population-based disease registries. In addition, medical record documentation of DES exposure, if available, was asked for. Also, the women were requested to complete a 16-page self-administered questionnaire with questions on reproductive risk factors for hormone-related cancers and medical history. For this analysis we used questionnaire data about education, age at first gynecological examination, age at discovery of being DES-exposed, certainty of DES exposure, and DES-related malformations (T-shaped uterus, cox comb, vaginal ridges, adenosis, squamous cell metaplasia). All reported malformations were medically verified by sending questionnaires to the treating physicians. The study was approved by the Institutional Review Board of the Netherlands Cancer Institute and the Surveillance Committees of PALGA and the Netherlands Cancer Registry (NCR). The Surveillance Committees granted us permission to extract data from population-based registries for both responders (62%) and non-responders (36%) under strict privacy procedures. Refusers were excluded from linkage and from this study (5%) (Figure 1).

To study the effectiveness of screening we used a nested case-control design. A total of 107 cases of invasive vaginal and cervical cancer were identified through linkage with the NCR and PALGA or by medical verification of self-reported cancer diagnosed before 1989. PALGA contains excerpts of all histopathology and cytopathology reports. Both registries have nationwide coverage since 1989. Follow-up ended on 30 November 2008. Because of incomplete national coverage by the PALGA database before 1989, resulting in incomplete 5-year screening histories of the early cases, we excluded 46 cases diagnosed before 1989 (28 women with vaginal cancer (26 CCA) and 18 cervical cancers (6 CCAs), with a mean age at diagnosis of 21.7 years (Figure 1).

Subsequently, each case (n=61) was individually matched to five controls (n=305) by date of birth (± 21 days) and **response status** (i.e. being a responder or non-responder to the questionnaire). We matched on response status because this might be a confounder of the association between cancer risk and screening behavior. Controls were assigned a date of pseudo-diagnosis at which they had exactly the same age as the corresponding case at date of diagnosis. Controls were not diagnosed with cancer and had had no hysterectomy before the date of pseudo-diagnosis.

In order to evaluate the effectiveness of screening, we restricted our analyses to women who had an opportunity to be screened, i.e. women who were eligible for the general population screening program or aware of their DES exposure at least one year before their date of (pseudo-)diagnosis (exclusion of 22 cases and 129 controls), leaving 39 cases and 186 controls for analyses.

Information on stage distribution (TNM-classification) of invasive cancer was derived from the NCR. FIGO stage was defined by the clinical TNM stage or if unknown, the pathological TNM stage.

Assessment of history of cytological screening and stage distribution

From the PALGA database we extracted information on all cervical and vaginal smears and biopsies taken from each woman from the date of the first occurrence in PALGA till date of (pseudo-)diagnosis or November 30th, 2008, whichever was first. Women were linked to PALGA by date of birth and the first four letters of their maiden name. Because this code was not always unique we did additional checks for responders using first initial and place of birth. We collected information on date, type of examination (smear or biopsy), localisation (vagina or cervix) and outcome (type of lesion, morphology).

To evaluate effectiveness of screening, we used two different measures to estimate the effectiveness of screening. Cases and controls were compared with respect to 1) having had a Pap smear (*vaginal* or *cervical*) within the 5-year interval preceding diagnosis and 2) the time since operationally last negative smear[15;16]. Operationally negative means that a negative smear is not preceded by an abnormal smear (Pap2+) within the previous 12 months[17]. Time since last operationally negative smear gives information about the sojourn time of the preclinical disease. Being tested within the recommended screening interval merely reflects screening behavior.

For the calculation of having had Pap smears during a 5-year interval, we excluded the first 0.5 years preceding diagnosis, similar to the analyses done by Andrae *et al.*[15] and as suggested by Weiss *et al.*[18]. Pap smears taken during the 0.5 years before diagnosis are likely to be diagnostic tests rather than screening tests and would therefore bias the observed association, with more cases being tested[18]. Thus, we used an interval of 0.5-5.5 years preceding diagnosis. Cervical and vaginal smears were defined as abnormal in case of borderline dyskaryosis (pap 2), mild dyskaryosis (pap3a1), moderate dyskaryosis (pap3a2) or severe dyskaryosis (pap3b and worse). For analysis purposes, borderline and mild dyskaryotic smears were combined into one category and referred to as BMD smears and moderate/severe dyskaryotic smears were grouped and referred to as MSD smears. We also conducted a sensitivity analysis in which we shifted the time window to one till six years prior to diagnosis, instead of 0.5-5.5 years. For the assessment of the second measure (time since operationally last negative smear) a Pap smear was considered as operationally negative when not preceded by an abnormal smear (Pap2+) within the previous 12 months[17].

Besides studying the effectiveness of screening, we assessed whether the follow-up after an abnormal smear, detected during the 5-year interval, was conducted according to the Dutch guidelines with respect to timing and type of examination. We defined the follow-up interval as the time between date of the abnormal smear and date of first follow-up examination. In case more than one abnormal smear was detected during the 5-year period we used the first one. For the definition of an adequate follow-up interval, we used three months as a cut-off point for the follow-up of MSD smears and one year for BMD smears.

Additionally, for each woman the numbers of vaginal and cervical smears taken during the 5-year interval were counted, as both types of smears are recommended by the DES screening guideline.

Furthermore, for women with MSD lesions during the recommended screening interval extra information on treatment was collected from medical files and from the PALGA pathology reports, with the limitation that information on destructive techniques (like cryo-coagulation or laser evaporation) was not available in PALGA.

Statistical analyses

Conditional logistic regression was used to estimate odds ratios (ORs) for invasive vaginal or cervical cancer associated with different screening parameters. When analyzing subgroups (e.g.

Table 1 Characteristics of 39 DES daughters with vaginal or cervical cancer and 186 age- and response matched controls

	Cases (n=39)		Controls (n=186)		p*
Characteristic	**No.**	**%**	**No.**	**%**	
Birth cohort					0.99
<1955	6	15.4	28	15.1	
1955-1959	6	41.0	80	43.0	
1960-1964	7	18.0	35	18.8	
1965-1969	6	15.3	29	15.6	
>=1970	4	10.3	14	7.5	
Year of registration at the DES Center					<0.01
1992	30	76.9	173	93.0	
1993-1999	3	7.7	7	3.8	
2000-2002	6	15.4	6	3.2	
Vaginal cancer	*Cases (n=11)*		*Controls (n=55)*		
Mean age at (pseudo) at diagnosis	38.3		38.3		1.00
SD	4.3		4.1		
Calendar year of (pseudo) diagnosis					1.00
1989-1992	1	9.1	5	9.1	
1993-1999	7	63.6	35	63.6	
2000-2008	3	27.3	15	27.3	
Morphology			---		
Clear cell adenocarcinoma	9	81.8			
Squamous cell	2	18.2			
FIGO stage			---		
I	7	63.6			
II or higher	2	18.2			
Missing	2	18.2			
Mean age at first gynecological examination*	29.3		30.6		0.50
SD	6.1		5.4		
Cervical cancer	*Cases (n=28)*		*Controls (n=131)*		
Mean age at (pseudo) at diagnosis	35.6		36.5		0.65
SD	7.9		7.0		
Calendar year of (pseudo) diagnosis					0.90
1989-1992	10	35.7	41	31.3	
1993-1997	8	28.6	40	30.5	
1998-2007	10	35.7	50	35.7	
Morphology			--		
Clear cell adenocarcinoma	5	17.9			
Squamous cell	15	53.6			
Other¶	8	28.6			
FIGO stage			---		
I	5	17.9			
IA	9	32.1			
IB	9	32.1			
II or higher	4	14.2			
Missing	1	3.6			
Mean age at first gynecological examination*	28.9		28.7		0.89
SD	6.9		7.2		

table continues on page 96

screened women only) we used unconditional logistic regression with adjustment for age as a continuous variable, because case-control sets were split up. Because screening is thought to be less effective in preventing adenocarcinoma compared to squamous cell carcinoma[19-21] and less effective in vaginal cancer compared to cervical cancer[7], we conducted analyses stratified by morphology (squamous cell, clear cell adenocarcinoma) and by topography (cervix, vagina). Furthermore, we calculated separated ORs for different age categories (<40,40+ years) and presence or absence of DES-related malformations, with the latter information only available for women with questionnaire data.

All analyses were processed with Stata/IC version 10. Differences between group means and distributions over categories were tested by Student's t-test or $\chi 2$ test.

4.4 Results

General characteristics of our study population are listed in table 1. Of the 39 cases 11 women had been diagnosed with invasive vaginal cancer and 28 with invasive cervical cancer. Clear cell adenocarcinoma was the most frequent morphology for patients with vaginal cancer (82%), while SCC was most frequent among cervical cancer patients (54%). The majority of women was born between 1955 and 1959. Mean age at diagnosis was 38.3 years among vaginal cancer cases and 35.6 years among cervical cancer cases. Among women with questionnaire data, slightly more cases than controls had medically confirmed DES-related malformations (51.5% versus 37.2%, respectively, p=0.31). Cases reported to have discovered being a DES daughter at a later age than controls (mean ages 27.0 and 24.7 years, respectively, p=0.14). Mother's medical file with confirmation of DES exposure was available for 10% of the women, with no difference between cases and controls. No differences between cases and controls were found with respect to mean age at first gynecological examination (25.4±7.7 and 24.3±6.3 years for cases and controls, p=0.47, respectively) and other characteristics.

No overall decreased risk of cervical and vaginal cancer was found for DES daughters who were screened during the 0.5-5.5 years preceding (pseudo-)diagnosis (OR 1.48, 95% CI 0.56-3.9) (table 2). A recent negative smear was associated with a non-statistically significant reduced risk when considering the period 0.5-1 year preceding (pseudo-)diagnosis (OR=0.35, 95%CI 0.07-1.61) and this risk gradually increased with increasing time interval.

Table 1, continued

Responders (women with questionnaire data)	**Cases (n=33)**		**Controls (n=156)**		**p***
Characteristic	**No.**	**%**	**No.**	**%**	
Confirmation of DES exposure					0.84
Medical file of the mother	3	9.1	16	10.3	
No medical file of the mother	30	90.9	140	89.7	
Self-reported certainty about DES exposure					0.55
Certain	22	66.7	109	69.9	
Probably	11	33.3	43	27.6	
Missing	0	0	4	2.6	
Mean self-reported age at discovery DES exposure†	27.0		24.7		0.14
SD	9.8		7.5		
Mean self-reported age at first gynecological exam‡	25.4		24.3		0.47
SD	7.7		6.3		
DES-related malformations§					0.31
No DES-related malformations	16	48.5	98	62.8	
Epithelial changes (VEC)	11	33.3	37	23.7	
Anatomical malformations and n.s.	6	18.2	21	13.5	
Highest educational level					0.36
Primary school	2	6.1	21	13.5	
Secondary school	21	63.6	69	44.2	
College or university	9	27.3	59	37.7	
Unknown or missing	1	3.0	7	4.5	

Abbreviations: SD standard deviation, *P* for T-test or chisq

* screened women only

† based on 32 cases and 148 controls because of missings

‡ based on 21 cases and 101 controls because of missings

§ adenosis, elaborate squamous cell metaplasia, cox comb, vaginal ridges, T-shaped uterus

¶ cervical cancer, other morfology: 7 adenocarcinoma, 1 adenosquamous cell carcinoma

With respect to vaginal cancer no protective effect of screening was observed as all vaginal cancer cases had been screened. Having had a vaginal smear did also not reduce the risk of vaginal cancer (OR=1.32, 95%CI=0.29-5.9). For cervical cancer we also did not observe reduced risks after screening (OR=1.0, 95%CI=0.36-2.9). Regarding the different morphologies, the results for SCC were quite similar to the results for cervical cancer overall (only 2 out of the 17 SCCs were located in the vagina). Likewise, the results for CCA were quite similar to the results for vaginal cancer (5 out of 14 CCA tumors were located in the cervix). A relatively high number of abnormal smears was detected during the 5-year interval among both cases and controls (64% and 28%, respectively). Among screened women, an abnormal smear increased the risk of cervical/vaginal cancer, compared to women with Pap 1 smears (OR=5.5, 95%CI=2.3-13.1), with a very high risk among women with a history of MSD smears (OR=29.3, 95%CI=7.5-115), and particularly when the morphology was squamous cell (OR=315, 95% CI=14.6-6799).

All abnormal smears among cases were followed by a smear or biopsy, whereas among controls 7 BMD smears were not monitored (table 3, appendix table 2). Thus, the risk of invasive cancer was not associated with type of follow-up examination after an abnormal smear (histology or smear) or delay in the timing of the first follow-up examination (> 3 months after the diagnosis of MSD or >1 year following diagnosis of BMD). Seven out of ten patients with MSD preceding the diagnosis were treated by conisation or large loop excision, and the other three were kept under intensive cytological surveillance (appendix table 2). Among the controls with MSD in the screening history, 3 women had a cytological follow-up and 1 woman had a large loop excision. Women with MSD lesions who were followed by biopsy (6 out of 7 MSDs appeared to be CIN2+) had a strongly increased risk of (cervical) cancer (OR=69.9, 95%CI=7.5-656).

Stratification by age revealed that screening exerted a small (non-significant) benefit against cervical cancer after age 40 (OR 0.35 (0.06-1.9) (results not shown) With respect to vaginal cancer screening efficacy did not differ between the two age groups.

Nearly all women with DES-related malformations had been screened (94% and 84% of the cases and controls, respectively) (table 4). Among women without malformations less women were screened, more resembling the 5-year screening coverage rates in the general population (76% and 69% of cases and controls, respectively). Among women without malformations screening during the 5-year interval preceding (pseudo)diagnosis was associated with a non-significantly reduced risk (OR=0.71, 95%CI=0.22-2.3), while we observed

Table 2 Risk of cervical and vaginal cancer according to screening characteristics, stratified by topography and morphology

	Total			Topography††					
	Cervical and vaginal cancer			Cervical cancer			Vaginal cancer		
	No.		OR (95% CI)	No.		OR (95% CI)	No.		OR (95% CI)
	Case	Control		Case	Control		Case	Control	
	39	186		28	131		11	55	
Screened within recommended interval*									
Not screened†	6	39	1.00	6	29	1.00	0	10	1.00
Screened†,‡	33	147	1.48 (0.56-3.9)	22	102	1.02 (0.37-2.9)	11	45	----
Screened, normal smear§¶	8	94	1.00	2	66	1.00	6	28	1.00
Screened, abnormal smear§,¶,||	25	53	5.5 (2.3-13.1)	20	36	18.2 (4.0-82.8)	5	17	1.37 (0.36-5.2)
Borderline/mild dyskaryosis§,¶,||	15	49	3.5 (1.4-8.9)	10	34	9.3 (1.9-44.8)	5	15	1.55 (0.40-5.9)
Moderate/severe dyskaryosis§,¶,||	10	4	29.3 (7.5-115)	10	2	172 (21.4-1391)	0	2	---
Screened, no vaginal smear	23	106	1.00	15	71	1.00	8	35	1.00
Screened, ≥1 vaginal smear	10	41	1.10 (0.48-2.5)	7	31	1.04 (0.38-2.8)	3	10	1.32 (0.29-5.9)
≥ 1 cervical and vaginal smear‡	9	40	1.58 (0.49-5.1)	6	30	1.02 (0.28-3.7)	3	10	---
≥ 1 vaginal smear, no cervical smear‡	1	1	4.96 (0.26-93.1)	1	1	3.49 (0.19-65.4)	0	0	---
≥ 1 cervical smear, no vaginal smear‡	23	106	1.42 (0.52-3.8)	15	71	0.99 (0.34-2.9)	8	35	---
Time since last negative smear**									
0.5- 1.0 years	2	22	0.35 (0.07-1.62)	0	16	---§§	2	6	5.59 (0.47-66.2)
1.0-2.0 years	5	41	0.43 (0.15-1.3)	4	30	0.33 (0.10-1.1)	1	11	1.90 (0.11-32.2)
2.0-5.0 years	15	59	0.93 (0.41-2.1)	8	41	0.47 (0.16-1.3)	7	18	7.54 (0.86-65.7)
>5.0 years or no (negative) smears	17	64	1.00	16	44	1.00	1	20	1.00

* Recommended screening interval: 5.5-0.5 years before diagnosis †Definition of screening: having had a vaginal or cervical pap-smear

‡ Reference group consists of non-screened women.

§ Definition of normal smear: pap1, abnormal smear: borderline dyskaryosis (pap 2), mild dyskaryosis (pap3a1), moderate dyskaryosis (pap3a2) or severe dyskaryosis (pap3b or worse). The maximum lesion during follow-up was counted and in case more than one abnormal smears the first occurrence of the most severe lesion was counted.

¶ Unconditional logistic regression analyses adjusted for age at diagnosis

|| Reference group consists of screened women with a normal smear

** Reference group consists of women with time last negative smear longer than 5 years, or no (negative) smear

†† Cervical cancer morphology: 5 clear cell adenocarcinomas, 15 squamous cell tumors, 7 adenocarcinomas and 1 adenosquamous cell tumor. Vaginal cancer morphology: 9 clear cell adenocarcinomas and 2 squamous cell tumors

Table 2, continued

	Morphology‡‡					
	Squamous cell			Clear cell adenocarcinoma		
	No.		OR (95% CI)	No.		OR (95% CI)
	Case	Control		Case	Control	
	17	83		14	58	
Screened within recommended interval[*]						
Not screened[†]	3	23	1.0	1	7	1.00
Screened[†,‡]	14	60	1.9 (0.46-7.7)	13	51	1.74 (0.19-16.1)
Screened, normal smear[§¶]	1	40	1.00	6	30	1.00
Screened, abnormal smear[§,¶,||]	13	20	26.0 (3.2-214)	7	21	1.6 (0.48-5.6)
Borderline/mild dyskaryosis[§,¶,||]	7	19	14.6 (1.7-128)	6	18	1.6 (0.45-5.9)
Moderate/severe dyskaryosis[§,¶,||]	6	1	315 (14.6-6799)	1	3	1.6 (0.14-18.2)
Screened, no vaginal smear	9	43	1.00	9	38	1.00
Screened, ≥1 vaginal smear	5	17	1.4 (0.41-4.8)	4	13	1.26 (0.33-4.8)
≥ 1 cervical and vaginal smear[‡]	5	16	2.55 (0.49-13.3)	3	13	2.79 (0.20-38.8)
≥ 1 vaginal smear, no cervical smear[‡]	0	1	---	1	0	---
≥ 1 cervical smear, no vaginal smear[‡]	9	43	1.76 (0.41-7.51)	9	38	1.62 (0.17-15.5)
Time since last negative smear[**]						
0.5- 1.0 years	0	9	<<	2	6	1.39 (0.21-9.1)
1.0-2.0 years	2	16	0.45 (0.08-2.5)	2	12	0.63 (0.10-4.0)
2.0-5.0 years	7	27	1.00 (0.29-3.5)	5	23	0.85 (0.19-3.8)
>5.0 years or no (negative) smears	8	31	1.00	5	17	1.00

‡‡ Topography squamous cell tumors: 15 located in the cervix, 2 located in the vagina. Topography clear cell adenocarcinomas: 5 located in the cervix and 9 located in the vagina

§§ Combined estimate 0.5-1 and 1.0-2.0 for cervical cancer: OR 0.22 (0.07-1.71)

Table 3 Follow-up of abnormal smears among cervical and vaginal cancer cases and controls screened within the recommended interval 5.5.-0.5 year preceding (pseudo)diagnosis

Screened within recommended interval*	**No. cases**	**No. controls**	**OR (95% CI)‡**
	33	147	
Normal smear†	8	94	1.00 (ref)
Abnormal smear†	25	53	5.5 (2.3-13.1)
Borderline/moderate dyskaryosis	**15**	**49**	**3.5 (1.4-8.9)**
Time interval next cytological/histological examination			
≤1 year	3	20	1.7 (0.42-7.1)
>1 year	12	29	4.8 (1.8-12.9)
Type of follow-up examination			
Cytology	14	38	4.3 (1.6-11.0)
Normal	*5*	*17*	
Dyskaryosis (borderline or worse)	*9*	*21*	
Histology	1	4	2.8 (0.28-28.6)
<CIN1	*0*	*1*	
CIN1	*1*	*1*	
CIN2+	*0*	*2*	
No cytological/histological follow-up	0	7	--
Moderate or severe dyskaryosis	**10**	**4**	**29.3 (7.5-115)**
Time interval next cytological/histological examination			
≤3 months	6	2	35.0 (6.0-202)
>3 months	4	2	23.7 (3.7-150)
Type of follow-up examination			
Cytology	4	3	15.8 (3.0-83.5)
Normal	*0*	*1*	
Dyskaryosis (borderline or worse)	*4*	*2*	
Histology	6	1	69.9 (7.5-656)
<CIN1	*1*	*0*	
CIN1	*0*	*0*	
CIN2+	*5*	*1*	

* Recommended screening interval: 5.5-0.5 years before diagnosis.
Definition of screening: having had a vaginal or cervical pap-smear

† Definition of normal smear: pap1, abnormal smear: borderline dyskaryosis (pap 2), mild dyskaryosis (pap3a1), moderate dyskaryosis (pap3a2) or severe dyskaryosis (pap3b or worse). The maximum lesion during follow-up was counted and in case more than one abnormal smears the first occurrence of the most severe lesion was counted

‡ Unconditional logistic regression analyses adjusted for age at diagnosis

a non-significantly increased risk in women with DES-related malformations (OR=3.10, 95%CI=0.36-26.7). Abnormal smears, and MSD in particular, were more commonly present among women with DES-related malformations than among women without malformations (45% versus 29%, respectively).

When we compared stage distributions between screened and non-screened cervical cancer patients, we found slightly more stage I tumors in screened versus non-screened individuals (87% versus 66% with stage I, p=0.22) and between screened individuals versus the general population (87% and 77%, p=0.15) (table 5). Women with vaginal cancer in our study had a similar stage distribution compared to the general population.

When considering the full screening history for all 39 cases (table 6), a remarkable finding was that the majority of cases had a history of highly frequent Pap smears. Despite all efforts (one cervical cancer case had undergone 25 vaginal and cervical smears and two biopsies during the 5-year interval preceding diagnosis), these women still developed invasive cancer. Furthermore, as noted earlier, all women with vaginal cancer had been screened before diagnosis.

4.5 Discussion

Despite the fact that nearly all DES daughters in our study had been screened, invasive cervical and vaginal cancer still developed. Our study shows that screening in DES daughters is not effective in preventing invasive vaginal and cervical cancer, although the efficacy of screening with respect to cervical cancer seemed to improve slightly at older ages. Screened cervical and vaginal cases had a slightly more favorable stage distribution compared to non-screened cases or the general population. Women with a history of abnormal smears (especially MSD) had a higher risk of cervical or SCC, although the treatment appeared to be adequate according to the Dutch treatment guidelines (ref: www.oncoline.nl, and appendix, table 2). Women with DES-related malformations more often had abnormal smears in their screening history.

An important feature of our study is that the screening histories for cases and controls were based on medical registry data and therefore much more complete and accurate than self-reported data. Furthermore, all cases were prospectively identified and medically verified. To our knowledge, this is the first comprehensive study to examine the effectiveness of

Table 4 History of screening among DES-exposed cases and controls stratified by medically verified DES-related malformations

	Presence of DES-related malformations (women with questionnaire data)					
	No malformations[¶]			DES-related malformation[||]		
	No. cases	No. controls	OR (95% CI) [§]	No. cases	No. controls	OR (95% CI) [§]
	16	98		17	58	
Screened in recommended interval[*]						
Not screened[†]	5	24	1.00	1	9	1.00
Screened[†]	11	74	0.71 (0.22-2.3)	16	49	3.10 (0.36-26.7)
Screened, normal smear[‡]	3	49	1.00	4	27	1.00
Screened, abnormal smear[‡]	8	25	5.1 (1.2-21.1)	12	22	3.7 (1.0-13.0)
Borderline/mild dyskaryosis	6	23	4.1 (0.93-17.8)	6	21	1.9 (0.47-7.6)
Moderate/severe dyskaryosis[**]	2	2	16.3 (1.7 (160)	6	1	43.9 (4.0-478)
Time since last negative smear						
0.5- 1.0 years	1	14	0.24 (0.03-2.1)	0	7	---
1.0-2.0 years	0	18	--	4	13	0.98 (0.23-4.2)
2.0-5.0 years	5	32	0.53 (0.16-1.7)	7	19	1.18 (0.33-4.2)
>5.0 years or no (negative) smears	10	34	1.00	6	19	1.00

[*] Recommended screening interval: 5.5-0.5 years before diagnosis

[†] Definition of screening: having had a vaginal or cervical pap-smear

[‡] Definition of normal smear: pap1, abnormal smear: borderline dyskaryosis (pap 2), mild dyskaryosis (pap3a1), moderate dyskaryosis (pap3a2) or severe dyskaryosis (pap3b or worse). The first occurrence of the lesion was counted

[§] Unconditional logistic regression

[¶] No malformations: 4 vaginal cancer (1 Scc, 3 CCA) and 12 cervical cancer (7 scc, 2 CCA, 3 adeno)

[||] Malformations, defined as T-shaped uterus, cox comb, vaginal ridges, adenosis, squamous cell metaplasia. Of the women with malformations 6 had vaginal cancer (1 scc, 5 CCA) and 11 cervical cancer (5 scc, 3 CCA,3 adeno)

[**] Of the 8 patients with moderate/severe dyskaryosis 5 women had DES-related adenosis/squamous metaplasia (in combination with/without structural malformations), 1 women unspecified malformations, 2 women no (verified) DES-related malformations

Table 5 Comparison of FIGO stage between screened cases versus non-screened cases and versus Netherlands Cancer Registry

	Non-screened cases	Screened cases	NCR[*]	X^2 screened versus non-screened	X^2 screened versus NCR
Cervical cancer	6	22		p=0.22	p=0.15
FIGO stage[†]					
I	2 (33%)	3 (14%)	7 %		
IA	0 (0%)	9 (41%)	31 %		
IB	2 (33%)	7 (32%)	39 %		
II+	2 (33%)	2 (9%)	22 %		
Missing	0 (0%)	1 (5%)	1 %		
Vaginal cancer	0	11		--	p=0.65
FIGO stage[†]					
I		7 (64%)	56%		
II		1 (9%)			
III		1 (9%)	44%		
Missing		2 (18%)			

[*] Reference data from the Netherlands Cancer Registry. Vaginal cancer: age category 25-44, period 1991-2004. Cervical cancer: age category 15-49, period 1989-2007

[†] FIGO= International Federation of Gynecology and Obstetrics, IA = microinvasive cancer, IB = localized cancer, II or higher = advanced cancer

screening among DES daughters on the risk of cervical and vaginal cancer in which all morphologies are included and which used a 5-year screening window.

There is sparse evidence from the literature on the effectiveness of screening among DES daughters, and the studies concerned only examined effectiveness of screening on CCA, not SCC [7;8]. The two only studies available were based on the US- and Dutch CCA registries, respectively [7;8] and included not only DES daughters but also unexposed women (28% and 42% unexposed patients, respectively)[7;8]. More importantly, these studies were only focused on the detection of *invasive* cancer and not on the detection (and treatment) of *pre-invasive* lesions; the time-window examined was limited to a maximum period of 3 years and 2 years before diagnosis for the US and Dutch study, respectively. Both studies found that the majority of CCAs (which predominantly occur in the vagina) can be detected by cytology at the time of diagnosis (73% and 76% in the US and Dutch study, respectively), but that the sensitivity was lower when (cervical and/or vaginal) smears had been taken in the 3 years before diagnosis (negative smears in 40% and 55% among symptomatic and asymptomatic women, respectively)[8]. The authors suggested that false negative smears at the time of diagnosis, might be due to inadequate sampling (cervix instead of vagina), the location of the tumor (submucosa) and difficulties in recognizing the tumor cells (which sometimes are highly differentiated or obscured by e.g. inflammatory cells)[8]. Thus, even in symptomatic women it appeared to be difficult to detect CCA.

In our study, we found that cytological examinations were ineffective in preventing invasive vaginal cancer (9 CCAs and 2 SCCs). All cases had been screened, but only three out of eleven had a history of vaginal smears, and these vaginal smears were classified as Pap1 (taken in the period 1-2 years before diagnosis). Despite the recommendations in the DES screening protocol, it appeared that relatively few vaginal smears had been taken in the total study group (23% of the women in our study had had at least one vaginal smear). All patients with vaginal cancer had had cervical smears, but obviously these are inappropriate to detect vaginal cancer[7]. Yet, the stage distribution among the vaginal cancer patients in our study seems to be more favorable than the general population although not statistically significant. It should be noted that, due to the rarity of the disease in the general population (65 incident cases of vaginal cancer in the period 1991-2004) and the relatively high number of patients in our study (11), this comparison was biased towards null.

Compared to studies on the efficacy of cervical cancer screening in the general population our results are rather disappointing. It has been estimated that screening reduces the risk of cervical cancer by 80%[1]. Risk reductions range from 30%-96%[15;17;21-23] depending on, among others, morphology, age, attendance rate and screening interval. The sensitivity of Pap smears to detect adenocarcinomas is generally assumed to be lower than for squamous cell carcinomas[19;21;24;25] due to the fact that Pap smears taken from the cervical transformation zone are not capable of detecting lesions in the endocervical canal, where adenocarcinomas mostly occur[20]. However, when we did a separate analysis for SCC (of which 89% was located in the cervix), no reduced risk of cancer among women who had had cytological examinations during the 5-year period preceding diagnosis was found either. On the other hand a recent negative smear (within two years preceding diagnosis) was associated with a reduced risk of cervical cancer (although not statistically significant), similar to other studies, with the reduced risk disappearing with increasing time interval[25-27].

A remarkable finding in our study is the high number of dyskaryotic smears found during the 5-year interval before diagnosis (with exclusion of the six months preceding diagnosis), not only among cases (64%) but also controls (28%). In other population-based case-control studies on effectiveness of cervical screening substantially fewer women with a history of abnormal smears were observed (ranges between 10%-16% for cases and 3%-5% for controls, respectively)[15;22;23;28].

The high number of BMD smears might be due to the annual cytological examinations in DES daughters as recommended by the DES screening guideline. A high frequency of screening has the potential for overdiagnosis of lesions that would otherwise regress spontaneously[2;29;30]. We found a high number of BMD lesions among *both* cases and controls (38% and 26%, respectively) indicating that many of these lesions, since they are mostly treated with a wait-and-see policy, have spontaneously regressed (controls did not develop cancer). However, with respect to non-transient high grade lesions (moderate and severe dyskaryosis), high screening frequency seems an unlikely explanation for an increased risk (unless DES-related tumors are rapidly growing tumors and the time window of detection of MSD is shortened). The high number of MSD lesions in our study may be the result of histological misclassification due to the common presence of vaginal and cervical epithelial changes (adenosis and a wider transformation zone, respectively) in DES daughters. Indeed, we observed that among cervical cancer patients six out of eight MSDs were found in women with

DES-related malformations (five women had adenosis and one woman had unspecified DES-related malformations; analysis restricted to women with questionnaire data on malformations) (table 4). On the other hand, it has also been suggested that the presence of glandular tissue (adenosis) and metaplastic squamous epithelium in the vagina and cervix might increase susceptibility to carcinogens[3;31], so the detected MSD may already be the first sign of the presence of precancer (OR=29.3, 95%CI 7.5-115). Remarkably, the average time interval between detection of the (first) MSD to the diagnosis of cancer was 4.8 years (results not shown), suggesting that these cancers might be slow developing tumors or that the progression from MSD to cancer is a rather slow process.

The fact that women with MSD lesions detected during screening had a strongly increased risk of cancer may reflect a failure of the screening system**,** by which enhanced surveillance and adequate treatment should decrease the risk of an invasive tumor after an abnormal smear, which is the rationale behind screening[15;32]. In our study population, however, we found that 60% and 50% of the cases and controls with MSD, respectively, were treated within the recommended time interval. Whether these subjects also received the proper treatment could not be completely evaluated since we missed information on colposcopic examinations. When colposcopy findings are normal, no biopsy is taken and cytological surveillance is adequate according to current Dutch Guidelines (www.oncoline.nl, appendix table 3). Therefore we could not evaluate whether the absence of histological examination in seven cases of MSD (four cases and three controls) was justified. The remaining seven women with MSD (six cases, one control) had histological examinations, with CIN confirmed in six women and inflammation diagnosed in one woman; all were treated according to the guidelines (table 2 and appendix, table 2). Women with BMD lesions were kept under intensive cytological surveillance (only four out of 64 women did not receive any follow-up examination after BMD, table 2). Thus, although management of MSD seemed to be according to the guidelines, it was not effective in preventing invasive cancer. Possibly an abnormal smear (even if this occurs five years preceding the diagnosis) already indicates the presence of invasive cancer.

Our study has a few limitations**.** In studying the effectiveness of screening, only true screening tests should be included and symptomatic tests be excluded, to avoid the effectiveness of screening being underestimated [33]. Unfortunately, we were not able to fully disentangle true

screening tests from symptomatic tests in our study, due to of lack of information on reasons for smear taking. We tried to minimize this problem by disregarding the smears taken during the half year preceding diagnosis, as has been done in other studies[15;27]. To investigate whether the chosen cutoff point of six months for defining the screening time window may have been too short to exclude all symptomatic tests, we conducted a sensitivity analysis excluding one year prior to the date of (pseudo-)diagnosis. The number of abnormal smears was unchanged (13 MSD lesions were detected instead of 14), suggesting that the MSD test results were not close to the diagnosis.

Weiss discussed another fallacy in defining the appropriate time window for screening[18]. He suggested that, to obtain unbiased estimates of the efficacy of screening, the time window should be restricted to the pre-invasive period with exclusion of the occult invasive phase[18]. Inclusion of the occult invasive phase would result in an underestimation of the number of screening tests among cases since a positive test in the occult period would be the diagnosis (shortening the period at risk for cases) and the protective effect of screening would be overestimated. Since cases in our study were quite as much screened as controls, the problem of underestimation of tests among cases does not seem to be a big issue in our study.
Another drawback of our study is the lack of documented DES exposure for the majority of participants. In our study all women considered themselves to be DES daughters and thus were eligible for DES screening even though the exposure of their mothers was not medically confirmed. The presence or absence of verified DES exposure might have influenced screening behavior. However, we adjusted for this, because we only included cases and controls who were aware of DES exposure (verified or suspected) before diagnosis, so that they were eligible for participation in the DES screening program, comprising of both cervical and vaginal cytological examinations. In an earlier paper we described our validation study among 115 participants in which we could verify DES exposure in 76% of the women. Yet, external validity might have been affected by the incompleteness of validated DES exposure, resulting in screening results that more resemble the general population.

Finally, although we used registered and validated medical information on screening, screening information for the early diagnosed cases might have been incomplete. Registration of smears and biopsies in PALGA did not reach near national coverage until 1989[34]. However, quite a number of pathology laboratories were already connected to PALGA before 1989, with the first laboratory connected in 1978[34]. When we restricted our analyses to cases and

matched controls diagnosed from 1996 onwards, similar associations were found, indicating that the information bias due to incomplete screening histories was at most non-differential.

In conclusion, screening of DES daughters seems to be less effective in preventing invasive vaginal/cervical cancer (neither squamous cell nor CCA) than generally assumed, although the majority of cancers was detected at stage I implying a good prognosis. As women with DES-related malformations more often present with dyskaryotic smears, which appear to be associated with an increased risk of vaginal/cervical cancer despite intensive surveillance, careful monitoring of DES daughters with malformations seems to be justified.

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Supplement Table 1 Dutch guidelines 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§	-	-

* 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)

Supplement Table 2 Follow-up of 14 women with HSIL cytology during screening

	Case/ control	Abnormal smear*	Follow-up of abnormal smear (time-window 5.5-0.5 years before diagnosis)	Initial follow-up examination		Initial treatment	
				Type	Timing	Type	Timing
1	Case	Moderate dyskaryosis	FIRST abnormal smear (outside time window): Biopsy from transformation zone: CIN3. Treated with laser therapy (destruction of the lesion), followed by yearly cytology	biopsy (CIN3)	<1month	laser	<1month
	Case	Moderate dyskaryosis	LAST abnormal smear: within 2 weeks LETZ treatment (CIN2), after 8 months curretment (CIN3)	biopsy (CIN2)	<1month	lis excision	<1month
2	Case	Carcinoma in situ	Biopsy: CIN2. Large loop excision, followed by yearly cytological follow-up (in past portio treated with cryo-coagulation)	LETZ (CIN2)	<1month	LETZ	<1month
3	Case	Severe dyskaryosis	Biopsy: CIN1/2 followed by conisation (CIN3). Susuquently yearly cytology (pap2)	biopsy (CIN1/2)	<1month	conus	<2months
4	Case	Carcinoma in situ	Biopsy: CIS, followed by conisation. Cytology each half year. Second conisation (after two years from the first CIS: CIN3)	biopsy (CIS)	<1month	curretment and conus	<2months
5	Case	Severe dyskaryosis	Biopsy from endocervix and vagina: infection, followed by cytology each half year	biopsy (inflammation)	1.7 monhts	no treatment, cytological fup	
6	Case	Moderate dyskaryosis	Cytology (after year) with HSIL, followed by large loop excision within 3 months (CIS), conisation (micro-invasive carcinoma), before time window biopsy	cytology (pap4)	13 months	LIS excision	>1 year
7	Control	Severe dyskaryosis	Biopsy, followed by 6-monthly cytology, in total 6 biopsies in a period of three years), persisting pap3a (large loop excision date unknown)	biopsy (CIN2)	<1month	cytological fup	<4 months
8	Control	Moderate dyskaryosis	No biopsy, 6-monthly cytology	cytology (Pap 2)	3.9 months	no treatment, cytological fup	
9	Control	Moderate dyskaryosis	Cytology (after month), biopsy (after 3 months from HSIL) followed by 6-monthly cytology first three years, then yearly	cytology (mild dyspl)	1.5 months	no treatment cytological fup	
10	Control	Moderate dyskaryosis	No biopsy, yearly cytology	cytology	11 months	no treatment, cytological fup	
11	Case	Carcinoma in situ	FIRST abnormal smear: Biopsy (CIN2) and conus cervix (CIN3), biopsy vagina (norm) and subsequently 3-monthly examinations (pap1, pap3b, pap2, pap3a)	cytology (pap4)	1.7 monhts	conus	<2 months
		Moderate dyskaryosis	LAST abnormal smear: followed by cytology at 3 months and at 7 months (from initial diagnosis)	cytology (pap2)	<3 months	no treatment, cytological fup	
12	Case	Moderate dyskaryosis	No biopsy, 6-monthly cytology (first two years) , ending with an interval of 3 years preceding diagnosis with no smears	cytology (pap2)	3.5 months	no treatment, cytological fup	
13	Case	Moderate dyskaryosis	FIRST abnormal smear: Cytology (twice a year), then yearly (pap3a), 2 years before diagnosis again a biopsy (CIN3), followed by cytology each year (before time window biopsy)	cytology (inflammation)	5.8 monhts	no treatment, cytological fup	
	Case	Moderate dyskaryosis	LAST abnormal smear followed by cytological examination (> 1 year)	cytology (pap4)	> 1year	no treatment, cytological fup	
14	Case	Moderate dyskaryosis	Cytology (3 months), biopsy (after 4 months), followed by exconisation (diagnosis)	cytology (severe dyspl)	3.2 months	exconisation	<7 months

* abnormal smear defined as moderate dyskaryosis or worse (≥pap3a2) † calculated from date of diagnosis abnormal smear

Supplement Table 3 Treatment guidelines of cervical intra-epithelial neoplasia in the Netherlands

Cytological result	Follow-up	Colposcopy	Colposcopic finding	Follow-up examination	Treatment of histological confirmed CIN
LSIL low grade intra-epithelial lesion	Repeated smears at 6 and 18 months			Cytological	
HSIL high grade intra-epithelial lesion	Immediate referral gynaecologist for colposcopy (within 6 weeks)	Sufficient colposcopy (complete transformation zone a vue)	Normal	Cytological follow-up by general practitioner (at 12 months)	CIN I: no treatment CIN II/III: large loop excision (or destruction) Suspected micro-invasive carcinoma or carcinoma in situ: conization
			Atypical transformation zone	Histology ectocervix	
		Insufficiënt colposcopy	Transformation zone not completely a vue	Histology ecto/endo cervix	

Sources:
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Supplement Table 4 Description screening history of all 39 patients, sorted by morphology, topography and age of diagnosis

									5- 2 yrs before diagnosis										
							Screened‡		SM		B		Outcome¶						
	Morf*	Topo	Age	Stage	D	Malformations†	Yr§	y/n	v	c	v	c	1	2	3	4	5	6	7
1	CCA	C	15-19	2A	no	No	0,0	no											
2	CCA	C	20-24	1A	no	epith changes	1,0	yes											
3	CCA	C	20-24	1B	no	epith/struct	1,5	yes											
4	CCA	C	30-34	2A	no	No	8,9	yes	0	2	0	0	pap2	pap2					
5	CCA	C	15-19	2A	no	epith changes	2,0	yes											
6	CCA	V	35-39		yes	No	7,8	yes	3	2	0	0	pap1	pap1	pap1	pap2	pap2		
7	CCA	V	35-39	1	no	No	3,1	yes	0	1	0	0	pap1						
8	CCA	V	35-39	1	no	No	17,8	yes	0	1	0	0	pap1						
9	CCA	V	35-39	1	no	epith changes	5,2	yes	0	1	0	1	pap1	metapl					
10	CCA	V	30-34	1	no	struct malf	13,2	yes	2	2	0	0	pap2	pap3a1	pap2	aden			
11	CCA	V	25-29	1	no	epith/struct	4,6	yes	0	4	1	0	pap1	pap1	aden	pap1	pap2		
12	CCA	V	35-39	1	no	epith/struct	2,8	yes	0	1	0	0	pap1						
13	CCA	VC	40-44	2A	no	struct malf	12,4	yes	0	1	0	0	pap1						
14	CCA	V	35-39	X	yes		16,5	yes	0	2	0	0	pap2	pap1					
15	SCC	C	35-39	1	no	No	0,0	no											
16	SCC	C	40-44	1B	no	struct malf	0,1	no											
17	SCC	C	40-44	2A	no	No	0	no											
18	SCC	C	30-34	1A	no	No	6,2	yes	0	1	0	0	pap3a1						
19	SCC	C	25-29	1A	no	No	11,7	yes	0	5	0	1	pap3a2	pap3a2	pap3a1	pap3a2	pap3a2	CIN3	
20	SCC	C	35-39	1A	no	epith changes	3,7	yes	0	1	1	0	pap1	norm					
21	SCC	C	35-39	1A	no	n.s.	5,2	yes	0	2	0	0	pap2	pap2					
22	SCC	C	50-54	1A	no	epith/struct	27	yes	2	3	0	1	pap1	pap1	pap1	norm	pap1	pap1	
23	SCC	C	35-39	1A	yes		8,6	yes	0	5	0	2	pap3b	pap3b	CIN2	CIN3	pap2	pap2	pap2
24	SCC	C	30-34	1B	no	n.s.	9,2	yes	9	10	1	1	pap4/pap1, CIN3/norm, pap1, pap1, pap1/pap1, pap3b, pap3a1, pap3a2/pap1, pap3a2, pap1, pap3b/pap1, pap3a2, pap3b/pap1, pap2/ pap2						
25	SCC	C	30-34	1B	no	No	0,9	yes											
26	SCC	C	25-29	1B	no	struct malf	4,4	yes	0	1	0	0	pap1						
27	SCC	C	25-29	1B	no		5,3	yes	0	5	0	2	pap1	pap4	in situ	in situ	pap1	pap1	pap1
28	SCC	C	30-34	1B	no		16,3	yes	1	1	0	0	pap1	pap3a1					
29	SCC	C	30-34	X	no	No	11,5	yes	2	2	0	0	pap1	aden	pap1	aden			
30	SCC	V	35-39	1	no	struct malf	12	yes	0	2	0	0	pap1	pap2					
31	SCC	V	40-44	4	no	No	3,4	yes	0	1	0	0	pap1						
32	OTH	C	40-44	1	no	epith changes	0,0	no											
33	OTH	C	30-34	1B	no	No	0,1	no											
34	OTH	C	30-34	1	no	No	7,4	yes											
35	OTH	C	40-44	1	yes		9,0	yes	0	2	0	0	pap1	pap3a1					
36	OTH	C	50-54	1	no		19,7	yes	0	1	0	0	pap1						
37	OTH	C	35-39	1A	no	No	3,3	yes	0	1	0	0	pap1						
38	OTH	C	35-39	1A	no	epith/struct	11,4	yes	0	4	0	0	pap3a2	pap3a1	pap1	aden			
39	OTH	C	25-29	1B	no	epith/struct	11,0	yes	1	0	0	1	pap3a2	in situ					

Abbreviations used: D=deceased,T=topography,M=morphology,SM=smear,B=biopsy,V=vagina, C=cervix, OUTC=maximum outcome, Dx=date of diagnosis. Underscored cell=examination at same date. *Morphology: CCA=clear cell adenocarcinoma, SCC=squamous cell carcinoma, OTH=adenocarcinoma and adenosquamous cell carcinoma. †Definition malformations. Struct malf=structural malformations: cox comb, vaginal ridges and T-shaped uterus. Epith=epithelial changes: adenosis, squamous metaplasia ‡Definition of screening : having had a vaginal or cervical pap-smear recommended screening interval: 5.5-0.5 years before diagnosis. §Screening years calculated as interval between first examination noted in PALGA (smear or biopsy) and date of diagnosis. ¶Cytological outcomes: Pap1, Pap2, Pap3a1 (mild dyskaryosis), MSD=moderate (pap3a2) or severe dyskaryosis (pap3b) or carcinoma in situ (cytology). Histological outcomes: Norm=normal, aden =adenosis, CIN= cervical intra-epithelial neoplasia. CIN1=Mild dyskaryosis, CIN2=moderate dyskaryosis, CIN3=severe dyskaryosis , CIS=cervical cancer in situ

Supplement Table 4, continued

	2 - 1 years before diagnosis								1-0,5 year before diagnosis						0,5 year before diagnosis - diagnosis							
	SM		B		Outcome¶				SM		B		Outcome¶		SM		B		Outcome¶			
	v	c	v	C	1	2	3	4	v	c	v	c	1	2	v	c	v	c	1	2	3	4
1															0	1	0	1	pap0		cancer	
2	0	1	0	0	pap3b				0	1	0	1	norm	pap1	1	2	0	1	pap1	pap3b	pap3b	CIN1
3	1	0	0	0	aden																	
4	0	1	0	0	pap2																	
5	0	1	0	0	pap1																	
6	1	1	0	0	pap1	pap1			1	1	0	0	pap1	pap1	1	0	0	0	pap0			
7																						
8																						
9	0	1	0	0	pap1																	
10	1	1	0	0	pap1	aden																
11															0	1	0	0	pap1			
12																						
13															0	1	0	0	pap3b			
14	1	1	0	1	pap1	norm	pap1		1	1	0	0	pap1	pap1								
15																						
16															0	1	0	1	pap4	CIN3		
17																						
18																						
19	0	1	0	0	pap3a2																	
20	0	1	0	0	pap3a2																	
21	0	1	0	0	pap2										1	2	1	0	aden	pap4	normal	MSD
22	1	0	0	0	pap1				0	1	0	1	pap4	CIN2	0	0	0	1	CIN3			
23	0	1	0	0	pap2										0	1	0	0	pap3a1			
24	2	1	0	0	pap2	pap2	pap2		1	2	0	0	pap3a1	pap3a2	2	2	0	0	pap3a2	pap1	pap3b	MSD
25									1	1	0	0	pap2	pap1								
26	0	2	0	0	pap3a1	pap3a1			0	0	0	1	norm		0	1	0	1	pap3a1	CIN3		
27	0	2	0	0	pap3b	pap4			0	0	0	1	in situ		0	1	0	1	pap3b	in situ		
28	1	2	0	0	pap0	pap1	pap1															
29	1	0	0	0	pap3a1				1	0	0	0	aden									
30	0	2	0	1	pap3a1	CIN1	pap1		0	1	0	0	pap2		0	1	0	0	pap3a2			
31									0	0	1	0	CIN2		0	0	1	0	cancer			
32																						
33															0	1	0	0	pap3b			
34									0	1	0	0	pap3a2		0	1	0	1	pap3b	CIN3		
35	0	2	0	0	pap3a1	pap1									0	1	0	0	pap4			
36																						
37	0	1	0	0	pap2										0	1	0	0	pap2			
38																						
39	2	2	0	0	pap1	pap1	pap1	pap1							1	1	0	0	pap3b	pap0		