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ORIGINAL RESEARCH ARTICLE

Benefits and potential harms of human papillomavirus (HPV)-based cervical cancer screening: A real-world comparison of HPV testing versus cytology

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Abstract

Introduction: Human papillomavirus (HPV) testing as the primary cervical cancer screening method is implemented in several countries. We report data from the first round of a large Danish pilot implementation of HPV-based screening. Our aim was to compare colposcopy referrals, detection of high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer, and positive predictive value (PPV) of colposcopy referral in HPV vs cytology-based screening.

Material and methods: From May 2017 to October 2018, women aged 30-59 years attending cervical cancer screening in the uptake area of the Department of Pathology, Vejle Hospital, Region of Southern Denmark were screened by primary HPV testing (n = 16 067) or primary cytology (n = 23 981) depending on municipality of residence. In the HPV group, women with HPV16/18, or other high-risk HPV types and abnormal cytology, were referred to immediate colposcopy. Women with other high-risk HPV types and normal cytology were invited for repeat screening with HPV test and cytology after 12 months. From a nationwide pathology register, we obtained information on screening results and subsequent histological diagnoses during up to 2.9 years after the first screen. PPVs included diagnoses within 1 year after referral.

Results: In the HPV group, 3.7% were referred to immediate colposcopy and 2.8% were referred at the 12-month repeat screening. The total referral to colposcopy was higher in the HPV (6.6%) than cytology group (2.1%) (age-adjusted relative referral = 3.05, 95% confidence interval [CI] 2.75-3.38). The detection of CIN3+ was higher in the HPV (1.5%) than the cytology group (0.8%) (age-adjusted relative detection = 1.88, 95% CI 1.56-2.28). The probability of $CIN3^+$ among women referred to colposcopy (= PPV) was lower in the HPV (21.1%; 95% CI 18.7%-23.7%) than the cytology group (34.6%; 95% CI 30.7%-38.9%). In the HPV group, the PPV was lower among women referred at repeat screening (12.1%) than among women referred immediately (27.8%).

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion; PPV, positive predictive value; RR, relative risk.

Conclusions: Compared with cytology-based screening, HPV-based screening provided a 90% increased CIN3+ detection at the cost of a threefold increase in colposcopy referrals, when considering complete data from the prevalence round. Our findings support implementation of HPV-based screening in Denmark, but modifications of screening algorithms may be warranted to decrease unnecessary colposcopy referrals.

KEYWORDS
cervical cancer, cervical intraepithelial neoplasia, HPV testing, human papillomavirus, prevention, screening

Key message

In a large Danish pilot implementation of human papillomavirus (HPV)-based cervical cancer screening, HPV-based screening detected more cases of cervical precancerous lesions compared with screening by cervical cytology, but also required approximately three times as many referrals to colposcopy.

1 | INTRODUCTION

For more than 75 years, prevention of cervical cancer has been possible through early detection of precancerous lesions using cervical cytology. More recently, the realization that virtually all cervical cancers are caused by high-risk types of human papillomavirus (HPV) has led to increasing use of HPV testing in cervical cancer screening.[1] Randomized trials show that HPV testing detects more cervical precancers[2,3] and prevents more cervical cancers[4] than cytology-based screening does. Consequently, several countries have implemented or are planning to implement HPV testing for primary cervical cancer screening of women aged ≥25-30 years.[5,6]

A drawback of HPV testing, however, is that most HPV infections are transient,[1] and therefore HPV-based screening has lower specificity[2] and may increase colposcopy referrals compared with cytology-based screening.[7,8] Unnecessary colposcopies should be avoided because they can be associated with psychosocial and physical discomfort[9,10] and may ultimately lead to overtreatment of regressive cervical intraepithelial neoplasia (CIN).[11]

As countries transition to primary HPV screening, there is an increasing need for evidence on the performance of HPV-based screening in a real-world setting, in contrast to a controlled research setting. Results from randomized trials may not be transferable to the routine clinical setting, due to selection bias in recruitment and stringent follow-up protocols in clinical trials.[12] Furthermore, since HPV prevalence and risk of cervical (pre)cancer differs between populations, policy-makers require national data to inform country-specific evidence-based screening algorithms.

In Denmark, nationwide organized cervical cancer screening has been in place since the mid-1990s, with cytology as the primary screening method.[13] In 2018, a working group under the National Board of Health recommended a gradual implementation of HPV-based screening for women aged 30-59 years.[14] This transition started on 1 January 2021, but limited national data are available to inform the introduction of HPV-based screening in Denmark.[15]

In 2017, we initiated the first Danish pilot implementation of HPV-based screening (HPV SCREEN DENMARK). We have previously reported the cross-sectional baseline results from the pilot.[15] In the present prospective study, we extend our analysis with a longer follow-up period, including results both of referral at baseline and after 12-month repeat screening. Our aim was to compare the proportion of women referred to colposcopy, the detection of high-grade CIN and cervical cancer, and the positive predictive value (PPV) of colposcopy referral in HPV vs cytology-based screening.

2 | MATERIAL AND METHODS

2.1 | Study population

The design of HPV SCREEN DENMARK has been described previously.[15] This implementation study has been ongoing since 29 May 2017 and is embedded in the routine screening program at the Department of Pathology, Vejle Hospital, Region of Southern Denmark. Women aged 30-59 years attending cervical cancer screening in the department's uptake area receive either HPV or cytology-based screening depending on their municipality of residence. Women from four municipalities receive HPV-based screening and women from nine municipalities receive cytology-based screening (Table S1). This analysis includes women screened from 29 May 2017 to 29 October 2018 (n = 40 146).

2.2 | Sample processing

Liquid-based cervical cytology samples (ThinPrep, Hologic) were obtained in routine clinical practice by general practitioners or

gynecologists and sent to Vejle Pathology Department for processing. HPV DNA testing was performed using the Cobas 4800 HPV test (Roche), which is a PCR-based assay detecting 14 high-risk HPV (hrHPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). Results for HPV16 and HPV18 are reported separately, whereas the 12 other hrHPV types are reported as a pooled result.[16] HPV mRNA testing was performed using the Aptima HPV test (Hologic), which detects E6/E7 mRNA of the same 14 hrHPV types.[17] Cytology was classified according to Bethesda.[18]

2.3 | Clinical management

In the HPV group (Figure 1A), women with HPV16/18 according to the Cobas test were referred to immediate colposcopy regardless of cytology. Women with one of the 12 other hrHPV types and a cytology result of atypical squamous cells of undetermined significance or worse (≥ASCUS) were also referred to colposcopy. Women with other hrHPV types and normal cytology were invited for repeat screening with HPV DNA testing and cytology after 12 months. At the repeat screen, they were referred to colposcopy if they were hrHPV DNA-positive (regardless of HPV type) or had ≥ASCUS.

In the cytology group, women were managed according to Danish national screening guidelines (Figure 1B).[14,19] Women with high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells – cannot exclude HSIL, atypical glandular cells or adenocarcinoma in situ were referred to immediate colposcopy. Women with low-grade squamous intraepithelial lesions (LSIL) received hrHPV mRNA testing, and those with hrHPV mRNA-positive LSIL were referred to immediate colposcopy; those with hrHPV mRNA-negative LSIL were invited for repeat cytology after 12 months. Women with ASCUS received hrHPV DNA testing, and those with hrHPV DNA-positive ASCUS were referred to immediate colposcopy, while those with hrHPV DNA negative ASCUS returned to routine screening. At the 12-month repeat screen, women with ≥ASCUS were referred to colposcopy.

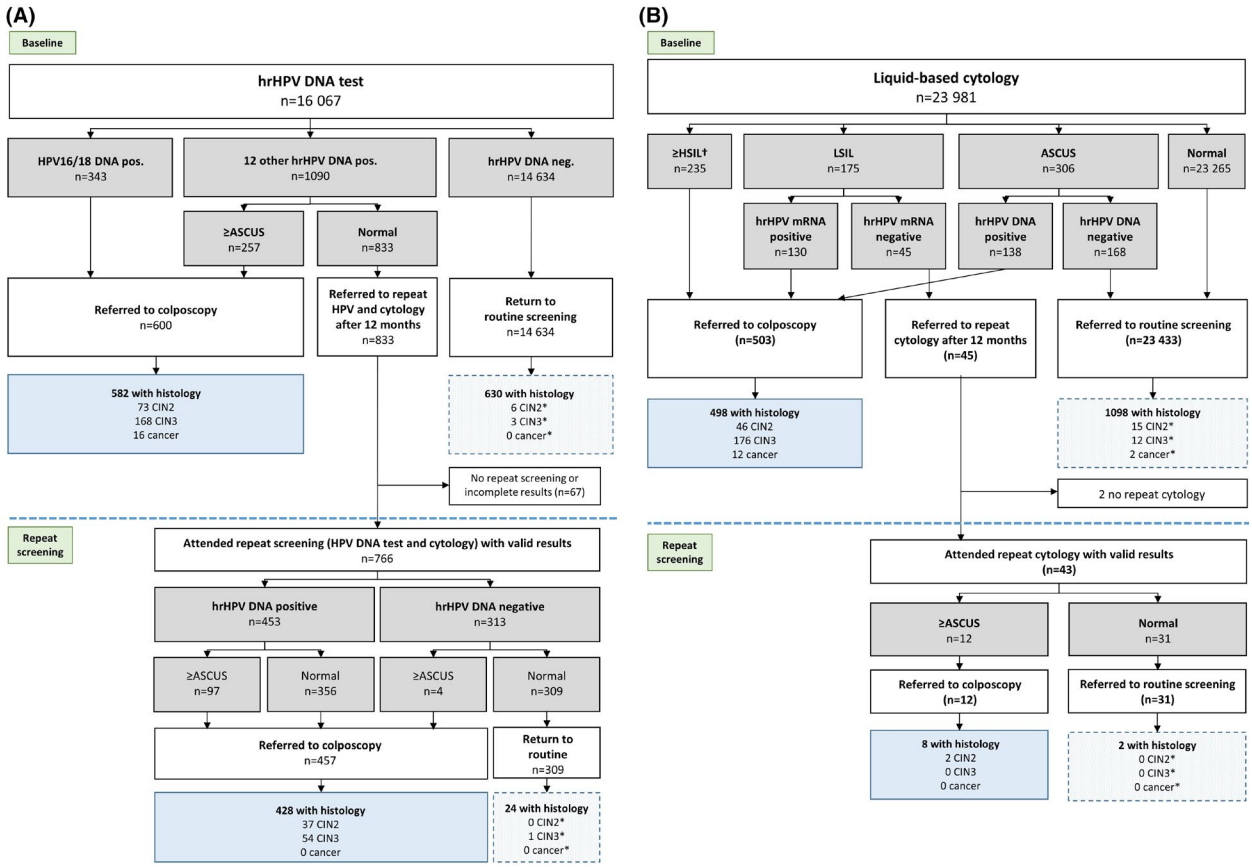

FIGURE 1 (A) Flowchart of women in the HPV group, included in HPV SCREEN DENMARK from May 2017 to October 2018. (B) Flowchart of women in the cytology group, included in HPV SCREEN DENMARK from May 2017 to October 2018. †≥HSIL includes HSIL, atypical squamous cells – cannot exclude HSIL, atypical glandular cells, adenocarcinoma in situ and cancer; *Shaded boxes with dashed borders indicate CIN2+ cases diagnosed outside protocol, i.e. in women not referred to colposcopy according to protocol. ASCUS, atypical squamous cells of undetermined significance; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; DNA, deoxyribonucleic acid; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion, mRNA, messenger ribonucleic acid [Color figure can be viewed at wileyonlinelibrary.com]

2.4 | Histological follow-up and retrieval of registry data

Colposcopies with biopsies and potential subsequent conizations or hysterectomies were performed in routine practice by hospital-based or practicing gynecologists. Histological diagnoses were classified by the CIN nomenclature. Results of screening visits and histologic follow-up were registered in the nationwide Pathology Databank, an online database used in daily clinical practice by all pathology departments in Denmark.[20] Test results and diagnoses are recorded under each woman's unique personal identification number, which is assigned to all residents in Denmark. For this analysis, we retrieved data from the Pathology Databank until 30 April 2020 (ie 18-35 months of follow-up for the included women).

2.5 | Statistical analyses

Baseline was defined as the date of first screening visit with a valid result during the study period. We calculated the proportion of women referred to colposcopy at baseline and after 12-month repeat screening in the HPV and cytology groups, overall and by age (30-39, 40-49, 50-59 years). Log-binomial regression models were used to estimate the relative risk (RR) with 95% confidence intervals (CIs) of colposcopy referral in the HPV compared with cytology group, adjusting for age as a linear variable. We also computed the absolute difference in number of referrals per 1000 women screened in the HPV vs cytology group.

We furthermore calculated the proportion of women with CIN grade 2 or more severe (CIN2+) or CIN grade 3 or more severe (CIN3+) diagnosed up to 35 months (~2.9 years) after baseline in the HPV compared with cytology group. We used the worst histological diagnosis during follow-up as outcome, and we included only "per-protocol detected" cases, that is, cases in women referred to colposcopy at baseline or after 12-month repeat screening. Cases diagnosed outside protocol are shown in Figure 1A,B. We considered CIN3+ the main outcome because CIN2 frequently regresses and has low reproducibility.[21,22] We included CIN2+ as a secondary outcome because it is less influenced by treatment than CIN3+. We used log-binomial regression to estimate the RR of CIN2+ or CIN3+ detection in the HPV compared with cytology group, adjusting for age. We also calculated the absolute difference in number of cases detected per 1000 women screened.

Finally, to evaluate the PPV of colposcopy referral, we used Kaplan-Meier's method to estimate the absolute risk of CIN2+ or CIN3+ among women referred to colposcopy at baseline or repeat screening in the HPV and cytology groups. We defined PPV as the absolute risk 12 months after referral, which we considered to be the risk of prevalent CIN2+/CIN3+ among women referred. In the Kaplan-Meier analysis, follow-up time was calculated from baseline or date of repeat screening until first occurrence of CIN2+/CIN3+ or end of follow-up (30 April 2020).

2.6 | Ethical approval

The implementation was considered by the Health Research Ethics Committee in the Region of Southern Denmark to be an implementation study in routine practice exempt from informed consent (S-20160146, 11 October 2016). Data collection was approved by the Data Protection Agency in the Region of Southern Denmark (18-21475). Retrieval of data from the Pathology Databank was approved by the National Board of Patient Safety (3-3013-2597).

3 | RESULTS

3.1 | Study population

A total of 40 146 women were screened at Vejle Pathology Department between 29 May 2017 and 29 October 2018 (HPV group: n = 16 079; cytology group: n = 24 067). We excluded 98 women with invalid screening tests, leaving 40 048 women (HPV group: n = 16 067; cytology group: n = 23 981). The median age was similar in the HPV (43 years; interquartile range [IQR] 37-49) and cytology groups (44 years; IQR 37-50). The distribution of time from baseline to end of follow-up (30 April 2020) was identical in the two groups (median 27 months, IQR 22-31 months, range 18-35 months).

Figure 1A,B provides an overview of screening results and histologic outcomes during follow-up. In the HPV group, 343 women (2.1%) had HPV16/18, and 257 (1.6%) had other hrHPV types and ≥ASCUS at baseline. In the cytology group, 235 women (1.0%) had ≥HSIL, 130 (0.5%) had hrHPV mRNA-positive LSIL, and 138 (0.6%) had hrHPV DNA-positive ASCUS. Of the women referred to colposcopy at baseline, the vast majority in both the HPV (582/600; 97%) and cytology groups (498/503; 99%) had a histology during follow-up, with a median time to first histology of 46 days (IQR, 29-74) in the HPV group and 34 days (IQR, 22-50) in the cytology group. Of the women referred to 12-month repeat screening, the majority in both the HPV (766/833; 92%) and cytology groups (43/45; 96%) attended. The median time from baseline to repeat screening was 385 days (IQR 347-463) in the HPV group and 391 days (IQR 311-462) in the cytology group. Of those referred to colposcopy after the repeat screen, a high proportion had a subsequent histology (HPV group: 428/457 [94%]; cytology group: 8/12 [67%]).

3.2 | Colposcopy referrals

Table 1 shows the proportion of women referred to colposcopy in the HPV and cytology groups. In the HPV group, 3.7% were referred at baseline and 2.8% at repeat screening, yielding a total colposcopy referral of 6.6%. In the cytology group, 2.1% were referred at baseline and 0.1% at repeat screening, yielding a total referral of 2.1%.

TABLE 1 Referrals to colposcopy at baseline and repeat screening, overall and according to age, in the HPV and cytology groups of HPV SCREEN DENMARK

	HPV group							Cytology group							HPV versus cytology		
		Referrals to colposcopy							Referrals to colposcopy								
		Baseline		Repeat screen		Total			Baseline		Repeat screen		Total		RR of colposcopy referral		Absolute difference[b]
Age	No. screened	n	%	n	%	n	%	No. screened	n	%	n	%	n	%	RR[a]	95% CI	
All	16 067	600	3.7	457	2.8	1057	6.6	23 981	503	2.1	12	0.1	515	2.1	3.05	2.75–3.38	44
30-39 years	5349	288	5.4	191	3.6	479	9.0	8023	252	3.1	2	0.0	254	3.2	2.83	2.45–3.29	58
40-49 years	6831	221	3.2	184	2.7	405	5.9	9896	180	1.8	8	0.1	188	1.9	3.12	2.63–3.70	40
50-59 years	3887	91	2.3	82	2.1	173	4.5	6062	71	1.2	2	0.0	73	1.2	3.64	2.79–4.80	32

Abbreviations: CI, confidence interval; $CIN2^+$, cervical intraepithelial neoplasia grade 2 or more severe; $CIN3^+$, cervical intraepithelial neoplasia grade 3 or more severe; HPV, human papillomavirus; RR, relative risk.

[a]Including colposcopy referrals at baseline and repeat testing, adjusted for age.

[b]Additional referrals per 1000 women screened.

The age-adjusted RR of referral was 3.05 (95% CI 2.75-3.38) in the HPV compared with the cytology group. In absolute numbers, an additional 44 women were referred in the HPV group for every 1000 women screened. The proportion of women referred was highest in younger women. In the HPV group, 9.0% of women aged 30-39 years were referred to colposcopy.

3.3 | Detection of CIN3+ and CIN2+

Table 2 shows the detection of CIN3+ and CIN2+ during up to 35 months of follow-up in each group. The detection of CIN3+ (RR = 1.88, 95% CI 1.56-2.28) and CIN2+ (RR = 2.19, 95% CI 1.86-2.59) was higher in the HPV than the cytology group. This pattern was consistent in all age groups, although the estimate for $CIN3^+$ in women aged 50-59 years was not statistically significant. Per 1000 women screened, seven more cases of CIN3+ and 12 more cases of CIN2+ were detected in the HPV group.

3.4 | Positive predictive value

Table 3 shows the estimated PPV of colposcopy referral in the HPV and cytology group, overall and separately for referrals at baseline or repeat screening. The overall PPV for CIN3+ was lower in the HPV (21.1%; 95% CI 18.7%-23.7%) than the cytology group (34.6%; 95% CI 30.7%-38.9%). The PPV decreased with age in both groups, but was lower in the HPV group at all ages. In the HPV group, the PPV for CIN3+ was higher among women referred at baseline (27.8%; 95% CI 24.4%-31.6%) than among women referred at repeat screening (12.1%; 95% CI 9.3%-15.5%). The lowest PPV was seen in women aged 50-59 referred after repeat screening in the HPV group, of whom <5% had CIN3+ within a year after referral. The patterns were similar for the outcome of CIN2+. The Kaplan-Meier curves used to estimate the PPVs are presented in Figures S1-S2, showing that the patterns between groups were similar irrespective of the selected time point during follow-up.

4 | DISCUSSION

This paper reports results from the first round of a regional Danish pilot implementation of HPV-based cervical cancer screening, including data on referrals and histological outcomes up to 2.9 years after the first screen. We found that HPV-based screening detected 90% more CIN3+ cases than cytology-based screening did, but this increased detection came at the cost of a threefold increase in colposcopy referrals. In absolute numbers, HPV-based screening detected seven additional CIN3+ cases and required 44 additional colposcopy referrals per 1000 women screened, compared with cytology-based screening.

In our first paper on HPV SCREEN DENMARK,[15] we reported similar trends when considering only immediate colposcopy referrals

TABLE 2 Detection of CIN3+ and CIN2+ up to 2.9 years of follow-up, overall and according to age, in the HPV and cytology groups of HPV SCREEN DENMARK[a]

		HPV group			Cytology group			HPV vs cytology		
Outcome	Age	Screened (n)	Screen positive (n)	%	Screened (n)	Screen positive (n)	%	RR	95% CI	Absolute difference[b]
CIN3+	All	16 067	238	1.5	23 981	188	0.8	1.88	1.56–2.28	7
	30-39 years	5349	141	2.6	8023	114	1.4	1.86	1.46–2.38	12
	40-49 years	6831	75	1.1	9896	52	0.5	2.08	1.47–2.97	6
	50-59 years	3887	22	0.6	6062	22	0.4	1.52	0.84–2.74	2
CIN2+	All	16 067	348	2.2	23 981	236	1.0	2.19	1.86–2.59	12
	30-39 years	5349	191	3.6	8023	134	1.7	2.15	1.73–2.67	19
	40-49 years	6831	124	1.8	9896	74	0.7	2.42	1.82–3.24	11
	50-59 years	3887	33	0.8	6062	28	0.5	1.81	1.09–3.00	4

Abbreviations: CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or more severe; CIN3+, cervical intraepithelial neoplasia grade 3 or more severe; HPV, human papillomavirus; RR, relative risk.

[a]Including only per-protocol detected CIN2+ and CIN3+, that is, cases detected among women referred to colposcopy at baseline or repeat screening.

[b]Additional cases of CIN2+ and CIN3+ detected per 1000 women screened.

at baseline and subsequent histological outcomes within 6 months. The present analysis shows that the differences between HPV and cytology-based screening are augmented when considering both results of referrals at baseline and after 12-month repeat screening.

The finding of increased CIN3+ detection with HPV-based screening is in line with results of randomized trials[2,3] and other European implementation studies.[7,8,23-27] The increased CIN detection in our study (≈90% for CIN3+, ≈220% for CIN2+) was higher than in the Dutch HPV-based program (30% increase in CIN2+)[8,23] and the English pilot (40% increase in CIN2+),[7] but similar to the increase in a Finnish implementation study (250% increase in CIN2+).[24]

In accordance with our results, other implementation studies also found increases in colposcopy referrals when HPV-based screening was introduced.[6-8,23,25-27] The overall colposcopy referral rate in our HPV group (6.6%) was higher than in the Dutch screening program (3.9%-4.2%),[8,23] although the hrHPV prevalence in the Dutch population (9.2%) was similar to ours (8.9%). Likewise, referral rates in the Australian, Finnish and Italian programs were lower than ours (2.6%-4.6%).[6,24,28] The high referral rate in our study is likely influenced by our highly sensitive and conservative screening algorithm: We referred all women with HPV16/18 (regardless of cytology) at baseline, and at 12-month repeat screening we performed co-testing and referred all women with hrHPV or ≥ASCUS. In contrast, most European programs refer only women with abnormal cytology at baseline, and at the repeat screen most programs perform only cytology (eg Netherlands) or only HPV testing (eg Italy).[5,8,28] It should be noted that we found a particularly high colposcopy referral rate in the youngest women (30-39 years) (9.0%). This is concerning, since these women may still be child-bearing and are thus at risk of obstetric-related complications, for example preterm birth, after treatment for high-grade CIN.[29]

We also found that the differences in referrals between HPV and cytology-based screening were especially pronounced at the repeat screen (HPV group: 2.1%; cytology group: 0.1%). More than 5% (833/16 067) of women screened in the HPV group were recommended for repeat screening, and 60% of those attending repeat screening (457/766) were referred to colposcopy. A recent English study showed that women referred to early recall after a screening result of "hrHPV-positive, normal cytology" exhibited increased levels of anxiety and worry.[30] This underlines the need for better triage algorithms in HPV-based screening, to reduce both the proportion of women requiring repeat screening and the proportion of colposcopy referrals at the repeat screen.

A further finding of our study was that the PPV of colposcopy referral was lower in the HPV group than the cytology group. This is consistent with some[23,25] but not all[7,24,26,27] previous implementations, reflecting that screening algorithms and underlying CIN3+ prevalences differ between countries. In our study, one of five (21.1%) women referred in the HPV group had CIN3+ detected within a year. The PPV in the HPV group was markedly lower for women referred at 12-month repeat screening (12.1%) than for those referred at baseline (27.8%), which reflects that women attending repeat screening were initially positive for other hrHPV types than HPV16/18; these types are known to carry a lower risk of CIN3+.[31]

Our observation that the PPV of HPV-based screening decreased with age is in agreement with findings from Norway,[32] Australia[33] and USA.[34] In our study, this was not explained by a differential HPV genotype distribution according to age, since the prevalence of HPV16/18 among women referred to colposcopy did not differ by age (data not shown). A potential explanation is that in well-screened populations such as ours, aggressive persistent HPV infections may be removed when women are screened at younger ages, and therefore hrHPV infections may be less likely to cause CIN3+ in older women than in younger women.[34] Another potential explanation is that changes in the transformation zone after menopause may make it more difficult to detect small CIN lesions during colposcopy in older women.[33] In our study, it was noteworthy that among women aged 50-59 years referred to colposcopy at repeat screening, only

TABLE 3 Positive predictive value of referral to colposcopy in the HPV and cytology group, overall and according to age, among women in HPV SCREEN DENMARK

		PPV at baseline					PPV at repeat screening					PPV total				
			CIN3+[a]		CIN2+[a]			CIN3+[a]		CIN2+[a]			CIN3+[a]		CIN2+[a]	
Age	Group	No. referred	%	(95% CI)	%	(95% CI)	No. referred	%	(95% CI)	%	(95% CI)		%	(95% CI)	%	(95% CI)
All	Cytology	503	35.4	(31.4–39.7)	45.3	(41.1–49.8)	12	0.0	_[b]	0.0	_[b]	515	34.6	(30.7–38.9)	44.3	(40.1–48.7)
	HPV	600	27.8	(24.4–31.6)	38.5	(34.7–42.5)	457	12.1	(9.3–15.5)	20.3	(16.9–24.4)	1057	21.1	(18.7–23.7)	30.7	(28.0–33.6)
30–39	Cytology	252	43.7	(37.8–50.0)	52.4	(46.4–58.7)	2	0.0	_[b]	0.0	_[b]	254	43.4	(37.5–49.7)	52.0	(46.0–58.3)
	HPV	288	33.7	(28.5–39.5)	45.1	(39.6–51.1)	191	18.6	(13.6–25.1)	25.2	(19.6–32.1)	479	27.7	(23.9–32.0)	37.4	(33.2–42.0)
40–49	Cytology	180	27.8	(21.8–34.9)	39.4	(32.7–47.0)	8	0.0	_[b]	0.0	_[b]	188	26.6	(20.9–33.5)	37.8	(31.3–45.1)
	HPV	221	24.4	(19.3–30.7)	35.7	(29.8–42.4)	184	8.8	(5.5–14.0)	21.1	(15.8–27.8)	405	17.4	(14.0–21.5)	29.1	(24.9–33.8)
50–59	Cytology	71	25.4	(16.8–37.2)	35.2	(25.4–47.5)	2	0.0	_[b]	0.0	_[b]	73	24.7	(16.3–36.2)	34.2	(24.6–46.3)
	HPV	91	17.6	(11.2–27.1)	24.2	(16.6–34.4)	82	4.6	(1.5–14.2)	7.4	(3.1–17.3)	173	11.4	(7.4–17.3)	16.0	(11.2–22.5)

Abbreviations: CI confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or more severe; CIN3+, cervical intraepithelial neoplasia grade 3 or more severe; HPV, human papillomavirus; PPV, positive predictive value.

[a]Includes cases diagnosed within 12 months after referral to colposcopy.

[b]Not computed due to no cases.

7% had CIN2+, and <5% had CIN3+. This is below commonly accepted risk-thresholds for colposcopy referral in Europe,[35] supporting that a less aggressive referral strategy at repeat screening may be warranted in this age group.

The strengths of this study include the population-based design and the embedment of the study into the routine Danish screening program, thus providing evidence on the real-life performance of HPV-based screening. All screening results and histologic outcomes were registered in a nationwide database,[20] which enabled us to include all subsequent cervical diagnoses throughout the country. Furthermore, we had a very high compliance rate with colposcopy (96%) and repeat screening (92%). Finally, since HPV screening was not yet implemented nationwide in Denmark, we were able to include a contemporary control group screened with cytology, rather than a historical comparison group, as some prior studies.[6,8,23,25-27]

A limitation of the study is that women were allocated to HPV or cytology-based screening based on geographic area rather than randomization. However, we have previously shown that the HPV and cytology groups were virtually identical in terms of socioeconomic characteristics and prior screening history.[15] Therefore, we consider it highly unlikely that our results are caused by underlying population differences. Another potential limitation is that colposcopy without biopsy is not registered in the Pathology Databank, and therefore we could not distinguish between women lost to follow-up and women attending colposcopy without biopsies taken. However, this should have limited impact on our results, since 96% of the women referred to colposcopy did have a histological sample. Finally, in the present analysis, we lacked statistical power to assess detection rates of CIN3+ in the HPV group according to HPV type and cytological diagnosis. Such analyses are important to inform management strategies for hrHPV-positive women[32,33] and will be reported from our study when more women have been included.

5 | CONCLUSION

This large pilot implementation supports the conclusion that introduction of HPV-based screening will lead to increased detection of CIN3+ and improved cervical cancer prevention in Denmark. However, adjustments of the screening algorithm are warranted to avoid excessive colposcopy referrals, which may cause unnecessary concern in women and can ultimately lead to overtreatment. Potential triage strategies include p16/ki-67 dual-staining[36] and delayed referral of women with non-16/18 hrHPV types and low-grade cytology.[6] Furthermore, continued monitoring of the clinical performance and cost-effectiveness of the program during the next screening round will be crucial, as both referrals and CIN detection rates are expected to decrease in the second round of HPV-based screening.[7,25]

CONFLICT OF INTEREST

Susanne K. Kjær has previously received speakers' fees and a research grant from Merck. All other authors declare no potential competing interests.

AUTHOR CONTRIBUTIONS

MW, SKK, LTT, DOE and CM initiated, planned and designed the study. MW, SKK and LTT obtained funding. MW organized the implementation of HPV-based screening. DOE was responsible for HPV testing and other laboratory procedures. LTT performed registry linkage, data management and statistical analyses. LTT wrote the first draft of the manuscript. All authors contributed to analysis and interpretation of results, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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