

# Clinical Performance of Human Papillomavirus (HPV) Testing versus Cytology for Cervical Cancer Screening: Results of a Large Danish Implementation Study

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**Background:** Human papillomavirus (HPV) testing is increasingly used as the primary cervical cancer screening test. In a large pilot implementation, we compared participation, referrals and detection of high-grade cervical intraepithelial neoplasia (CIN) in HPV- versus cytology-based cervical cancer screening.

**Methods:** The implementation was embedded into the routine screening program at Lillebaelt Hospital, Department of Pathology, Vejle, Denmark. Based on the area of residence, women aged 30–59 years were screened by either HPV testing (with HPV16/18 genotyping and cytology triage) or cytology (with HPV triage for minor abnormalities). Our analysis includes women invited or screened during May 2017–May 2018 (invited: n=35,081; screened: n=28,352) with 6 months of follow-up. Information on screening results and sociodemographic characteristics were obtained from registers. Using logistic regression, we estimated odds ratios (ORs) with 95% confidence intervals (CIs) of participation, referral and CIN3+–detection in HPV- versus cytology-based screening, adjusting for sociodemographic characteristics.

**Results:** Participation was virtually identical in the HPV- and cytology group (58.4% vs 58.8%; OR<sub>adjusted</sub>=0.97, 95% CI, 0.93–1.01). Referral to colposcopy was more common in the HPV- than cytology group (3.8% vs 2.1%; OR<sub>adjusted</sub>=1.88, 95% CI, 1.63–2.17). More cases of CIN3+ were detected in the HPV- than cytology group (1.0% vs 0.7%, OR<sub>adjusted</sub>=1.47; 95% CI, 1.13–1.91).

**Conclusion:** Participation did not differ between HPV- and cytology-based screening. HPV-based screening detected more cases of CIN3+, but in this initial screening round also led to more colposcopies than cytology-based screening.

**Keywords:** human papillomavirus, cervical cancer, cervical intraepithelial neoplasia, screening, HPV testing, cytology, secondary prevention

## Introduction

Screening for cervical cancer using cervical cytology has reduced the incidence of this disease in many countries.<sup>1</sup> Randomised trials have shown that testing for high-risk human papillomavirus (HPV) is more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN).<sup>2–8</sup> Therefore, HPV-based screening can prevent more cervical cancers.<sup>9</sup> HPV testing is, however, less specific than cytology.<sup>10</sup>

Several countries are currently implementing HPV-based screening for women aged >25–35 years.<sup>11–17</sup> On this basis, data on the performance of HPV-based screening in

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a real-world clinical setting, in contrast to a randomised research setting, are beginning to emerge.<sup>12,15,18-28</sup> However, only few of these studies compared HPV-based screening with a contemporary control group screened by cytology.<sup>12,18,19</sup>

In Denmark, national guidelines recommend screening every 3 years in women aged 23–49 and every 5 years in women aged 50–64.<sup>29</sup> Women aged 23–59 years are screened by cytology, while HPV-based primary screening is only recommended for women aged 60–64.<sup>29</sup> A National Steering Group for Cervical Cancer Screening was established in 2018 to plan a gradual implementation of HPV-based screening for women aged 30–59.<sup>30</sup> However, currently, no Danish data are available to inform these implementation efforts.

In 2017, we initiated the first Danish pilot implementation of HPV-based screening. This paper presents the first results from the pilot. We aimed to compare participation, proportions of unsatisfactory screening tests, colposcopy referrals and detection of high-grade CIN and cervical cancer in women screened with HPV testing compared with cytology.

## Methods

### Organization of Screening

Cervical cancer screening in Denmark is organized by the five regions that are responsible for implementing the screening guidelines from the National Board of Health. Screening is free of charge, and smears are obtained by general practitioners or gynecologists. A nationwide register, the Pathology Databank, contains information on screening invitations, reminders, and all cervical cytologies, biopsies, cones and hysterectomies. The register is virtually complete from the mid-1990s, but most pathology departments have added historical data dating back to the 1970s.<sup>31</sup> Women are invited for screening if they do not have a screening sample registered within the recommended interval. Women who do not participate receive reminders 90 and 180 days after the invitation.<sup>29,32</sup>

### Design and Study Population

HPV SCREEN DENMARK is a real-world implementation study embedded into the routine screening program at the Department of Pathology, Vejle Hospital, Lillebaelt Hospital, Region of Southern Denmark. This department processes around 50,000 cervical cytology samples annually (approximately 12% of all smears in Denmark).<sup>32</sup> The implementation targets women aged 30–59 years residing in the department's uptake area. During the implementation, women are offered HPV- or cytology-based screening depending on the

municipality of residence. Women residing in four municipalities (Vejle, Fredericia, Kolding, Middelfart) are offered HPV-based screening, while women residing in nine other municipalities (Assens, Faaborg-Midtfyn, Kerteminde, Nyborg, Odense, Svendborg, Nordfyns, Langeland, Ærø) are offered cytology-based screening. The screening invitations describe the screening method offered to each woman. The choice of municipalities for each screening method was made to achieve an approximate 40–60% distribution of women in the HPV- and cytology groups and was also based on administrative considerations relating to different IT- and invitation systems in the two geographical areas. Before initiation of the implementation, general practitioners and gynecologists in the region were informed about the study at meetings, by e-mail and through a webpage.

HPV-based screening was initiated on 29 May 2017. In this paper, the analysis of participation included women invited during the first year ( $n=35,081$ ). The analysis of referral rates and high-grade CIN detection included women screened during the first year ( $n=28,352$ ), including women screened after invitation and those screened at their own initiative.

### Ethics

The study was considered by the Ethics Committee in the Region of Southern Denmark to be an implementation project in clinical practice exempt from informed consent (S-20160146). The study was approved by the Data Protection Agency in the Region of Southern Denmark (18/21475) and the National Board of Patient Safety (3-3013-2597).

### Sample Collection and Processing

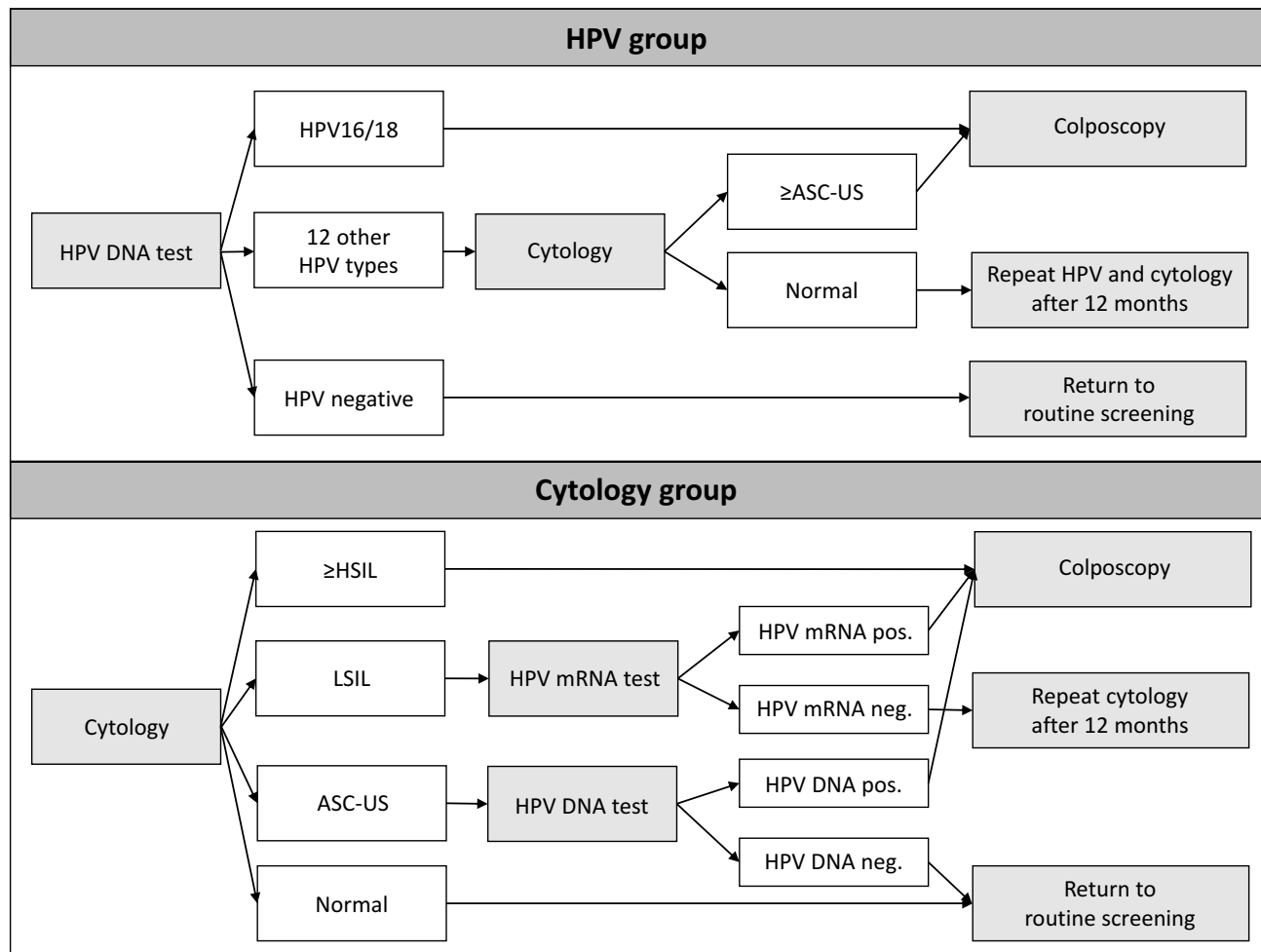
Liquid-based cytology samples (ThinPrep, Hologic) were collected by general practitioners or gynecologists and sent to Vejle Pathology Department for processing. HPV DNA testing was performed using the Cobas HPV test (Roche) providing individual detection of HPV16 and 18, and pooled detection of 12 other oncogenic HPV types (31,33,35,39,45,51,52,55, 56,58,66,68). HPV mRNA testing was performed using the Aptima HPV mRNA test (Hologic) which detects E6/E7 mRNA of 14 oncogenic HPV types (16,18,31,33,35,39,45,51, 52,55,56,58,66,68). Cytologic diagnoses were classified according to the 2001 Bethesda classification. Slides were read by biotechnicians, and abnormal diagnoses were confirmed by a pathologist.

## Clinical Management

Figure 1 shows the screening algorithms. In the HPV group, women with HPV16/18 were referred to colposcopy. Women with 12 other oncogenic HPV types received cytology triage. If they had atypical squamous cells of undetermined significance (ASCUS) or worse, they were referred to colposcopy, and if they had normal cytology, they were invited for repeat screening after a year. Cytology was read with knowledge of HPV status. In the cytology group, women were managed according to national guidelines.<sup>29</sup> Women with high-grade squamous intraepithelial lesions (HSIL), atypical glandular cells or adenocarcinoma in situ (AIS) were referred to colposcopy. Women with low-grade squamous intraepithelial lesions (LSIL) were tested for HPV mRNA, and HPV mRNA positive women were referred to colposcopy, while HPV mRNA negative women were referred to colposcopy, while HPV mRNA negative

women were invited for repeat screening after a year. Women with ASCUS were tested for HPV DNA, and HPV DNA positive women were referred to colposcopy, while HPV DNA negative women returned to routine screening.

Colposcopy was performed in routine practice by private or hospital-based gynecologists. Danish guidelines recommend that colposcopy-guided biopsies are supplemented with random biopsies from quadrants without visible changes.<sup>33</sup> The vast majority of histologies were evaluated at pathology departments in the Region of Southern Denmark (Vejle Pathology Department: 50%; Odense Pathology Department: 49%), whereas 1% were evaluated at other pathology departments in the country. Histological diagnoses were classified according to the CIN nomenclature. In the analysis, CIN grade 2 or worse (CIN2+) included histological diagnoses of CIN2, CIN3,



**Figure 1** Screening algorithms in the HPV- and cytology group of HPV SCREEN DENMARK.

**Abbreviations:** ASC-US, atypical squamous cells of undetermined significance; DNA, deoxyribonucleic acid; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; mRNA, messenger ribonucleic acid.

AIS and cervical cancer, and CIN grade 3 or worse (CIN3+) included CIN3, AIS and cervical cancer.

## Registry Linkages

All residents in Denmark have a unique personal identification number which is used in all national registries. Using this personal identification number, we retrieved information from the Pathology Databank on screening invitations, cervical cytological and histological diagnoses and HPV results. Information from the Pathology Databank was available until 27 Nov 2018, ie all women had at least 6 months' follow-up from the screening date. Furthermore, we retrieved individual-level data on educational level, employment and country of origin from registries in Statistics Denmark,<sup>34,35</sup> and information on individual HPV vaccination status from the National Prescription Register<sup>36</sup> and the National Health Service Register.<sup>37</sup>

## Statistical Analysis

In the analysis of screening participation, baseline was defined as the date of first screening invitation during the study. We used Kaplan-Meier's method to estimate the probability of participation by time since the invitation. Logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) of participation within 6 months of invitation in the HPV- compared with the cytology group. Estimates were reported crude (model 1); adjusted for age, education, country of origin and employment (model 2); and further adjusted for HPV vaccination, previous history of CIN2+, and whether or not the woman was screened in the previous round (model 3). Adjustment variables were selected *à priori*.

In the analysis of referrals and detection of CIN2+/CIN3+, baseline was defined as the date of the first screening test during the study. We calculated the proportion of women with unsatisfactory screening tests, and the proportions referred to colposcopy or repeat screening at baseline. Furthermore, we calculated the proportion of women with CIN2+ or CIN3+ diagnosed within 6 months. This analysis included CIN2+/CIN3+ diagnosed as a result of clinical follow-up after the baseline screen, whereas the results of follow-up after repeat testing at 12 months were not yet available. Logistic regression was performed to assess ORs of unsatisfactory tests, colposcopy, repeat screening and detection of CIN2+/CIN3+ in the HPV versus cytology group. Furthermore, we calculated the positive predictive value (PPV) of colposcopy referral as the proportion of women with CIN2+/CIN3+ among those referred to colposcopy. We

did not calculate the negative predictive value, because <3% of the women with a negative screening test (HPV negative or normal cytology) had a cervical cytology or histology within the 6-month follow-up period in the study.

## Results

### Screening Participation

We invited 35,081 women for screening during the first year (HPV group:  $n=14,104$ ; cytology group:  $n=20,977$ ). The age distribution was similar in women invited for HPV- and cytology-based screening (median, 43 years; interquartile range [IQR], 37–49 years). Women invited for HPV-based screening were slightly more likely than those invited for cytology-based screening to be employed (77.6% vs 73.4%). All other characteristics were similar in the two groups (Table 1).

At 6 months after invitation, participation was virtually identical in women invited for HPV- and cytology-based screening (58.4% [95% CI, 57.6–59.2%] versus 58.8% [95% CI, 58.1–59.4%]). The same pattern was seen 1 year after invitation (69.9% [95% CI, 69.1–70.7%] versus 70.0% [95% CI, 69.3–70.6%]) (Supplementary Figure S1).

The odds of participation did not differ in women invited for HPV- compared with cytology-based screening, neither in the crude model ( $OR_{\text{crude}}=0.99$ , 95% CI, 0.94–1.03) or when adjusting for sociodemographic characteristics ( $OR_{\text{adj.}}=0.97$ , 95% CI, 0.93–1.01) (Table 2). There were no statistically significant differences in participation between HPV and cytology-based screening at any age. When we additionally adjusted for HPV vaccination, history of CIN2+, and previous screening participation, OR estimates were virtually identical (data not shown).

### Referrals and Histological Outcomes

A total of 28,352 women were screened during the first year (HPV group:  $n=11,339$ ; cytology group:  $n=17,013$ ). Of these, 21,268 (75.0%) had been invited for screening within a year before the screening date (HPV group: 71.5%; cytology group: 77.3%), whereas the remaining women had not been invited within the past year. The median age was 43 (IQR, 37–49) in the HPV group and 44 (IQR, 37–50) in the cytology group. A slightly higher proportion in the HPV than cytology group were employed (81.4% vs 77.4%). Other characteristics were similar in the two groups (Table 1).

**Table 1** Characteristics of Women in HPV and Cytology Groups in the HPV SCREEN DENMARK Study

Characteristic	A. Invited population(n=35,081)				B. Screened population(n=28,352)			
	HPV group		Cytology group		HPV group		Cytology group	
	(n=14,104)		(n=20,977)		(n=11,339)		(n=17,013)	
	n	%	n	%	n	%	n	%
<b>Age<sup>a</sup></b>								
30–34	2,190	15.5	3,196	15.2	1,705	15.0	2,685	15.8
35–39	2,609	18.5	3,791	18.1	2,065	18.2	3,005	17.7
40–44	3,097	22.0	4,345	20.7	2,429	21.4	3,440	20.2
45–49	3,093	21.9	4,589	21.9	2,378	21.0	3,601	21.2
50–54	1,462	10.4	2,430	11.6	1,493	13.2	2,230	13.1
55–59	1,653	11.7	2,626	12.5	1,269	11.2	2,052	12.1
Median (IQR)	43 (37–49)		43 (37–49)		43 (37–49)		44 (37–50)	
<b>Educational level</b>								
Basic	2,228	15.8	3,438	16.4	1,510	13.3	2,356	13.8
Medium	5,560	39.4	8,332	39.7	4,443	39.2	6,727	39.5
High	6,001	42.5	8,831	42.1	5,247	46.3	7,752	45.6
Missing	315	2.2	376	1.8	139	1.2	178	1.0
<b>Country of origin</b>								
Denmark	11,994	85.0	17,835	85.0	9,911	87.4	14,918	87.7
Immigrant	1,965	13.9	2,925	13.9	1,362	12.0	2,005	11.8
Descendant	56	0.4	115	0.5	46	0.4	75	0.4
Missing	89	0.6	102	0.5	20	0.2	15	0.1
<b>Employment</b>								
Employed	10,945	77.6	15,403	73.4	9,232	81.4	13,176	77.4
Unemployed	2,824	20.0	5,006	23.9	1,905	16.8	3,428	20.1
Students	239	1.7	454	2.2	181	1.6	389	2.3
Missing	96	0.7	114	0.5	21	0.2	20	0.1
<b>HPV vaccinated</b>								
Yes	1,234	8.8	1,915	9.1	1,247	11.0	1,928	11.3
No	12,870	91.3	19,062	90.9	10,092	89.0	15,085	88.7
<b>Screened in previous screening round<sup>b</sup></b>								
Yes	11,103	78.7	16,616	79.2	9,904	87.3	14,788	86.9
No	3,001	21.3	4,361	20.8	1,435	12.7	2,225	13.1
<b>History of cervical dysplasia</b>								
No prior CIN2+	13,249	93.9	19,660	93.7	10,322	91.0	15,584	91.6
Prior CIN2+	855	6.1	1,317	6.3	1,017	9.0	1,429	8.4

**Notes:** <sup>a</sup>For invited population: age at invitation; For screened population: age at screening. <sup>b</sup>For women aged 30–49 years: Screened during the past 4 years; For women aged 50–59 years: Screened during the past 6 years.

**Abbreviations:** CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, human papillomavirus, IQR, interquartile range.

Figure 2 provides an overview of baseline referrals and histological outcomes during up to 6 months. Of those referred to colposcopy, 92% (403/436) in the HPV group and 98% (354/362) in the cytology group had a histology during follow-up. Few histological samples were taken outside protocol, ie among those referred to 12-month repeat (HPV group: 36/566=6.4%; cytology group: 1/30=3.3%) or

routine screening (HPV group: 278/10,329=2.7%; cytology group: 415/16,504=2.5%). In the HPV group, we observed 51 CIN2 cases, 93 CIN3 cases, 12 AIS cases and 5 cancers (3 squamous cell carcinomas and 2 adenocarcinomas). In the cytology group, we observed 41 CIN2 cases, 104 CIN3 cases, 6 AIS cases and 7 cancers (5 squamous cell carcinomas and 2 adenocarcinomas).

**Table 2** Odds Ratio of Screening Participation Within Six Months of Invitation in HPV Group and Cytology Group, Overall and by Age

Age	Group	N Invited for Screening	n (%) Screened		Model 1 (Crude)		Model 2 (Adjusted*)	
					OR	(95% CI)	OR	(95% CI)
All	Cytology	20,977	12,327	(58.8)	1	(ref)	1	(ref)
		14,104	8241	(58.4)	0.99	(0.94–1.03)	0.97	(0.93–1.01)
30–39	Cytology	6987	3703	(53.0)	1	(ref)	1	(ref)
		4799	2477	(51.6)	0.95	(0.88–1.02)	0.93	(0.86–1.00)
40–49	Cytology	8934	5405	(60.5)	1	(ref)	1	(ref)
		6190	3800	(61.4)	1.04	(0.97–1.11)	1.01	(0.95–1.09)
50–59	Cytology	5056	3219	(63.7)	1	(ref)	1	(ref)
		3115	1964	(63.0)	0.97	(0.89–1.07)	0.95	(0.86–1.05)

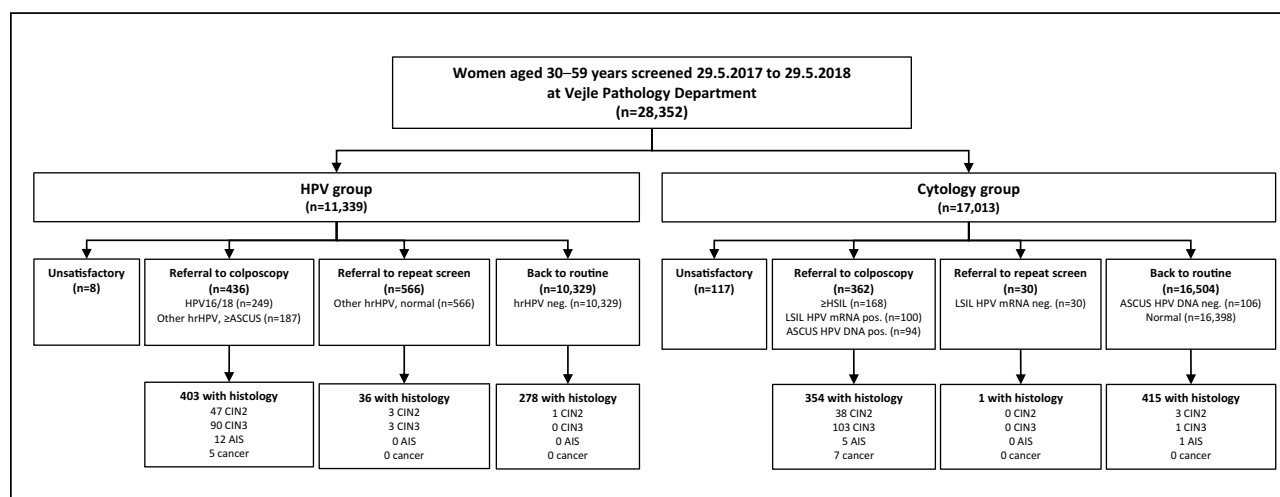
**Notes:** \*Adjusted for age, educational level, country of origin and employment status. A total of 691 women (2.0%) with missing values of adjustment variables excluded from model.

**Abbreviations:** CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

Fewer women in the HPV (0.1%) than cytology group (0.7%) had an unsatisfactory screening test ( $OR_{crude}=0.10$ ; 95% CI, 0.05–0.21) (Table 3). Of the eight women with unsatisfactory screening tests in the HPV group, four had an invalid HPV result while four had unsatisfactory cytology triage (data not shown). The proportion of women referred to colposcopy was higher in the HPV (3.8%) than cytology group (2.1%) ( $OR_{crude}=1.84$ ; 95% CI, 1.60–2.12) (Table 3). The proportion referred to colposcopy decreased with age in both groups. The proportion of women referred to 12-month repeat screening was higher in the HPV (5.0%) than cytology group (0.2%) ( $OR_{crude}=29.73$ ; 95% CI, 20.58–42.96), and this was seen in all age groups. All OR estimates were virtually

unchanged when adjusting for sociodemographic characteristics. Furthermore, all estimates were similar when further adjusting for HPV vaccination, history of CIN2+, and screening during the past round (data not shown).

The proportion of women with CIN2+ was higher in the HPV (1.4%) than cytology group (0.9%) ( $OR_{crude}=1.54$ ; 95% CI, 1.23–1.92) (Table 4). This was seen in all age groups. The proportion of women with CIN3+ was also higher in the HPV (1.0%) than cytology group (0.7%) ( $OR_{crude}=1.42$ ; 95% CI, 1.09–1.84). When adjusting for sociodemographic characteristics, OR estimates were virtually unchanged. Further adjustment for HPV vaccination, history of CIN2+ and screening during the past round yielded

**Figure 2** Flowchart of referrals and clinical outcomes among women screened by HPV testing or cytology during year I of the HPV SCREEN DENMARK study.

**Abbreviations:** AIS, adenocarcinoma in situ; 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.

**Table 3** Proportions and Odds Ratios of Referral to Colposcopy, Repeat Screening and Routine Screening in HPV and Cytology Groups, Overall and According to Age

Referral at Baseline	Age	HPV Group n=11,339		Cytology Group n=17,013		OR for HPV versus Cytology Group			
						Model 1		Model 2	
						(Crude)		(Adjusted*)	
		n	(%)	n	(%)	OR	(95% CI)	OR	(95% CI)
New screen due to unsatisfactory test	All	8	(0.1)	117	(0.7)	0.10	(0.05–0.21)	0.11	(0.05–0.22)
	30–39	3	(0.1)	47	(0.8)	0.10	(0.03–0.31)	0.10	(0.03–0.33)
	40–49	3	(0.1)	40	(0.6)	0.11	(0.03–0.35)	0.11	(0.04–0.36)
	50–59	2	(0.1)	30	(0.7)	0.10	(0.03–0.43)	0.11	(0.03–0.44)
Colposcopy	All	436	(3.8)	362	(2.1)	1.84	(1.60–2.12)	1.88	(1.63–2.17)
	30–39	199	(5.3)	182	(3.2)	1.69	(1.37–2.07)	1.73	(1.41–2.13)
	40–49	162	(3.4)	128	(1.8)	1.88	(1.49–2.38)	1.94	(1.53–2.46)
	50–59	75	(2.7)	52	(1.2)	2.27	(1.59–3.25)	2.22	(1.55–3.17)
Repeat screening in 12 months	All	566	(5.0)	30	(0.2)	29.73	(20.58–42.96)	29.85	(20.65–43.14)
	30–39	236	(6.3)	12	(0.2)	31.59	(17.66–56.49)	31.44	(17.56–56.28)
	40–49	227	(4.7)	13	(0.2)	26.79	(15.30–46.91)	27.11	(15.48–47.48)
	50–59	103	(3.7)	5	(0.1)	33.12	(13.48–81.34)	32.78	(13.33–80.63)
Back to routine screening	All	10,329	(91.1)	16,504	(97.0)	0.32	(0.28–0.35)	0.31	(0.27–0.34)
	30–39	3332	(88.4)	5449	(95.8)	0.34	(0.29–0.40)	0.32	(0.28–0.38)
	40–49	4415	(91.8)	6860	(97.4)	0.30	(0.25–0.36)	0.29	(0.24–0.35)
	50–59	2582	(93.5)	4195	(98.0)	0.30	(0.23–0.39)	0.30	(0.23–0.39)

**Notes:** \*Adjusted for age, educational level, country of origin and employment status; 317 women with missing values of adjustment variables excluded from models.

**Abbreviations:** CI, confidence interval; HPV, human papillomavirus; OR, odds ratio, PPV, positive predictive value.

similar results (data not shown). When we only included cases of CIN2+/CIN3+ diagnosed among women referred to colposcopy (“per protocol-detected” cases), we still found more CIN2+ (1.4% vs 0.9%; OR<sub>crude</sub>=1.52; 95% CI, 1.21–1.90) and CIN3+ (0.9% vs 0.7%; OR<sub>crude</sub>=1.40; 95% CI, 1.08–1.82) in the HPV than cytology group (data not shown).

The PPV of colposcopy referral was lower in the HPV than cytology group, both for CIN2+ (35.3% [95% CI, 30.8–39.8] versus 42.3% [95% CI, 37.2–47.4]) and CIN3+ (24.5% [95% CI, 20.5–28.6] versus 31.8% [95% CI, 26.9–36.6]) (Table 4). The PPV decreased with age in both groups, but was lower for HPV than cytology screening at all ages.

## Discussion

In this large Danish pilot implementation, HPV-based screening provided greater detection of high-grade CIN than cytology-based screening. This is in line with the findings of randomised trials<sup>2–8</sup> and supports the higher sensitivity of HPV testing than cytology. Moreover, our finding is in line with previous implementation studies from England,<sup>12</sup> Netherlands,<sup>28</sup> Italy<sup>21,22,25</sup> and Finland.<sup>18</sup> In our study, the CIN3+ detection was 40–50% higher in the HPV than

cytology group. Notably, this improved detection was seen already after 6 months of follow-up, without including the additional cases of CIN2-3 that may be detected after 12-month repeat screening of women with non-HPV16/18 oncogenic HPV and normal cytology at baseline.

Our analysis also showed that HPV screening resulted in more colposcopy referrals at baseline than cytology, in line with previous results.<sup>12,18,21,22,25,28</sup> Excessive colposcopy referrals are problematic because they may lead to overtreatment of regressive CIN2,<sup>4</sup> and CIN treatment may increase the risk of pregnancy-related morbidity.<sup>38</sup> In our study, approximately 80% more women were referred to immediate colposcopy in the HPV than cytology group (3.8% versus 2.1%). Our referral rate in the HPV group (3.8%) was similar to the baseline referral rate in the English pilot (4.2%),<sup>12</sup> but slightly higher than in the Dutch HPV-based screening program (3.0%).<sup>28</sup> In our study, we used a highly sensitive HPV-based screening strategy, referring all women with HPV16/18 regardless of cytology. This corresponds to algorithms in the USA<sup>39</sup> and Australia,<sup>16</sup> whereas most European countries only refer HPV-positive women with abnormal cytology.<sup>12,15,18,19,28</sup> In the cytology group, we employed

**Table 4** Detection of CIN2+ and CIN3+ Within Six Months of Follow-Up, in HPV and Cytology Group

Outcome	Age	% with CIN2+ or CIN3+ Detected						Odds Ratio of CIN2+ and CIN3+ Detection in HPV vs Cytology Group				PPV of Colposcopy Referral			
		HPV Group		Cytology Group		Model 1 (Crude)		Model 2 (Adjusted*)		HPV Group		Cytology Group			
		N	n	(%)	N	n	(%)	OR	(95% CI)	OR	(95% CI)	n/N	%	(95% CI)	
CIN2+	All	11,339	161	(1.4)	17,013	158	(0.9)	1.54	(1.23–1.92)	1.60	(1.28–2.00)	154/436	35.3	(30.8–39.8)	
	30–39	3770	86	(2.3)	5690	89	(1.6)	1.47	(1.09–1.98)	1.54	(1.14–2.08)	83/199	41.7	(34.8–48.6)	
	40–49	4807	57	(1.2)	7041	55	(0.8)	1.52	(1.05–2.21)	1.60	(1.09–2.33)	53/162	32.7	(25.4–40.0)	
	50–59	2762	18	(0.7)	4282	14	(0.3)	2.00	(0.99–4.03)	1.97	(0.97–3.96)	18/75	24.0	(14.1–33.9)	
CIN3+	All	11,339	110	(1.0)	17,013	117	(0.7)	1.42	(1.09–1.84)	1.47	(1.13–1.91)	107/436	24.5	(20.5–28.6)	
	30–39	3770	62	(1.6)	5690	69	(1.2)	1.36	(0.96–1.92)	1.43	(1.01–2.03)	60/199	30.2	(23.7–36.6)	
	40–49	4807	37	(0.8)	7041	37	(0.5)	1.47	(0.93–2.32)	1.52	(0.95–2.42)	36/162	22.2	(15.8–28.7)	
	50–59	2762	11	(0.4)	4282	11	(0.3)	1.55	(0.67–3.59)	1.52	(0.66–3.51)	11/75	14.7	(6.5–22.9)	

**Notes:** \*Adjusted for age, educational level, country of origin and employment status; 317 (1.1%) women with missing values of adjustment variables excluded from models.

**Abbreviations:** CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; HPV, human papillomavirus; OR, odds ratio; PPV, positive predictive value.

a conservative strategy, where women with LSIL were only referred to colposcopy if they were HPV mRNA positive. This is consistent with Danish guidelines<sup>29</sup> which recommend HPV mRNA testing for LSIL triage, because it has been shown that HPV mRNA testing of LSIL provides higher specificity and lower referral rates than HPV DNA testing of LSIL, with similar sensitivity.<sup>40</sup> These choices concerning screening algorithms have likely augmented the difference in referrals between HPV and cytology screening in our study. Furthermore, the referral rate in our study reflects a baseline round of HPV testing. In the Canadian FOCAL trial<sup>2</sup> and in Italian<sup>21,22</sup> and English<sup>12</sup> pilot implementations, immediate referral rates at the second round of HPV screening were markedly lower than in the first round, likely reflecting that prevalent HPV infections are “screened out” in the first round. Therefore, we expect the referral rate to decrease in the next round of HPV screening in Denmark.

We also found that HPV-testing of clinician-taken samples did not affect the attendance rate. In line with this, most previous implementation studies found similar<sup>18,19,21,22</sup> participation in HPV and cytology-based screening. Accordingly, a Norwegian survey found that women’s intentions to participate in screening did not differ between women invited for HPV- or cytology-based screening.<sup>41</sup> In the Netherlands, participation decreased slightly when HPV-based screening was introduced, but the authors argued that this was likely due to concurrent changes in the invitation procedure.<sup>28</sup> While it is reassuring that in our study introduction of HPV screening did not decrease attendance, the participation rates in both groups of our study (~70% after 1 year) were lower than the national target for the screening program (75% after 1 year).<sup>32</sup> Thus, increasing participation remains an important priority for the Danish program.<sup>30</sup>

A further finding of our study was that HPV-based screening resulted in fewer unsatisfactory screening tests (0.1%) than cytology (0.7%). During recent years, substantial efforts have been made in Denmark to decrease the rate of unsatisfactory smears, including introduction of liquid-based cytology, instruction of general practitioners with high unsatisfactory rates, and annual feedback at regional and hospital levels.<sup>32</sup> This has led to a marked decrease in the national proportions of unsatisfactory smears from 3.1% in 2009<sup>42</sup> to 0.7% in 2017,<sup>32</sup> consistent with the proportion in the cytology group in our study. Despite this, HPV screening still performed better than cytology. Since the proportions of unsatisfactory cytology in some screening programs may be as high as 3–10%,<sup>43</sup> the gains from introducing HPV screening in such settings may be even higher.



This implementation study was embedded into the routine screening program in a large Danish pathology department, allowing us to assess the real-world clinical performance of HPV-based screening. The strengths of the study include the population-based design and the follow-up in a nationwide pathology register with virtually 100% coverage, meaning that we had information on all CIN2+ cases diagnosed in Denmark. A potential limitation is that allocation to screening method was based on the area of residence instead of randomization. However, we compared the HPV and cytology groups by retrieving extensive, individual-level information from nationwide registers of high validity. There were no marked differences in sociodemographic characteristics or previous CIN2+-history between the two groups, and adjustment for these factors did not change our results. Another limitation is that we had only 6 months of follow-up, meaning that we could not take into account the results of clinical management after 12-month repeat screening. In the HPV arm, 5% of women were referred to repeat screening, and both referral rates and CIN2+ detection are expected to increase when results of this early recall are available.<sup>12,21,22,24,25</sup>

In conclusion, the results of this large pilot implementation confirm that HPV-based screening provides greater detection of high-grade CIN and cervical cancer than cytology-based screening. These results support ongoing efforts to implement HPV-based screening in Denmark<sup>30</sup> and elsewhere. Although referral rates were high in this initial screening round, referrals are expected to decrease in subsequent rounds. Furthermore, several triage methods in HPV-positive women are currently being evaluated, including p16/Ki-67 dual-staining, host or viral methylation and extended HPV genotyping.<sup>44</sup> Such triage methods may further optimize the management of HPV-positive women, thereby taking full advantage of the benefits of HPV-based screening while minimizing potential harms.

## Abbreviations

AIS, adenocarcinoma in situ; 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 2 or worse; DNA, deoxyribonucleic acid; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion; mRNA, messenger ribonucleic acid; OR, odds ratio, PPV, positive predictive value.

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## 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 linkages, data management and statistical analyses. KF assisted with the 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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## Disclosure

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