



A daunting challenge: Human Papillomavirus assays and cytology in primary cervical screening of women below age 30 years



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Abstract We compared cytology with Hybrid Capture 2 (HC2), cobas, CLART and APTIMA Human Papillomavirus (HPV) assays in primary cervical screening at age 23–29 years based on data from the Danish Horizon study. SurePath samples were collected from 1278 women undergoing routine cytology-based screening. Abnormal cytology was managed according to the routine recommendations, and women with cytology-normal/HPV-positive samples were invited for repeated cytology and HPV testing in 1.5 years. Loss to follow-up was similar between HPV assays. \geq CIN3 was detected in 44 women. The sensitivity of HC2 for \geq CIN3 was 95% (95% confidence interval (CI): 85–99), of cobas 98% (95% CI: 88–100), of CLART 100% (95% CI: 92–100), of APTIMA 82% (95% CI: 67–92), and of cytology 59% (95% CI: 43–74). Specificity for \geq CIN3 varied between 61% (95% CI: 59–64) for cobas and 75% (95% CI: 73–78) for APTIMA, and was 94% (95% CI: 93–96) for cytology. Similar results were observed for \geq CIN2 ($N = 68$). HPV screening with cytological triage doubled the number of colposcopies compared to cytology screening, and increased the frequency of repeated testing by four (APTIMA) to seven (cobas) times. The positive predictive value of a referral for colposcopy was relatively high for all screening tests ($\geq 30\%$ for \geq CIN3, and $\geq 50\%$ for \geq CIN2). CIN1 was detected by cytology in $\sim 1\%$ of women, and in $\sim 2\%$ by any of the four HPV assays. Although highly sensitive, HPV-based screening of

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young Danish women should be approached cautiously, as it resulted in large reductions in specificity, and increased the demand for additional testing.

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1. Introduction

Human Papillomavirus (HPV) infections are frequent in young women, but the majority clears spontaneously. To avoid false-positive test results, HPV-based primary cervical screening has been considered primarily for women aged ≥ 30 years [1–4]. Nevertheless, some studies suggested that certain HPV assays, particularly those based on detection of HPV mRNA rather than DNA, might be suitable for screening at younger ages. In the French FASE (French APTIMA Screening Evaluation) study, for example, 1109 women aged 20–29 years attending routine cervical screening were tested with ThinPrep liquid-based cytology (LBC), Hybrid Capture 2 (HC2) HPV DNA assay, and APTIMA HPV mRNA assay [5]. In that study, APTIMA detected as many cases of high-grade cervical intraepithelial neoplasia (CIN) as HC2, whereas its specificity for high-grade CIN was similar to that of LBC. However encouraging, these findings should ideally be confirmed by data from other studies. Unfortunately, other studies comparing APTIMA with HPV DNA assays in screening populations did not present data specifically for this age group [6] or did not ascertain histological results in women with positive HPV tests and normal cytology [7].

Here, we presented data from the Danish Horizon study using samples from 1278 women aged 23–29 years attending primary cervical screening. All samples were tested with SurePath LBC and four HPV assays (APTIMA HPV Test (APTIMA; Hologic, San Diego, CA), HC2 (QIAGEN, Gaithersburg, MD), cobas HPV Test (cobas; Roche Diagnostics, Pleasanton, CA), and CLART HPV2 Assay (CLART; Genomica, Madrid, Spain)). We used these data to study the impact of the five tests on screening sensitivity, specificity, proportions of women with false-positive tests, and on colposcopy referral rates in primary screening of young women.

2. Materials and methods

2.1. Study design

The design of the Horizon study was described in detail previously [8–12]. Consecutive SurePath samples from 5034 women arriving for routine LBC analysis at the Department of Pathology of Copenhagen University Hospital, Hvidovre, in June–August 2011 were tested with the four HPV assays. By linkage to the national Pathology Data Bank (Patobank) [13],

primary screening samples were defined as those without a: previous cervical cancer, CIN in ≤ 3 years, atypical squamous cells of undetermined significance (ASCUS) or non-CIN cervical biopsy in ≤ 15 months, or a more severe cytological abnormality, inadequate cytology, or a positive HPV test in ≤ 12 months. Approximately 10% of women aged 23–29 years living in the then-catchment area of the laboratory were vaccinated against HPV [14].

Danish women are recommended for routine cervical screening every 3 years from age 23 onwards. Women included in the Horizon study were managed in line with their cytology and HPV test results, so that this setup where cytology was the basis for routine clinical management also mimicked primary screening with HPV testing and cytology triage. Women with abnormal cytology, regardless of their HPV status, were managed according to the routine Danish guidelines (repeated cytology if ASCUS or low-grade squamous intraepithelial lesions (LSIL), referral for colposcopy otherwise). Women with a positive test result on at least one of the four HPV assays at baseline and normal cytology (i.e. triage-negative) were invited, for study purposes, for repeated cytology and HPV testing in November 2012, approximately 1.5 years after the baseline. A reminder was sent in March 2013.

Women who responded to the study follow-up invitation had two SurePath samples taken. Those with abnormal cytology or a positive HC2 test result (corresponding to the routine HPV testing in the laboratory at the time of the study) were recommended for colposcopy. Histology in women with follow-up outside of the study was included in the analysis. All follow-up outcomes were retrieved from the Patobank in December 2013. This means that all histology was ascertained in approximately 2.5 years after the baseline testing, i.e. throughout most of the recommended 3-year screening interval. Colposcopies were performed following routine protocols recommending biopsies from all suspicious areas, or random biopsies from the four quadrants if lesions were not visible.

2.2. Cytology

Routine cytological evaluation was undertaken first by FocalPoint Slide Profiler (BD, Burlington, NC). Blinded to the outcomes of HPV testing, samples were thereafter evaluated by cytoscreeners using FocalPoint GS Imaging System (BD), and abnormal findings were adjudicated by pathologists.

2.3. HPV testing

All assay testing was undertaken in strict accordance with the protocols agreed upon with all manufacturers prior to the study, described in detail previously [8–12]. HC2 testing was undertaken on the post-quot LBC material; cobas, CLART and APTIMA testing were undertaken on the original residual material, diluted approximately 1:1 in SurePath. HC2 detects 13 high-risk HPV genotypes [15] collectively. It is based on hybridisation of HPV DNA to a high-risk HPV RNA probe cocktail. No re-test range was used. Cobas is a real-time PCR analysis detecting the 13 high-risk HPV genotypes and HPV66. The assay separately identifies HPV16 and HPV18, while the remaining 12 genotypes are detected collectively. CLART is a PCR-based low density array assay individually reporting 35 defined genotypes including the 13 high-risk. APTIMA detects E6/E7 mRNA expression of the 13 high-risk HPV types and HPV66 collectively.

2.4. Ethical considerations

Baseline testing of residual material was undertaken as a quality development study. In Denmark, such studies do not require ethical approval. Invitation to follow-up of HPV-positive/cytology-normal women was approved by the Ethics Committee of the Danish Capital Region (H-4-2012-120). It was not permitted to reveal the results of the baseline testing in the invitation letter, but women could obtain them from their general practitioner (GP). The study was notified to the Danish Data Inspection Agency (notification number 2010-41-5594).

2.5. Statistical analysis

A positive HPV test result was defined as relative light units per cut-off value ≥ 1.0 for HC2; cycle threshold values ≤ 40.5 , ≤ 40.0 and ≤ 40.0 for cobas's channels 16, 18 and other high-risk, respectively; signal to cut off ≥ 0.5 for APTIMA; and detection of ≥ 1 of the 13 high-risk genotypes by CLART. Abnormal cytology was defined as \geq ASCUS.

A screening test's clinical sensitivity was calculated as the proportion of women with high-grade CIN (detected at any point during the study) having a positive screening test result at baseline, and clinical specificity as the proportion of women who did not test positive among those without high-grade CIN. A false-positive test result was defined as a positive test result at baseline not followed by a diagnosis of high-grade CIN. We calculated the relative sensitivity, specificity and the proportion of false-positive tests by comparing the values to those of the reference screening test (HC2 or cytology). The 95% confidence intervals (CI) for sensitivity and

specificity were calculated assuming binomial distribution; those for the relative indicators were calculated assuming lognormal distribution. Persistent infection was defined as a (pooled) high-risk positive HPV test result at baseline and at follow-up on the same HPV assay. We calculated the positive predictive value (PPV) of a persistent infection as the proportion of women with a persistent infection having high-grade CIN.

3. Results

3.1. Study population

At baseline, 1278 women had screening samples at age 23–29 years (Table 1, Fig. 1). Ninety-five (7%) had abnormal cytology. Whereas 681 (53%) had normal cytology and negative test results on all four HPV assays, 495 (39%) had normal cytology with a positive test result on at least one assay. Test results were positive on HC2 for 406 (32%) of women, on cobas for 519 (41%), on CLART for 481 (38%) and on APTIMA for 342 (27%).

Of the 95 women with abnormal baseline cytology, six (6%) were lost to follow-up (Table 2). For 495 cytology-normal/HPV-positive women, this was 247 (50%); 158 (32%) had study follow-up (Table 3) and 90 (18%) had other follow-up. The completeness of follow-up did not differ substantially by HPV assay.

Of the 158 women with repeated testing in 1.5 year within the study (mean = 18.0 months, SD = 1.4 month), 15 (9%) developed abnormal cytology. Persistent infections, i.e. a positive (pooled) high-risk HPV test result followed by another (pooled) high-risk positive HPV test result in 1.5 years, were observed in 69 (62%) of 112 women with a baseline positive HC2 result, 85 (61%) of 140 women positive on cobas, 63 (48%) of 130 positive on CLART and 45 (48%) of 94 women positive on APTIMA. PPV for \geq CIN2 of persistent HPV infections were ~ 30 – 40% , and $\sim 50\%$ for incident abnormal cytology.

3.2. Sensitivity and specificity for high-grade CIN

Of the 44 \geq CIN3, HC2 detected 42 (sensitivity: 95%, 95% CI: 85–99; Table 4). Cobas detected 43, sensitivity: 98% (95% CI: 88–100), CLART 44 (100%, 95% CI: 92–100), APTIMA 36 (82%, 95% CI: 67–92) and cytology 26 (59%, 95% CI: 43–74). APTIMA and cytology detected statistically significantly fewer \geq CIN3 than HC2, cobas and CLART. Specificity for \geq CIN3 varied between 61% (95% CI: 59–64) for cobas and 75% (95% CI: 73–78) for APTIMA; it was 94% (95% CI: 93–96) for cytology. The differences were significant, with lower specificity for cobas and CLART, and higher for APTIMA, compared to HC2. The results for \geq CIN2 ($N = 68$) were similar.

Table 1
Description of the 1278 women aged 23–29 years with screening samples at baseline.

Characteristics at baseline	N (%)
Total	1278 (100%)
<i>Age (years)</i>	
23	339 (27%)
24	92 (7%)
25	123 (10%)
26	246 (19%)
27	138 (11%)
28	135 (11%)
29	205 (16%)
<i>Cytological diagnosis</i>	
Inadequate	6 (<1%)
Normal	1177 (92%)
ASCUS	16 (1%)
LSIL	49 (4%)
AGC, ASC-H, AIS, HSIL, or carcinoma	30 (2%)
<i>HPV test results</i>	
Positive on HC2	406 (32%)
Positive on cobas	519 (41%)
Positive on CLART	481 (38%)
Positive on APTIMA	342 (27%)
<i>Combined cytology and HPV results</i>	
Cytology normal and all 4 HPV test results negative	681 (53%)
Cytology normal and at least one HPV test result positive ^a	495 (39%)
Cytology abnormal, regardless of HPV test results	95 (7%)
Other ^b	7 (1%)

Abbreviations: AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASCUS, atypical squamous cells of undetermined significance; ASC-H, atypical squamous cells cannot exclude HSIL; HC2, Hybrid Capture 2; HPV, Human Papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

^a Invited for repeated testing at 18 months according to the study protocol.

^b Inadequate cytology, or normal cytology with at least one invalid HPV test result and no positive HPV test result.

3.3. HPV testing compared with cytology

In total, 7% of women were recommended for follow-up because of abnormal cytology (Table 5). With stand-alone HPV testing, this proportion was between 27% with APTIMA and 41% with cobas, i.e. about four to six times higher than with cytology.

With cytology-based screening, 5% of women were referred for colposcopy and 2% needed only repeated testing. With HPV assays combined with cytological triage, about twice as many had colposcopy. The PPV of a referral for colposcopy was relatively high for all five screening tests (~30% for \geq CIN3, ~50% for \geq CIN2, Table 6). The frequency of repeated testing was four times higher with APTIMA and HC2 compared to cytology, six times higher with CLART and seven times higher with cobas.

CIN1 was detected by cytology in ~1% of women, and by any of the four HPV assays in ~2%; HC2, cobas

and CLART led to a statistically significant increase in CIN1 detection compared to cytology. Detection of \geq CIN3 was between 60% and 70% higher and of \geq CIN2 around 90% higher for the three HPV DNA assays compared to cytology. APTIMA detected more high-grade CIN than cytology (38%), but the difference was not significant.

As 2.0% of women screened with cytology had \geq CIN3 detected, 5% had a false-positive cytology test. Using any of the four HPV assays, this proportion was statistically significantly higher, and ranged from 24% with APTIMA to 37% with cobas.

4. Discussion

4.1. General findings

Follow-up of young, mostly unvaccinated, Danish women with normal cytology and positive HPV test results on HC2, cobas, CLART or APTIMA substantially increased the overall detection of both \geq CIN2 (by 70–90%) and \geq CIN3 (by 40–70%) compared to cytology alone. Not surprisingly, the increased sensitivity was mirrored by a substantial decrease in screening specificity. Even with cytological triage of women with positive HPV tests, about twice as many were referred for colposcopy, and between four and seven times as many had a false-positive test compared to cytology alone. To this end, the lowest increases in the detection of CIN, false-positive tests and referrals to colposcopy were observed for the HPV mRNA assay APTIMA.

4.2. Strengths and weaknesses

Horizon was one of the few studies comparing several HPV assays in the same women while also ascertaining the clinical outcomes after cytology-normal/HPV-positive test results. The studied women were representatives for the routine screening population [10], and the samples were tested while fresh [9,11].

Of women with cytology-normal/HPV-positive test results at baseline, 50% had follow-up. Had the follow-up been complete, more high-grade CIN lesions would have been detected on account of HPV testing in cytology-normal women. This would increase the calculated differences in the sensitivity of HPV assays and cytology. However, the gap between the proportion of women with positive screening test results and the detection of high-grade CIN was so large that the incomplete follow-up cannot explain the high proportions of false-positive tests (data not reported).

4.3. Comparison with the literature

The substantially higher sensitivity for high-grade CIN in young women for HPV testing compared to

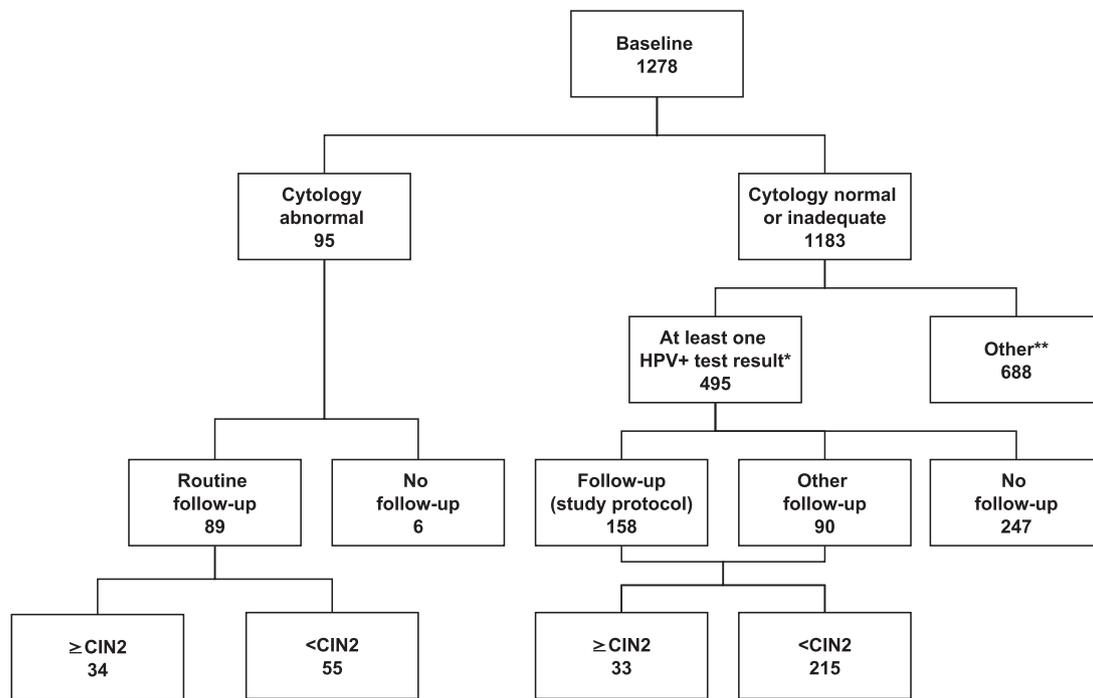


Fig. 1. Description of the study. *Women with normal cytology. **Other: Human Papillomavirus (HPV) negative on all four tests or with inadequate cytology. There were six women with inadequate cytology, of whom four had normal cytology in follow-up, and two had normal histology. One woman with normal cytology and four negative HPV test results had CIN2 in follow-up.

cytology was demonstrated previously [16]. In randomised controlled trials comparing primary HPV-based screening (using HC2) with cytology-based screening, the proportions of women with false-positive test results for \geq CIN3 varied between 12–17% at 25–34 years in Italy and Finland, and 31% at 20–29 years in the United Kingdom (UK) [17]. Proportions of women referred for colposcopy were generally not reported, except in the Italian NTCC Phase 1 trial [4,18]. There, the management at age 25–34 years after a positive HC2 test result was similar as in our study, with repeated testing in 12 months. Women were about twice as often referred for colposcopy with HC2 than with cytology. They also had three times as many false-positive tests with HC2, and were 11 times more likely referred for repeated testing [19]. These NTCC data were consistent with ours in pointing to a higher average number of health care contacts per woman screened with HPV testing than with cytology, at least in the initial HPV screening round.

One of the most frequently reported indicators of specificity from the trials was the PPV of a colposcopy referral. Like in our study, the PPV of HPV-based screening with cytological triage tended to be similar to that of cytology-based screening [20]. Yet, about twice as many women without high-grade CIN had colposcopy following HPV testing than following cytology screening alone.

Detection of HPV mRNA instead of DNA has been considered a viable and most likely a safe strategy to

reduce the high numbers of false-positive HPV screening tests [21]. The use of APTIMA in primary screening was evaluated in several studies [5–7,22,23]. As mentioned earlier, APTIMA was compared to LBC and HC2 in 1109 women aged 20–29 years in the FASE study [5]. Contrary to our findings, in French women APTIMA was as sensitive for high-grade CIN as HC2, but as specific as cytology. Among French women, 13.5% had abnormal cytology, whereas 23.5% tested positive on HC2, and 15.6% on APTIMA (relative proportion of positive test results for APTIMA versus HC2: 0.66). This difference between APTIMA and HC2 was less pronounced in our study, where the relative proportion of positive test results was 0.84. The FASE study used ThinPrep samples, and women with inadequate cytology (10.5%) were excluded from the analysis. Moreover, any difference in the background risk for cervical cancer, however unmeasured, between the French study and ours may have played a role, and suggested that the trade-offs between the detection and the burden by the different screening technologies may be population-specific.

In our study, APTIMA tended to detect fewer high-grade CIN than the three DNA assays. As the lower detection was confined to young women [24], a plausible interpretation might be a lower degree of over-diagnosis compared to HPV DNA assays [4,25]. mRNA assays are in principle designed to detect transcriptionally active HPV infections. By measuring only transcriptionally active/integrated HPV infections, transient, recent

Table 2

Follow-up outcomes for women with positive screening test results at baseline.

Baseline screening test		Total N at baseline (column%)	Worst outcome during follow-up (row%)							
Cytology test result	HPV test result		No follow-up	No histology		Histology				
					Normal cytology and/or negative HPV testing	Abnormal cytology and/or positive HPV testing	Inadequate histology	No CIN (CIN 0)	CIN1 ^a	CIN2
Normal or abnormal (total)	Negative or positive (total)	1278 (100%)	804 (63%)	279 (22%)	32 (3%)	6 (<1%)	61 (5%)	28 (2%)	24 (2%)	44 (3%)
<i>Cytology</i>										
Abnormal	Any	95 (7%)	6 (6%)	22 (23%)	4 (4%)	2 (2%)	16 (17%)	11 (12%)	8 (8%)	26 (27%)
Normal	≥1 Positive	495 (39%)	247 (50%)	137 (28%)	23 (5%)	4 (1%)	34 (7%)	17 (3%)	15 (3%)	18 (4%)
<i>HC2</i>										
Normal or abnormal	Positive	406 (32%)	152 (37%)	101 (25%)	22 (5%)	6 (1%)	36 (9%)	25 (6%)	22 (5%)	42 (10%)
Normal	Positive	314 (25%)	146 (46%)	80 (25%)	18 (6%)	4 (1%)	20 (6%)	14 (4%)	14 (4%)	18 (6%)
Abnormal	Positive	91 (7%)	6 (7%)	21 (23%)	4 (4%)	2 (2%)	15 (16%)	11 (12%)	8 (9%)	24 (26%)
<i>Cobas</i>										
Normal or abnormal	Positive	519 (41%)	215 (41%)	138 (27%)	27 (5%)	6 (1%)	41 (8%)	27 (5%)	22 (4%)	43 (8%)
Normal	Positive	432 (34%)	211 (49%)	117 (27%)	23 (5%)	4 (1%)	29 (7%)	16 (4%)	14 (3%)	18 (4%)
Abnormal	Positive	87 (7%)	4 (5%)	21 (24%)	4 (5%)	2 (2%)	12 (14%)	11 (13%)	8 (9%)	25 (29%)
<i>CLART</i>										
Normal or abnormal	Positive	481 (38%)	199 (41%)	127 (26%)	24 (5%)	6 (1%)	35 (7%)	23 (5%)	23 (5%)	44 (9%)
Normal	Positive	396 (31%)	194 (49%)	109 (28%)	20 (5%)	4 (2%)	23 (6%)	13 (3%)	15 (4%)	18 (5%)
Abnormal	Positive	85 (7%)	5 (6%)	18 (21%)	4 (5%)	2 (2%)	12 (14%)	10 (12%)	8 (9%)	26 (31%)
<i>APTIMA</i>										
Normal or abnormal	Positive	342 (27%)	128 (37%)	82 (24%)	19 (6%)	6 (2%)	27 (8%)	22 (6%)	22 (6%)	36 (11%)
Normal	Positive	261 (20%)	123 (47%)	64 (25%)	15 (6%)	4 (2%)	15 (6%)	12 (5%)	14 (5%)	14 (5%)
Abnormal	Positive	81 (6%)	5 (6%)	18 (22%)	4 (5%)	2 (2%)	12 (15%)	10 (12%)	8 (10%)	22 (27%)

Abbreviations: CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; HPV, Human Papillomavirus.

^a Includes atypia on histology and CIN not otherwise specified.^b No woman had cervical cancer.

Table 3

Women with normal cytology and at least one positive HPV test result at baseline ($n = 495$): 1.5-year persistence of infection, and final histology in 158 women with repeated cytology and HPV testing, by HPV assay.

Screening test	Cytology normal and HPV positive at baseline		Women with persistent infections/ incident abnormal cytology		
	Total	With 18-month follow-up (%)	N (% of those with follow-up)	\geq CIN2 (% of those with persistent infection)	\geq CIN3 (% of those with persistent infection)
Cytology	495 ^a (100%)	158 (32%)	15 (9%)	8 (53%)	4 (27%)
HC2	314 (100%)	112 (36%)	69 (62%)	26 (38%)	13 (19%)
Cobas	432 (100%)	140 (32%)	85 (61%)	≥ 25 ($\geq 29\%$) ^b	≥ 13 ($\geq 16\%$) ^b
CLART	396 (100%)	130 (33%)	63 (48%)	≥ 25 ($\geq 40\%$) ^b	≥ 12 ($\geq 19\%$) ^b
APTIMA	261 (100%)	94 (36%)	45 (48%)	≥ 20 ($\geq 44\%$) ^b	≥ 11 ($\geq 19\%$) ^b

Abbreviations: CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; HPV, Human Papillomavirus.

^a A positive test result on any of the four HPV assays.

^b Only women with abnormal cytology and/or a positive HC2 test result were referred for colposcopy.

Table 4

Sensitivity and specificity of HPV assays and cytology, and relative sensitivity and specificity compared to Hybrid Capture 2, for detection of \geq CIN3 and \geq CIN2, in women aged 23–29 years undergoing primary cervical screening.

	Sensitivity (95% CI)	Relative sensitivity (95% CI)	Specificity (95% CI)	Relative specificity (95% CI)
<i>Endpoint: \geq CIN3</i>				
HC2	95% (85–99)	1.00 (Ref)	71% (68–73)	1.00 (Ref)
Cobas	98% (88–100)	1.02 (0.95–1.11)	61% (59–64)	0.87 (0.82–0.92)
CLART	100% (92–100)	1.05 (0.98–1.12)	65% (62–67)	0.92 (0.87–0.97)
APTIMA	82% (67–92)	0.86 (0.74–1.00)	75% (73–78)	1.07 (1.02–1.12)
Cytology	59% (43–74)	0.62 (0.48–0.80)	94% (93–96)	1.34 (1.29–1.39)
<i>Endpoint: \geq CIN2</i>				
HC2	94% (86–98)	1.00 (Ref)	72% (69–74)	1.00 (Ref)
Cobas	96% (88–99)	1.02 (0.94–1.10)	62% (60–65)	0.87 (0.82–0.92)
CLART	99% (92–100)	1.05 (0.98–1.12)	66% (63–68)	0.92 (0.87–0.97)
APTIMA	85% (75–93)	0.91 (0.81–1.02)	77% (74–79)	1.07 (1.02–1.12)
Cytology	50% (38–62)	0.53 (0.42–0.68)	95% (94–96)	1.32 (1.27–1.37)

Abbreviations: CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2.

and cytoplasmatic and/or transcriptionally inactive HPV infections are theoretically avoided. It can be hypothesised that CIN missed by APTIMA but detected by an HPV DNA assay tended to be those that would have spontaneously regressed if left untreated, which is often the case in younger women [26]; however, this hypothesis is hard to prove. For now, the relative progression potential of HPV mRNA-negative/HPV DNA-positive high-grade CIN is unknown, and leaving these lesions untreated would be unethical.

4.4. Clinical implications

Despite screening since the 1960's, the incidence rate in Danish women aged 25–29 years remains around 12/100,000 women [27]. Although highly sensitive HPV assays would probably improve the protection from cervical cancer, they would unlikely eradicate the disease at young age [28]. Furthermore, there is a downside to detecting CIN lesions particularly in women of child-bearing age, as their treatment may be associated with an increased risk of poorer pregnancy outcomes [29–31]. This is particularly problematic given that many high-grade CIN detected at young age do not tend to

progress to cervical cancer [26,32]. Furthermore, a positive HPV test result induces a psychological burden [33,34], and may reduce sexual satisfaction [35].

The challenge, therefore, is to detect and triage to colposcopy only those HPV infections that have a real chance of progressing to cervical cancer. Although a careful selection of women for a referral for colposcopy can on its own not resolve the problem of low specificity of the screening test, colposcopy referral should be optimised with respect to its frequency and CIN detection. Based on the data from the Horizon study, we made some basic post hoc comparisons of the proportions of referred women and CIN detection at baseline for likely triage strategies. In interpreting the outcomes of this post hoc analysis, it should be taken into account that HPV-positive women were in reality managed through cytology triage, and also that in a true routine HPV-based screening setting the performance of cytology as a triage test might change. With cytology triage of HPV-positive women, 6–7% of all screened women would be referred for colposcopy at baseline (Table 7), and the remainder with positive HPV test results for follow-up with repeated testing (which would include a referral in case of abnormal findings). This strategy

Table 5

Consequences of replacing cytology with either of the four HPV assays combined with cytology triage in primary cervical cancer screening of women below age 30 years ($N = 1278$).

	Abnormal/positive test results	Follow-up procedures ^a		Detection of CIN			False-positive test results	
		Colposcopy ^b	Repeated testing only	CIN1 ^c	≥CIN2	≥CIN3	Endpoint: ≥CIN2	Endpoint: ≥CIN3
<i>Absolute numbers, N (%)</i>								
Cytology	95 (7%)	63 (5%)	26 (2%)	11 (0.9%)	34 (2.7%)	26 (2.0%)	61 (5%)	69 (5%)
HC2	406 (32%)	131 (10%)	123 (10%)	25 (2.0%)	64 (5.0%)	42 (3.3%)	342 (27%)	364 (28%)
Cobas	519 (41%)	139 (11%)	165 (13%)	27 (2.1%)	65 (5.1%)	43 (3.4%)	454 (36%)	476 (37%)
CLART	481 (38%)	131 (10%)	151 (12%)	23 (1.8%)	67 (5.2%)	44 (3.4%)	414 (32%)	437 (34%)
APTIMA	342 (27%)	113 (9%)	101 (8%)	22 (1.7%)	58 (4.5%)	36 (2.8%)	284 (22%)	306 (24%)
<i>Relative risk (95% CI)</i>								
Cytology	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
HC2	4.27 (3.47–5.27)	2.08 (1.56–2.78)	4.73 (3.12–7.17)	2.27 (1.12–4.60)	1.88 (1.25–2.83)	1.62 (1.00–2.62)	5.61 (4.32–7.28)	5.28 (4.13–6.74)
Cobas	5.46 (4.45–6.70)	2.21 (1.66–2.94)	6.35 (4.23–9.53)	2.45 (1.22–4.93)	1.91 (1.27–2.87)	1.65 (1.02–2.67)	7.44 (5.76–9.61)	6.90 (5.43–8.77)
CLART	5.06 (4.12–6.22)	2.08 (1.56–2.78)	5.81 (3.86–8.74)	2.09 (1.02–4.27)	1.97 (1.31–2.96)	1.69 (1.05–2.73)	6.79 (5.25–8.78)	6.33 (4.97–8.07)
APTIMA	3.60 (2.91–4.46)	1.79 (1.33–2.42)	3.88 (2.54–5.94)	2.00 (0.97–4.11)	1.71 (1.13–2.59)	1.38 (0.84–2.28)	4.66 (3.57–6.07)	4.43 (3.46–5.69)

Abbreviations: CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2.

^a The difference between the total number of women with positive screening test results and women with either colposcopy or repeated testing only were women who had no follow-up.

^b Measured as registered with a biopsy in the Patobank.

^c Includes CIN1, histological atypia and CIN NOS.

Table 6

Positive predictive value of colposcopy.

Screening test	Colposcopy (%)	Endpoint: ≥CIN2				Endpoint: ≥CIN3			
		<i>N</i> detected (PPV of colposcopy)	Relative PPV (95% CI)	<i>N</i> FP colposcopy (% of all screened women)	RR of FP colposcopy (95% CI)	<i>N</i> detected (PPV of colposcopy)	Relative PPV (95% CI)	<i>N</i> FP colposcopy (% of all screened women)	RR of FP colposcopy (95% CI)
Cytology	63 (100%)	34 (54%)	1 (ref)	29 (2%)	1 (ref)	26 (41%)	1 (ref)	37 (3%)	1 (ref)
HC2	131 (100%)	64 (49%)	0.91 (0.68–1.21)	67 (5%)	2.3 (1.5–3.5)	42 (32%)	0.78 (0.53–1.14)	89 (7%)	2.4 (1.7–3.5)
Cobas	139 (100%)	65 (47%)	0.87 (0.65–1.16)	74 (6%)	2.6 (1.7–3.9)	43 (31%)	0.75 (0.51–1.10)	96 (8%)	2.6 (1.8–3.8)
CLART	131 (100%)	67 (51%)	0.95 (0.71–1.26)	64 (5%)	2.2 (1.4–3.4)	44 (34%)	0.81 (0.56–1.19)	87 (7%)	2.4 (1.6–3.4)
APTIMA	113 (100%)	58 (51%)	0.95 (0.71–1.27)	55 (4%)	1.9 (1.2–3.0)	36 (32%)	0.77 (0.52–1.15)	77 (6%)	2.1 (1.4–3.1)

Abbreviations: CI, confidence interval; CIN, cervical intraepithelial neoplasia; FP, false-positive; PPV, positive predictive value; RR, relative risk.

Table 7
Number of women referred for colposcopy at baseline depending on the results of cytology or genotyping, and detected \geq CIN2 at baseline.

Screening test result	Triage test	Triage test cut-off	Screening test							
			HC2		Cobas		CLART		APTIMA	
			N (%) ^a	\geq CIN2 (%) ^b	N (%) ^a	\geq CIN2 (%) ^b	N (%) ^a	\geq CIN2 (%) ^b	N (%) ^a	\geq CIN2 (%) ^b
HPV positive	None	–	406 (32%)	64 (100%)	519 (41%)	65 (100%)	481 (38%)	67 (100%)	342 (27%)	58 (100%)
HPV positive	Cytology	\geq ASCUS	91 (7%)	32 (50%)	87 (7%)	33 (51%)	85 (7%)	34 (51%)	81 (6%)	30 (52%)
HPV positive	Genotyping	HPV 16 and/or 18	Not relevant	Not	201 (16%)	37 (57%)	182 (14%)	38 (57%)	Not	Not
HPV positive	Genotyping	HPV 16, 18, 31 and/or 33	Not relevant	Not	Not	Not	297 (23%)	60 (90%)	Not	relevant
HPV positive	Genotyping and cytology	HPV 16 and/or 18, and \geq ASCUS	Not relevant	Not	34 (3%)	18 (28%)	36 (3%)	18 (27%)	Not	relevant
HPV positive	Genotyping and cytology	HPV 16, 18, 31 and/or 33, and \geq ASCUS	Not relevant	Not	Not	Not	60 (5%)	29 (43%)	Not	relevant
HPV positive	HPV test	HPV positive ^c	312 (24%)	56 (88%)	323 (25%)	57 (88%)	313 (24%)	58 (87%)	312 (24%)	56 (97%)

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, Human Papillomavirus.

^a Number of women referred for colposcopy at baseline (proportion of all screened women).

^b Number of women with \geq CIN2 detectable at baseline (proportion of all \geq CIN2 detectable by the screening assay).

^c For HC2, cobas and CLART as primary screening assays; triage with APTIMA. For APTIMA as primary screening assay: triage with HC2.

would detect just over half of all \geq CIN2 already at baseline, leaving the remaining half to be detected through repeated testing. If, instead, triage were undertaken using limited genotyping for HPV16 and 18, 16% of all young screened women would be referred for colposcopy at baseline. This triage strategy would detect slightly more high-grade CIN at baseline than cytological triage. In young Danish women with normal cytology, progression to \geq CIN3 was most frequent for women persistently infected with HPV16 (cumulative 12-year \geq CIN3 incidence: ca. 27%), HPV18, HPV31, or HPV33 (15–20%) [36]. If triage was undertaken by genotyping for these four genotypes on CLART, 23% of screened women would be referred, however, approximately 90% of all high-grade CIN could be detected already at baseline. Combining genotyping with cytology as triage tests would refer to colposcopy relatively few women at baseline, but leave relatively large proportions of CIN to be detected at follow-up. Finally, triaging positive HPV DNA test results with a positive HPV mRNA test result or vice versa would refer to colposcopy about a quarter of all young screened women but would detect close to 90% of all high-grade CIN at baseline. A more detailed analysis of optimal triage strategies is on-going, and will be reported separately.

In conclusion, HPV-based screening of young Danish women with DNA or mRNA assays should be approached with caution. The gains in the detection of high-grade CIN tended to be obtained through large reductions in screening specificity, and increases in the demand for additional testing.

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Genomica, the manufacturer of the CLART HPV2 Assay; Hologic, the manufacturer of the APTIMA HPV Assay; Roche, the manufacturer of the cobas HPV Test; and QIAGEN, the manufacturer of the Hybrid Capture 2 Assay, provided tests, instrumentation, and limited co-funding for laboratory material. According to the contract between the manufacturers, Hvidovre University Hospital, and University of Copenhagen, all four manufacturers had the right to comment on a draft version of this manuscript, but had no editorial rights. MR and SP were funded by the Danish Strategic Research Council (Grant No.: 10-092793). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The researchers worked independently of the funders.

Contributors

Design of the study: MR, JB, EL.
Laboratory work: SP, DE, JB.
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Drafting of the manuscript: MR, JB.

Critical revisions of the manuscript: all authors.

Decision to submit: all authors.

All authors had full access to all of the data in the study.

Conflict of interest statement

All authors have attended meetings with manufacturers of HPV assays.

Matejka Rebolj and her former employer received honoraria from QIAGEN for lectures on her behalf.

Jesper Bonde used to serve as a paid advisor to Roche and Genomica, and received honoraria from Hologic, Roche, QIAGEN, Genomica, and BD Diagnostics for lectures. He is principal investigator on studies funded by BD Diagnostics.

Ditte Møller Ejegod received honoraria from Genomica and QIAGEN for lectures, and is project manager on studies funded by BD Diagnostics.

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