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Research paper

Clinical performance of high-risk HPV testing on self-samples versus clinician samples in routine primary HPV screening in the Netherlands: An observational study

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ABSTRACT

Background: High-risk human papillomavirus (hrHPV) testing on self-collected samples has potential as a primary screening tool in cervical screening, but real-world evidence on its accuracy in hrHPV-based screening programmes is lacking.

Methods: In the Netherlands, women aged 30–60 years invited for cervical screening can choose between sampling at the clinician's office (Cervex Brush) or self-sampling at home (Evalyn Brush). HrHPV testing is performed using Roche Cobas 4800. We collected screening test results between January 2017 and March 2018 and histological follow-up until August 2019. The main outcome measures were mean cycle threshold (Ct) value, cervical intraepithelial neoplasia (CIN) grade 3 or cancer (CIN3+) and CIN grade 2 or worse (CIN2+).

Findings: 30,808 women had a self-collected and 456,207 had a clinician-collected sample. In hrHPV-positive women with adequate cytology, Ct values were higher for self-collection than clinician-collection with a mean Ct difference of 1·25 (95% CI 0·98–1·52) in women without CIN2+, 2·73 (1·75–3·72) in CIN2 and 3·59 (3·03–4·15) in CIN3+. The relative sensitivity for detecting CIN3+ was 0·94 (0·90–0·97) for self-collection versus clinician-collection and the relative specificity was 1·02 (1·02–1·02).

Interpretation: The clinical accuracy of hrHPV testing on a self-collected sample for detection of CIN3+ is high and supports its use as a primary screening test for all invited women. Because of the slightly lower sensitivity of hrHPV testing on a self-collected compared to a clinician-collected sample, an evaluation of the workflow procedure to optimise clinical performance seems warranted.

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Research in context

Evidence before this study

HPV self-sampling has been recently implemented as primary screening method in routine screening. Some countries offer HPV self-sampling to non-attendees (e.g. Australia, Malaysia, Denmark) and a few others offer it as an opt-in alternative for women who feel uncomfortable with a clinician-collected cervical smear (e.g. Argentina, the Netherlands). Primary HPV testing on self-collected samples in routine screening is a new promising screening approach, but it is only acceptable when its accuracy is similar to that of primary HPV testing on clinician-collected samples. We searched PubMed with the terms ("self-sampling" OR "self-collected samples") AND ("HPV" OR "human papillomavirus") for studies published in English up to December 2020. A meta-analysis of HPV self-sampling studies, published in December 2018, showed that HPV PCR testing on self-collected and clinician-collected samples have similar clinical accuracy. Most studies in the meta-analysis were conducted in underscreened or rural populations. After the meta-analysis, five additional studies were conducted in colposcopy referral populations. Sensitivity of HPV testing for detection of CIN2+ in those studies was similar or slightly lower for self-collection as compared to clinician-collection. Evidence on the performance of primary HPV screening on self-collected samples in regular screening populations has been collected in an implementation trial in the Jujuy province in Argentina and a randomised population-based non-inferiority trial (IMPROVE) in the Netherlands.

Added value of this study

The present study is the first to report on the real-world performance of primary HPV testing on self-collected versus clinician-collected samples within a national, organised screening programme. This means that all women invited for routine screening were offered a choice between self-sampling at home and clinician-sampling at the general practitioner's office. This study shows that PCR-based HPV testing on a self-collected sample instead of a clinician-collected sample leads to a higher cycle threshold (Ct) value. This slightly increases the specificity and decreases the sensitivity for detection of CIN3+ but consensus guidelines for the clinical sensitivity and specificity of HPV DNA testing in regular screening are still met. This study also shows that Ct value is strongly associated with CIN3+ risk, in particular for clinician-collected samples.

Implications of all the available evidence

This study confirmed that HPV testing on self-collected cervicovaginal material is an accurate alternative to HPV testing on clinician-collected samples in primary HPV-based screening programmes. This finding, together with results from focus groups and questionnaires showing that women have a positive attitude towards self-sampling, supports the use of self-sampling in routine screening. When HPV self-sampling is used as a primary screening instrument, the slight decrease in sensitivity of HPV testing on a self-collected sample compared to a clinician-collected sample seems to warrant an evaluation of the workflow to optimise clinical performance. The strong relation between the Ct value and CIN3+ risk supports the potential use of Ct in the management of HPV-positive results.

1. Introduction

In several countries, high-risk human papillomavirus (high-risk HPV, hrHPV) testing has been implemented as a primary test in cervical cancer screening. Some countries, including Argentina, Australia, Denmark, Malaysia, and the Netherlands, also offer hrHPV testing on self-collected cervicovaginal material (hrHPV self-sampling) as a screening option. Self-sampling is associated with less shame, anxiety, discomfort and pain than clinician-sampling and may increase the coverage of the screening programmes by lowering the barrier for underscreened women [1-3]. Offering hrHPV self-sampling as a primary test also greatly reduces the number of visits to the general practitioner (GP) and can be offered as a primary screening test to shorten the delay in screening attendance caused by the current Covid19 pandemic [4]. However, so far, hrHPV self-sampling has only played a limited role in screening programmes; in the Netherlands, for example, only 8% of participants has requested a self-sampling kit since the introduction 3 years ago [5].

The use of hrHPV self-sampling for detection of cervical precancerous lesions is supported by diagnostic studies and randomised participation trials. A meta-analysis of hrHPV self-sampling studies in underscreened and rural populations showed that hrHPV PCR testing on self-collected and clinician-collected samples have similar clinical sensitivity and specificity for detection of cervical intraepithelial neoplasia (CIN) grade 3 or cancer (CIN3+) and CIN grade 2 or worse (CIN2+) [6,7]. These results were confirmed in a recent Dutch study where women invited for routine hrHPV screening were randomised to home-based self-sampling or clinician-sampling (IMPROVE trial) [8]. The encouraging results on the accuracy of hrHPV self-sampling have prompted the implementation of self-sampling in the Netherlands as an alternative for all women invited for HPV primary screening. However, in the first year, a significantly lower hrHPV prevalence was observed in self-collected as compared to clinician-collected samples (7% versus 9%) [9]. This has raised concerns about the reliability of a negative self-sampling result in the Dutch hrHPV screening programme with a screening interval of 5−10 years.

The aim of the present study is to evaluate the accuracy of primary hrHPV self-sampling for detection of CIN3+ and CIN2+ in the Dutch cervical screening programme. For this purpose, we compared the cycle threshold (Ct) values of the hrHPV PCR test (Cobas 4800 HPV Test) in self-collected and clinician-collected samples in the first 14 months following the start of the new national HPV-based screening programme. We linked the Ct values to histology, reported cumulatively over at least 17 months after an hrHPV-positive test, to assess the clinical importance of a difference in Ct values. Finally, we assessed whether the CIN3+ and CIN2+ risks after a positive hrHPV test depends on the Ct value in which case the Ct value may be considered for additional stratification of hrHPV-positive women.

2. Methods

2.1. Dutch cervical screening programme

In the Netherlands, women are invited for hrHPV testing in the calendar year in which they turn 30, 35, 40, 45, 50, 55 and 60. Women with a negative hrHPV test at age 40 or 50 will be re-invited after 10 years [10]. Women who prefer not to undergo cervical sampling at the GP's office can request a device for self-collection at home. Both self-collected and clinician-collected samples are sent to a screening laboratory for hrHPV DNA testing. Women with a positive hrHPV result on a clinician-collected sample are triaged by reflex cytology, whereas women with an hrHPV-positive result on a self-collected sample are invited for cytological testing at the GP's office. Women with normal cytology are re-invited for repeat cytology after 6 months and women with abnormal cytology

are referred to the gynaecologist for colposcopic examination. HrHPV genotype information is currently not used in the management of hrHPV-positive results.

2.2. HrHPV testing, cytology and histology

Clinical cervical samples were taken at the GP's office by trained personnel using a Cervex Brush (Rovers Medical Devices B.V., Oss, NL) and placed in a vial containing 20 ml PreservCyt medium (Hologic, Marlborough, MA, US). Cervicovaginal self-samples were collected at home using the Evalyn Brush (Rovers Medical Devices B.V., Oss, NL) and also placed in 20 ml PreservCyt medium. In case the self-sample was hrHPV-positive, women were asked for a cervical smear to be taken at the GP's office. HrHPV DNA was assessed with the fully automated Cobas HPV Test (Cobas 4800 System, Roche Molecular systems, Branchburg, NJ, US) [11]. The Cobas HPV Test is a PCR technology which provides Ct values for three separate channels, i.e. HPV16, HPV18, and a pool of 12 other hrHPV types (HPV 31/33/35/39/45/51/52/56/58/59/66/68; hereafter referred to as "non-16/18 hrHPV"). An overall test result is also given, hereafter referred to as "(overall) hrHPV". More details are given in the Supplementary appendix.

Cytology was classified according to the CISOE-A framework used in the Netherlands, which can be translated to the Bethesda system [12]. An abnormal cytological outcome includes all categories ASC-US or worse. During the gynaecological visit, colposcopy-directed biopsies were taken from suspected areas on the cervix for histological examination according to standard procedures in the Netherlands [13]. Histology was examined locally and classified as normal, CIN grade 1, 2, 3, or invasive cancer, according to international criteria [14]. Adenocarcinoma in situ was added to CIN grade 3.

2.3. Data collection

We collected data of women invited from 1 January 2017 until 1 March 2018 and from whom a sample was received by the laboratory between 1 January 2017 and 9 March 2018 (ScreenIT RIVM, Bilthoven, NL). Data were extracted for 487,015 women, including 30,808 women with a self-collected sample and 456,207 women with a clinician-collected sample. All records included information on age group (30–34, 35–39, 40–44, 45–49, 50–54, 55–59, ≥60 years), laboratory region, method of collection (self-collected or clinician-collected), and hrHPV status (negative or positive). Amongst hrHPV-positive women, only women with adequate cytology were included. Ct values were collected for overall hrHPV, HPV16, HPV18, and non-16/18 hrHPV. Histological results were retrieved from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA, Houten, NL). In case of multiple histological results, the most severe diagnosis was taken. Follow-up was collected until 11 August 2019 so that the minimum follow-up time after the baseline hrHPV test was at least 17 months for all subjects. Cytological and histological history up to 10 years prior to baseline hrHPV testing was also collected. We defined women as being "not underscreened" when they had a previous screening invitation (i.e. they were aged 35 years and older) and the time to previous cytology and/or histology was not more than 7 years [8].

2.4. Statistical analyses

Our study focuses on the accuracy of hrHPV testing on a self-collected sample and clinician-collected sample (index tests) for the detection of histologically confirmed CIN3+ (reference standard). Amongst women with an hrHPV-positive result, we calculated the mean difference in Ct values between self-collected and clinician-collected samples, stratified by age category, cytology and histology. We applied linear regression to check whether the mean difference in Ct values between self-collected and clinician-collected samples was mediated by age, histological outcome, and screening history.

We estimated the relative clinical sensitivity and specificity of hrHPV self-sampling versus clinician-based hrHPV testing for detection of CIN3+. We applied two different methods for estimating the relative sensitivity (Method I and II) which use independent pieces of information provided by the study. A detailed description of Method I and II is given in the Supplementary appendix. In brief, Method I is based on a proposal for unpaired screen-positive designs [15] and estimates the relative sensitivity of the two hrHPV screening tests (index tests) at detecting CIN3+ (reference standard) by comparing the detected CIN3+ proportions in the two study groups. This method can only be used when the prevalence of underlying CIN3+ is the same in the two study groups. Since screening non-attendance is a main risk factor for CIN3+ and previous non-attendance is expected to be different in the self-collection and clinician-collection group, we only selected women who had received a previous screening invitation (i.e. age >34) and attended screening in the previous round. We also adjusted for the proportion of hrHPV-positive women without adequate cytology in the self-collection group and clinician-collection group. Method II estimates the clinical sensitivity directly from the tail of the distribution of Ct scores in women with CIN3+. Ct values are transformed such that they follow a normal distribution. We checked normality by a Quantile-Quantile (QQ) plot, skewness and kurtosis. We applied Method II to the whole study population, to the subgroup of women who were not underscreened, and to the complement of the latter subgroup. The relative sensitivity is the ratio of the estimates for the self-collection and clinician-collection group. The relative specificity can be estimated by comparing the proportion of hrHPV positives in the two study groups after excluding CIN3+ cases. Similar as in Method I, we only selected women who were not underscreened. The accuracy of the hrHPV test on self-collected and clinician-collected samples for detecting CIN3+ was visualised by receiver operating curves (ROC). The estimation of CIN3+ risks and construction of ROC were repeated for Ct values of channels HPV16, HPV18 and non-16/18 hrHPV.

Finally, we estimated the risk of CIN3+ in strata of women with Ct values between 40·5 and 35, between 35 and 30, and below 30. We also estimated the risk of CIN3+ against the continuous Ct value by a monotone quadratic I spline function with knots at Ct values 25, 30 and 35 [16]. All analyses were repeated for CIN2+.

Consensus criteria for non-inferiority of the clinical sensitivity of a test are that the relative sensitivity for detection of CIN2+ should be at least 0·90 and the relative specificity should be at least 0·98 [17]. About 400,000 women are needed to achieve a power of 80% for assessing non-inferiority of clinician sensitivity by Method I. The sample size calculation assumes that 9·2% of the hrHPV tests are positive [9], that the positive predictive value of the hrHPV test for CIN2+ is 25% [18], and that the sample size ratio is 1:15 for self-collection versus clinician-collection [9].

Throughout the analyses, statistical significance of differences between proportions and between continuous values were assessed by chi-square testing and z-testing, respectively. Ninety-five percent confidence intervals (95% CI) of proportions were calculated using Wilson method, 95% CI of means of continuous variables were calculated assuming a normal distribution, 95% CI of relative risks and relative sensitivities were calculated using the delta method (Supplementary appendix). A finding was considered statistically significant when the two-sided p-value was below 0·05. Statistical analyses were performed in Stata/SE 14.1, Microsoft Excel and R software version 3.6.1. We followed the STROBE guidelines for reporting of observational studies.

2.5. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

3. Results

3.1. Study population

Women who opted for self-collection (N = 30,808) had a mean age of 46·7 years and were slightly younger than women with a clinician-collected sample (N = 456,207) whose mean age was 47·8 years. Amongst women with a previous screening invitation (aged 35 years and older), the proportion of women attending the previous round were 69·4% and 73·6% amongst hrHPV-positive and hrHPV-negative women in the self-collection group and 88·0% and 92·3% amongst hrHPV-positive and hrHPV-negative women in the clinician-collection group.

Figure 1 shows the flowchart of women who opted for self-collection and clinician-collection with an hrHPV-negative and hrHPV-positive test result, together with cytological and histological follow-up after a positive hrHPV test. The stratification by age is shown in Table 1. The proportion of women with a positive hrHPV test result was 7·4% in the self-collection and 9·3% in the clinician-collection group. Stratified for age groups 30–39, 40–49, and ≥50, the proportion of positive hrHPV results was 12·4%, 6·8%, and 4·1% in the self-collection and 16·6%, 8·7%, and 5·8% in the clinician-collection group, respectively. The median time between baseline cytology and histology was 1·3 months (range 0–23) in the self-collection group and 1·5 months (range 0–29) in the clinician-collection group.

3.2. Ct values in hrHPV-positive samples

Ct values could be retrieved for 39,810 of 44,555 (89·4%) hrHPV-positive samples with adequate baseline cytology, including 1,755 of 2,031 (86·4%) women from the self-collection group and 38,055 of 42,524 (89·5%) women from the clinician-collection group.

Table 2 shows the mean Ct values in the self-collection and clinician-collection group and for subgroups defined by age category, cytology, and histology. Mean Ct values were slightly higher for the self-collection group than the clinician-collection group. The difference in mean Ct value was 1·58 (95% CI 1·34–1·82) between self-collected samples and clinician-collected samples, with no marked differences between age cohorts. The difference in mean Ct was larger in women with abnormal cytology than in women with normal cytology and was also larger in women with CIN2+ and CIN3+ than in women without CIN2 (Table 2, Figure S1).

The effect of self-collection on the Ct score, adjusted for age and histology, was 1·74 (95% CI 1·49–1·98) (Table S1). A similar effect (1·82, 95% CI 1·51–2·11) was found when confining the analysis to women 35 years and older and additionally adjusting for screening history (Table S1). We did not find significant interaction effects between collection method and age and screening history (not tabulated).

3.3. Sensitivity and specificity of hrHPV testing on self-collected and clinician-collected sample

The relative sensitivity of hrHPV self-sampling versus clinician-based hrHPV testing for detection of CIN3+ was 0·88 (95% CI 0·72–1·08) for Method I and 0·94 (0·90–0·97) for Method II (Table 3). Regarding Method II, a square-square root transformation was applied to the Ct values to obtain normally distributed scores (Table S2, Figure S2). The Ct-based relative sensitivity (Method II) was fairly robust against the choice of the normalising transformation and the screening history. The relative sensitivity for detection of CIN3+ was 0·92 for log-transformed Ct values, 0·96 for crude untransformed Ct values, 0·91 when computed for the subgroup of women

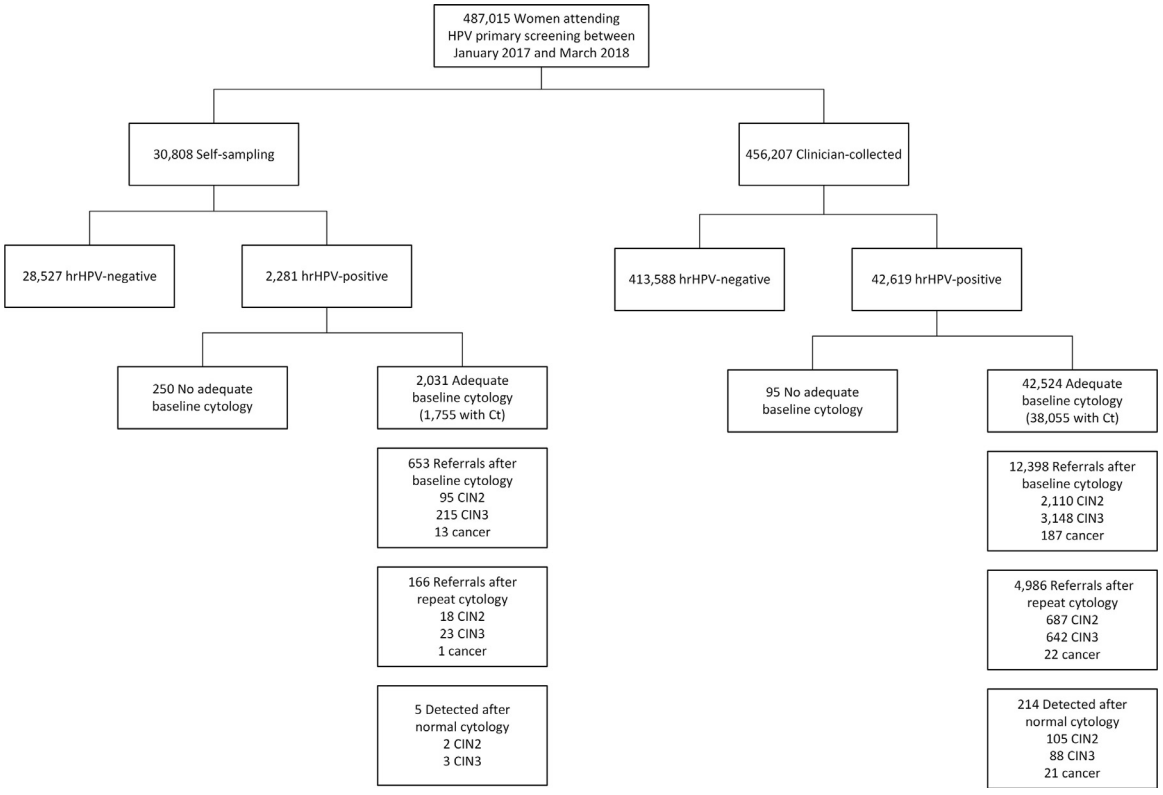

Figure 1. Flowchart of women attending national HPV primary screening in the Netherlands between 1 January 2017 and 9 March 2018, with histology follow-up until 11 August 2019.

Table 1
Screening results of all women (*N* = 487,015) stratified by age, collection method, and hrHPV test result, including histology of women with adequate cytology.

	Self-collected						Clinician-collected					
	hrHPV-neg	hrHPV-pos					hrHPV-neg	hrHPV-pos				
		Adequate cytology		CIN2	CIN3	Cancer		Adequate cytology		CIN2	CIN3	Cancer
		no	yes					no	yes			
Total	28,527	250	2,031	134	281	16	413,588	95	42,524	3,263	4,389	256
Age (years)												
30–34	4,740	71	725	52	117	5	44,647	16	11,600	1,086	1,669	75
35–39	3,827	48	371	24	56	2	49,911	11	7,180	585	972	48
40–44	3,345	25	242	17	45	2	54,238	9	5,603	433	591	45
45–49	3,839	22	237	16	27	1	65,164	11	5,824	479	490	48
50–54	4,178	37	197	13	18	4	69,842	12	5,306	375	334	20
55–59	4,310	27	135	2	11	1	70,566	19	4,167	197	210	8
≥60	4,288	20	124	10	7	1	59,220	17	2,844	108	123	12

CIN2/3: cervical intraepithelial neoplasia grade 2/3; hrHPV: high-risk human papillomavirus; neg: negative; pos: positive.

Table 2
Mean Ct values of hrHPV-positive samples with standard deviations and mean differences with 95% confidence intervals,
stratified by collection method, age, cytology, and histology.

	Self-collected		Clinician-collected		Self-collected versus clinician-collected
	N	Mean Ct (SD)	N	Mean Ct (SD)	Mean difference (95% CI)
Total	1,755	32·73 (4·98)	38,055	31·15 (5·30)	1·58 (1·34–1·82)
Age (years)					
30–39	943	32·51 (4·77)	16,815	30·66 (5·18)	1·84 (1·53–2·16)
40–49	402	32·47 (5·24)	10,209	31·09 (5·40)	1·37 (0·85–1·90)
≥50	410	33·50 (5·12)	11,031	31·93 (5·29)	1·56 (1·05–2·07)
Cytology					
NILM	1,102	33·81 (4·58)	25,657	32·80 (4·70)	1·01 (0·73–1·28)
ASC-US/LSIL	380	30·76 (5·24)	8,173	27·87 (5·15)	2·90 (2·36–3·44)
HSIL	273	31·10 (4·90)	4,225	27·44 (4·01)	3·67 (3·07–4·26)
Histology					
<CIN2	1,385	33·10 (4·99)	31,045	31·85 (5·26)	1·25 (0·98–1·52)
No histology	1,110	33·45 (4·79)	24,191	32·57 (4·98)	0·87 (0·58–1·16)
CIN0/1	275	31·71 (5·51)	6,854	29·31 (5·46)	2·40 (1·74–3·07)
CIN2+	370	31·33 (4·71)	7,010	28·03 (4·20)	3·31 (2·81–3·80)
CIN2	115	30·88 (5·28)	2,902	28·15 (4·55)	2·73 (1·75–3·72)
CIN3+	255	31·54 (4·42)	4,108	27·95 (3·93)	3·59 (3·03–4·15)
CIN3	241	31·59 (4·43)	3,878	27·97 (3·93)	3·62 (3·05–4·20)
Cancer	14	30·65 (4·39)	230	27·61 (3·94)	3·04 (0·46–5·61)

ASC-US: atypical squamous cells of undetermined significance; CI: confidence interval; CIN: cervical intraepithelial neoplasia; Ct: cycle threshold; hrHPV: high-risk human papillomavirus; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; NILM: negative for intraepithelial lesion or malignancy; SD: standard deviation.

Table 3
Relative sensitivity and specificity with 95% confidence intervals of hrHPV testing on self-collected as compared to clinician-collected samples for detection of CIN3+ and CIN2+.

	CIN3+	CIN2+
Relative sensitivity		
Method I	0·88 (0·72–1·08)	0·79 (0·67–0·92)
Method II	0·94 (0·90–0·97)	0·91 (0·88–0·96)
Relative specificity	1·02 (1·02–1·02)	1·02 (1·01–1·02)

A description of Method I and II is given in the Methods section; CIN2/3+: cervical intraepithelial neoplasia grade 2/3 or worse.

who were not underscreened, and 0·95 when computed for the complement of the latter subgroup.

The relative specificity estimate was 1·02 (95% CI 1·02–1·02). For end-point CIN2+, the relative sensitivity was slightly lower as compared to end-point CIN3+ (Table 3).

Figure 2 shows the ROC of hrHPV testing on self-collected and clinician-collected samples for clinical endpoints CIN3+ and CIN2+. The curves of the two hrHPV tests were similar up to the point where the ROC of the self-sampling test reached the system's cut-off of 40·5 cycles. ROC curves of HPV16, HPV18, and non-16/18 hrHPV types were also similar for up to the system's cut-off (Figures S3-S5). The test sensitivities differed at the system's cut-offs, in particular for HPV18 and non-16/18 hrHPV, but there was a lot of uncertainty around the sensitivity estimates because of small sample sizes.

3.4. Risk of CIN3+ against Ct value

The risks of CIN3+ in hrHPV-positive women against Ct category are presented in Table 4. The CIN3+ risks were similar for self-collected and clinician-collected samples for category Ct value <30, but differed between the two collection methods for categories Ct value 30–35 and Ct value >35. In Figure 3, the CIN3+ risk is displayed against the continuous Ct value showing that CIN3+ risks start to diverge when the Ct value exceeds 28. The clinician-collected samples showed a

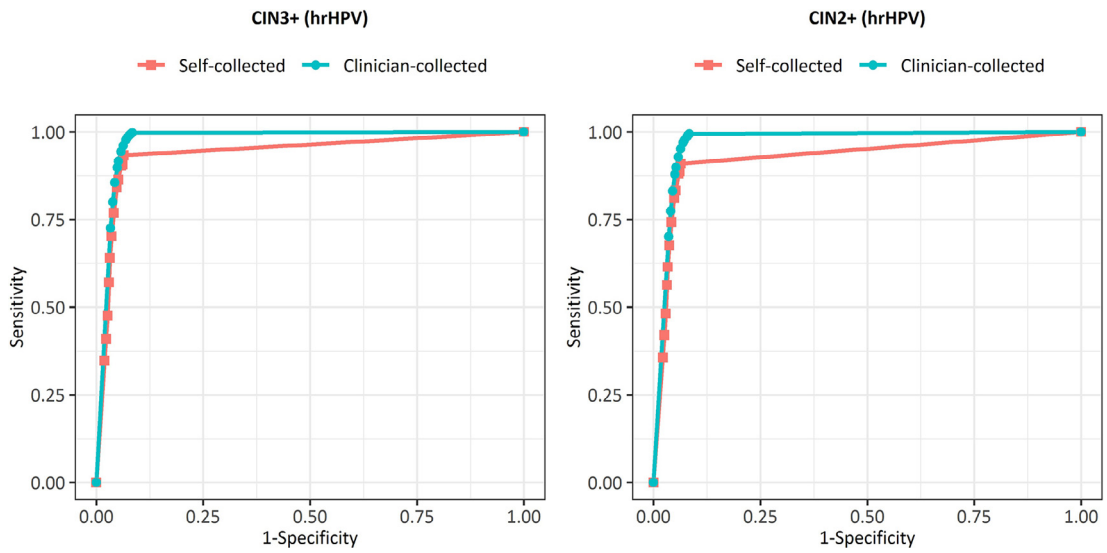

Figure 2. ROC of overall hrHPV Ct values on self-collected (red) and clinician-collected (blue) samples for clinical endpoint CIN3+ (left) and CIN2+ (right).

Table 4
Risks of CIN3+ and CIN2+ against overall Ct value of the hrHPV test and collection method.

Overall hrHPVCt value	N total	CIN3+		CIN2+	
		N	Risk % (95% CI)	N	Risk % (95% CI)
Self-collected					
>35	708	60	8·5 (6·6–10·8)	91	12·9 (10·6–15·5)
≤35	1,047	195	18·6 (16·4–21·1)	279	26·6 (24·1–29·4)
30–35	503	100	19·9 (16·6–23·6)	134	26·6 (23·0–30·7)
<30	544	95	17·5 (14·5–20·9)	145	26·7 (23·1–30·5)
Clinician-collected					
>35	10,589	204	1·9 (1·7–2·2)	433	4·1 (3·7–4·5)
≤35	27,466	3,904	14·2 (13·8–14·6)	6,577	23·9 (23·4–24·5)
30–35	11,185	917	8·2 (7·7–8·7)	1,637	14·6 (14·0–15·3)
<30	16,281	2,987	18·3 (17·8–18·9)	4,940	30·3 (29·6–31·1)

CI: confidence interval; CIN2/3+: cervical intraepithelial neoplasia grade 2/3 or worse; Ct: cycle threshold; hrHPV: high-risk human papillomavirus.

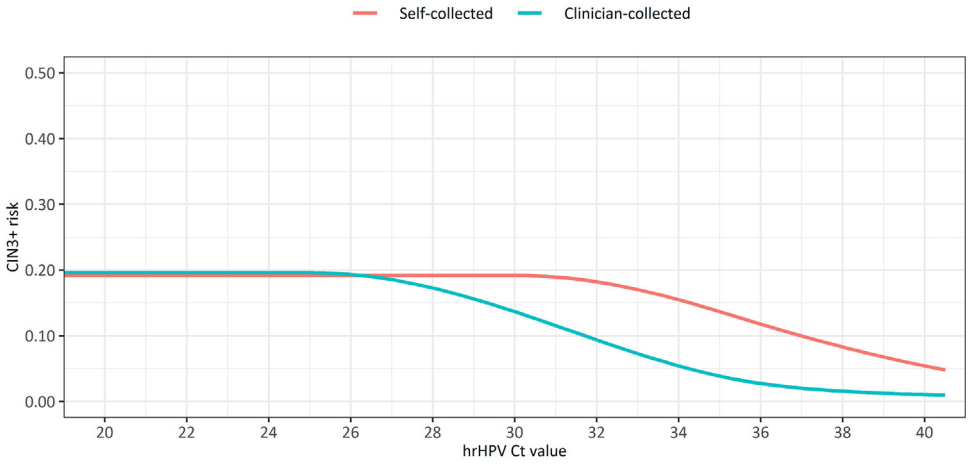

Figure 3. Risks of CIN3+ against hrHPV Ct value. The risk functions are quadratic I splines with knots at Ct 25, 30, and 35.

sharper decline in CIN3+ risk than the self-collected samples when Ct values were high. The CIN3+ risks were also calculated for each of the three genotype channels (Tables S3-S5, Figures S6-S8) and they were highest for the HPV16 channel. The risk of CIN3+ was associated with the Ct value amongst clinician-collected samples for all three channels, but amongst self-collected samples a strong association was observed only for the HPV16 channel. The CIN3+ risk could not be reliably estimated in women with an HPV18 positive self-sampling result.

4. Discussion

We studied the first round of a national hrHPV primary screening cohort in which women could opt for hrHPV self-sampling as an alternative to sampling at the clinician's office. Ct values of the hrHPV test were higher in self-collected samples translating into a 2% increase in specificity at the cost of a 6% lower sensitivity for detection of CIN3+. A high Ct value was associated with a low CIN3+ risk, in particular in women with a clinician-collected sample, supporting the use of Ct value as a risk stratifier in the management of hrHPV-positive results.

The main strength of our study is that it is the first study that reports on the real-world performance of primary hrHPV testing on self-sampled cervicovaginal material in comparison to hrHPV testing on clinician-collected material within a national, organised screening programme. This means that all women invited for screening, not only underscreened women, could opt for home testing and that self-collected samples were sent via regular mail. It further means that screening laboratories have facilities for high-volume hrHPV testing and a quality control system in place and cyto-technicians have been trained for reading hrHPV-positive slides. Another recent study with both hrHPV self-sampling and clinician-sampling as part of programmatic screening was the evaluation of a regional hrHPV screening programme in Argentina [19]. However, that study had a smaller sample size and used a signal amplification test for self-collected samples, which is known to be inferior to target amplification [7]. Another strength of our study is that through an established linkage with the nationwide network and registry of histo- and cytopathology (PALGA), we had at least 17 months of follow-up as well as information about age and screening history of the women. Our study has a number of limitations. Firstly, in a real-world setting, the performance of hrHPV self-sampling can only be compared to that of hrHPV clinician-sampling in an unpaired fashion. Hence, the study outcomes may be influenced by unmeasured confounders. Although we excluded underscreened women from our unpaired comparison of detected CIN3+ cases (Method I), a potential difference in CIN3+ risk between study groups remains a point of concern. Therefore, we also applied a method which utilises the Ct value of the hrHPV PCR test (Method II). We showed that Method II was robust against changes in Ct transformations and the screening history of the participants and yielded an estimated relative sensitivity with a much lower standard error than Method I. Secondly, in about 10% of the women with an hrHPV-positive test result, the Ct value was not available. The most likely reason for this is that Ct values are not used for screening management and some Ct values have not been adequately stored in the Cobas software. This conjecture is supported by the observation that missingness varied strongly across laboratories with percentages ranging from 6% in the South-West region to 17% in the East region. Thirdly, we did not collect results on colposcopic impression. However, 75% of the women in the self-collection group and 76% of the women in the clinician-collection group had an histological result after colposcopy referral so that the impact of loss to follow-up after colposcopy referral on the results is expected to be limited. Fourthly, only 6% of the women in our population opted for self-collection. This raises concerns about the generalisability of the results. We think that results also hold when a larger proportion of the screening population opts for self-collection since the relative sensitivity, estimated by Method II, was similar in the total population and in the subgroups of women who were underscreened and not underscreened. Hence, our estimates were robust against a change in the composition of the screening population. Fifthly, we did not have information on cellularity and cellular composition, which may differ by sampling method [20] and may influence the Ct value.

We observed a 6% lower sensitivity for CIN3+ and 2% higher specificity of hrHPV self-sampling as compared to clinician-based hrHPV testing. Because the lower bound of the 95% confidence interval of the relative sensitivity is not below the 90% margin, the hrHPV self-sampling test meets consensus criteria defined for evaluating newly developed clinician-collected hrHPV DNA tests in cervical screening [17]. The most likely explanation for the slight difference in sensitivity is that a sample obtained by vaginal self-sampling contains fewer representative cells for diagnosis than a sample obtained by clinician-based cervical sampling as reflected by the difference in mean Ct levels in CIN3+ cases. This is supported by diagnostic studies with Cobas PCR testing showing relative sensitivities for CIN3+ between 0·92 and 0·98 [20-23]. Similar findings were observed for other hrHPV tests [7]. Besides, for dry samples, a relative sensitivity of 0.96 was reported based on seven studies [7]. However, in our study the ROC of hrHPV self-sampling and clinician-based hrHPV testing showed a strong overlap up to the point where hrHPV self-sampling Ct value reached the system's cut-off. This suggests that, in theory, a similar performance hrHPV self-sampling and clinician-based hrHPV testing can be achieved by optimisation of the workflow procedure. An optimisation step that may be considered is to increase the volume-equivalent of the original self-collected sample used for hrHPV testing, for instance by decreasing the Preservcyt suspension volume. In this regard, a higher hrHPV prevalence was observed for self-sampling compared to clinician-sampling in a Dutch screening study where the self-collected brush was immersed in 4·5 ml, instead of 20 ml, PreservCyt [24]. The total impact of screening on the detection of cervical lesions does not only depend on the sensitivity of the test but also on compliance with follow-up procedures. In our data, 10% of women with a positive hrHPV self-sampling test did not show up for cytology testing which lowers the impact of screening. Therefore, alternative triage methods, such as HPV genotyping and HPV DNA methylation testing, may be considered which do not require an extra visit to the general practitioner [25].

An implication of the slight decrease in sensitivity of hrHPV self-sampling is that the 10-year screening interval may be reconsidered in women aged 40 or 50 who opted for self-sampling and had an hrHPV-negative test result. To determine the screening interval, the extra number of cancers prevented by shortening the interval should be weighed against the screening-related harms and costs of one or two extra screens.

The proportion of screened women opting for hrHPV self-sampling was below 10% in the first two years of the new Dutch hrHPV-based screening programme. A main reason for the low uptake of self-sampling is that women need to opt-in and actively have to request a self-sampling kit. During the Covid19 pandemic, the invitation letter was revised and the proportion of women opting for hrHPV self-sampling increased to at least 20% [26]. A different positioning of self-sampling, for instance by switching from opt-in to opt-out, may lead to a further increase in uptake in the future. The test sensitivity and specificity and precancer risks reported in our study could be included in educational campaigns to inform women about self-sampling and to facilitate informed decision-making by the women.

Our study furthermore showed a strong relation between CIN3 + risk and Ct value, in particular in clinician-collected samples. A similar finding was reported in populations from China [27,28] and further research is needed to determine whether suitable triage options for hrHPV-positive women with high Ct values exist, possibly in combination with hrHPV genotyping and other triage markers such as and DNA methylation analysis [25]. As Ct values are assay-specific, the CIN3+ risk should be determined separately for every new PCR assay.

In conclusion, our study confirmed that hrHPV testing on self-collected cervicovaginal material is an accurate alternative to hrHPV testing on clinician-collected samples in primary hrHPV-based screening programmes. Self-sampling can be used for targeting underscreened women, as a more convenient primary screening tool, and as an alternative for women during the current Covid19

pandemic [29]. When hrHPV testing on self-collected samples is used as a primary instrument in routine screening, the slight decrease in sensitivity of hrHPV testing on a self-collected sample compared to a clinician-collected sample seems to warrant an evaluation of the workflow procedure. To achieve international consensus on the requirements of hrHPV testing on a self-collected specimen, clinical criteria should be defined specifically for use in primary cervical screening.

Contributors

JB designed the study. AJCvdB, WJGM, AM, JWJH, HGMN, and AGS provided the data. FI and JB verified the underlying data, performed the statistical analysis and drafted the manuscript. All authors contributed to the data interpretation and revision of the manuscript giving their contribution to improve it, read and approved the final version.

Data sharing

The data that support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

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DAMH is minority shareholder of Self-screen B.V., a spin-off company of VUmc, which develops, manufactures and licences hrHPV and methylation marker assays for cervical cancer screening and holds patents on these tests. DAMH has been on the speakers' bureau of Qiagen and serves occasionally on the scientific advisory boards of Pfizer and Bristol-Myers Squibb.

All other authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lanepe.2021.100235.

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