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Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: the FASE study

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The APTIMA® HPV Assay (AHPV) allows detection of 14 high-risk human papillomavirus (HPV) RNA types in cervical specimens. Until present, the assay has been compared to HPV DNA tests only in triage settings. Herein, we compare AHPV with a DNA assay (Hybrid Capture® 2; HC2) and liquid-based cytology (LBC; using PreservCyt® ThinPrep liquid Pap) in a screening setting (French APTIMA screening evaluation [FASE] study). Women (N = 5,006) aged 20–65 were screened by gynecologists in 17 private practices in Paris, France. One cervical specimen was collected and tested with LBC, AHPV and HC2 assays. Women were referred to colposcopy if they were ASC-US+ in LBC or HPV positive in either HPV assay. To control for verification bias, a random group (14%) with normal LBC and dually HPV negative tests underwent colposcopy. Data from 4,429 women were analyzed. Sensitivity, specificity and predictive values were calculated for the three tests. AHPV and HC2 were highly sensitive for CIN2+ (92.0% and 96.7%) and CIN3+ (95.7% and 95.3%) detection and much more sensitive than LBC (69.1% for CIN2+ and 73.3% for CIN3+). Specificity of AHPV was higher than that of HC2, but similar to that of LBC ($p < 0.001$). Combining LBC with either HPV test slightly increased sensitivity but compromised specificity. AHPV assay is both specific and sensitive for the detection of high-grade precancerous lesions and may be considered as an option for routine cervical cancer screening for women over 20 years of age.

Invasive cervical cancer (ICC) is the second most frequent female cancer worldwide,[1] with an estimation of 493,000 cases annually. ICC incidence and mortality rates have dramatically declined over the past five decades in developed countries, largely due to screening programs based on conventional cervical Papanicolaou (Pap) smears.[2,3] Conventional Pap smear screening, however, has limited sensitivity, positive predictive value (PPV) and reproducibility, which limits its use for primary screening.[3,4–7] Liquid-based cytology (LBC) has been shown to be more sensitive than conventional

Key words: cervical cancer screening, HPV RNA test, HPV DNA test, liquid-based cytology, high-grade CIN

Abbreviations: ACG: atypical glandular lesion; ADC: adenocarcinoma; AHPV: APTIMA® HPV; ASC-US: atypical cells of undetermined significance; ASC-H: atypical squamous cells: cannot rule out a high grade lesion; CIN: cervical intraepithelial neoplasia; HC2: Hybrid Capture® 2; HPV: human papillomavirus; HR-HPV: high-risk HPV; HSIL: high-grade cytological abnormalities; ICC: invasive cervical cancer; LBC: liquid-based cytology; LSIL: low-grade cytological abnormalities; NPV: negative predictive value; Pap: Papanicolaou; PPV: positive predictive value; RLU/CO: relative light units to control cut-off; S/CO: signal to cut-off; SIL: squamous intra-epithelial lesion; TZ: transformation zone

Conflict of interest: J. Monsonego has received funding to conduct studies related to the FASE study from Gen-Probe Inc. and related to HPV vaccines from Merck and GlaxoSmithKline, and has participated to Steering Committees at Merck and to the Advisory Board of Sanofi Pasteur MSD, Gen-Probe, Qiagen, and Roche Diagnostics. J. Smith has received research grants or contracts, honoraria and consulting fees during the last three years from Qiagen or Genprobe. F. Ruiz is an employee of ClinSearch. K. Syrjänen, M. Hudgens, P. Halfon, L. Zerat and J.C. Zerat have no conflict of interest to report.

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cytology for the detection of atypical cells of undetermined significance (ASC-US) and squamous intraepithelial lesion (SIL).[8] However, a recent meta-analysis showed that LBC is neither more sensitive nor more specific than conventional cytology for histologically confirmed high-grade cervical intraepithelial neoplasia (CIN2 or 3).[9]

High-risk human papillomavirus (HR-HPV) types are related to virtually all ICC cases and most high-grade cervical precancer.[10] For CIN2 or greater (CIN2+) detection at one time point, HPV testing is more sensitive and has a higher negative predictive value (NPV) than cytology.[11–14] ICC screening using HPV DNA detection allows earlier detection of high-grade CIN and cancer.[15] Further, a single negative HPV DNA test reliably predicts a low risk of subsequent CIN2+, thus justifying longer screening intervals.[16–18] In the United States, DNA HPV tests have been recommended and approved (*i*) to triage patients with ASC-US cytology results to determine the need for referral to colposcopy and (*ii*) when used adjunctively to cytology, to assess the presence/absence of HR-HPV types in women 30 years and older to help determine proper patient management.[19,20] HPV DNA testing has not been approved yet in Europe for primary screening or for cotesting purposes.

HPV RNA testing is based on the detection of HR-HPV E6 and E7 mRNA. Given that the oncogenic potential of HPV infection depends on the production of viral E6/E7 oncoproteins, detection of E6/E7 mRNA transcripts may provide a more specific test in detecting clinically significant disease. The APTIMA® HPV Assay (AHPV; Gen-Probe, San Diego, CA)[21,22] detects 14 high-risk types of HPV E6/E7 mRNA and has been compared to DNA tests in patients referred for colposcopy due to an abnormal Pap smear.[21,22–25] In this setting, AHPV is equally sensitive but more specific than the HC2 HPV DNA test (Qiagen, Gaithersburg, MD) for CIN2+ and CIN3+ detection.[22–25] AHPV has not yet been validated in screening settings.

The present diagnostic accuracy study is the first to compare AHPV with HC2 and ThinPrep LBC (Cytyc Corp., Bedford, MA), in a population-based screening for high-grade CIN. We adjusted for verification bias by referring a subset of screened women with negative cytology and HPV test results to colposcopy. We evaluated the performance of all three tests in the whole cohort and stratified by age.

Material and Methods

Study patients and conduct

From April 2008 to February 2009, women who were seen for their annual exam in 17 private gynecology practices in Paris, France, were invited to participate in this voluntary screening. In France, cervical cancer screening is recommended every 3 years, but most often is conducted every 1.5–2 years at the physician's discretion. The number of patients who declined participation is unknown, but informal survey at the gynecology centers (where women were enrolled) revealed that very few women did not consent. The study was coordinated by a steering group headed by the principal investigator (JM) and monitored by a Contract Research Organization (ClinSearch, Bagneux, France). This protocol was conducted in accordance with the Declaration of Helsinki and approved by an Independent Ethics Committee (Pitié Salpétrière University Hospital).

Women in the age group of 20–65 years were enrolled after signing an informed consent. Women were not eligible if they had undergone total hysterectomy, were pregnant or had an abnormal cytology in the past 6 months. Demographic, reproductive and sexual history data were recorded at screening.

LBC sample collection and analysis

One cervical sample from each patient was collected by the gynecologist during a routine gynecological examination. Cervical samples were collected from the transformation zone (TZ) using a Cervex-Brush® (Rovers Medical Devices, Oss, The Netherlands), which was rinsed into PreservCyt® medium (Cytyc Corp., Marlborough, MA). Cytology (LBC) was performed using the ThinPrep liquid Pap test (Cytyc Corp.) in PreservCyt medium, according to the manufacturer's instructions (Fig. 1). All LBC samples were analyzed by a central laboratory (Laboratoire Lavergne, Paris, France) and classified according to the 2001 Bethesda System (TBS 2001). Cytopathologists were blinded to the HPV test results. An independent external reviewer (KS) blindly double-read the cytology samples with abnormal cytology results and a random selected group of women (14%) with normal LBC samples and negative HPV tests (adjudicated cytology). The high (10.5%) rate of unsatisfactory ThinPrep results was due to poor celullarity, thick preparations and obscuring blood/debris, and the high proportion of post-menopausal women (20%) with poor cervical cellularity; women with unsatisfactory cytology results were not included in the data analyses. Final analyses were based on the adjudicated cytology results as HPV test performance was the same as that observed in the first cytology reading (data not presented).

HPV testing

The LBC sample was then divided into two equal aliquots. For the first 2,500 samples, the first LBC aliquot was tested for HC2 and the second aliquot for AHPV; for the next 2,500 samples, the first aliquot was tested for AHPV and the second for HC2. Individuals performing HPV testing were blinded to LBC results.

The HC2 DNA assay is based on qualitative detection of L1 in 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68).[20,26] Specimens for HC2 testing were collected in LBC/ThinPrep and transported to the central laboratory at 2–30°C. Specimens were tested according to the manufacturer's instructions. Samples were considered positive using the relative light units to control cut-off (RLU/CO) of 1.0 pg/mL.

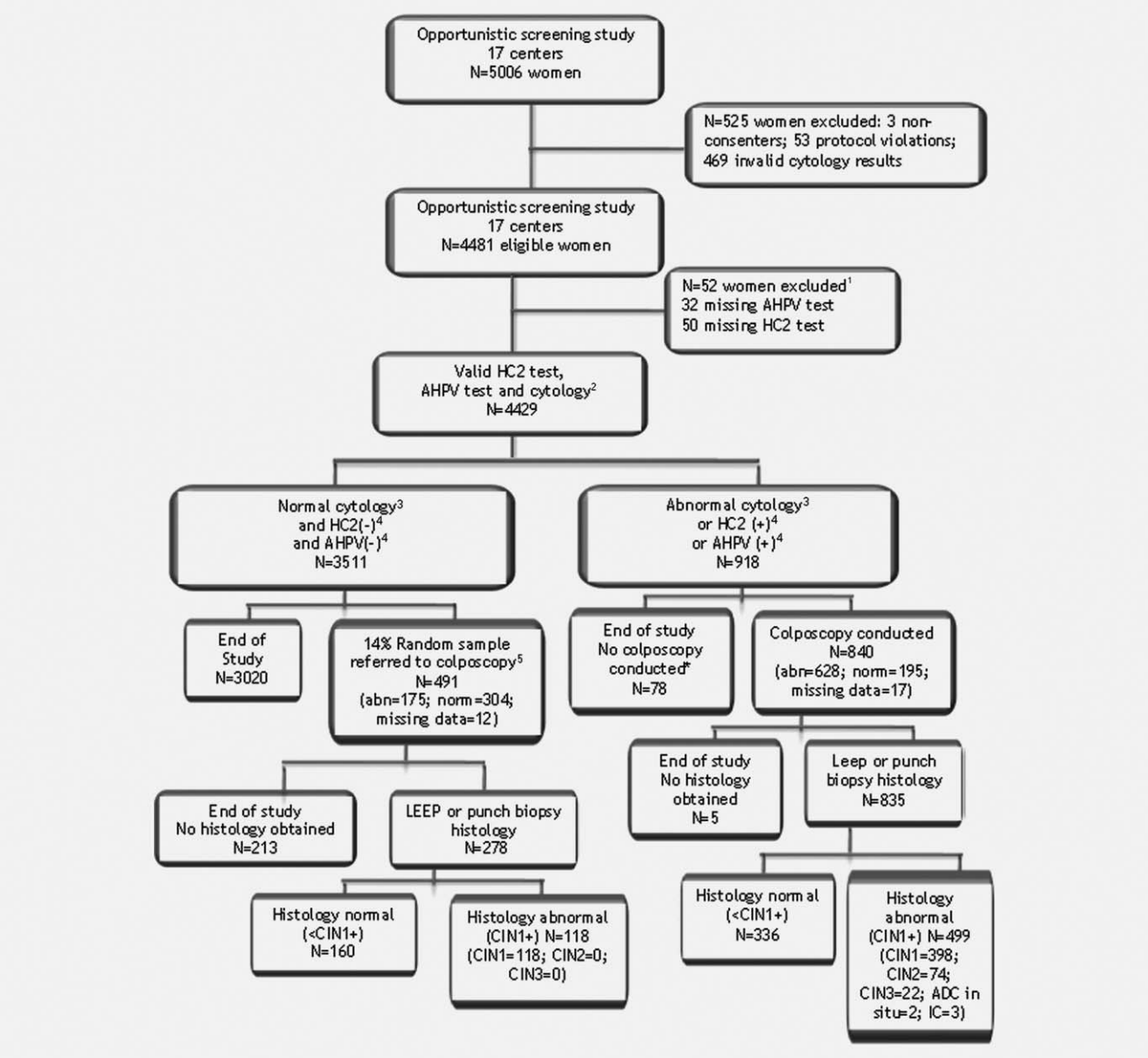

Figure 1. Study flowchart. Altogether, 5,006 women were examined by the 17 centers; 525 women were excluded: three were nonconsenting, 53 had protocol violations (were not between the ages of 20 and 65) and 469 women had uninterpretable cytology results. Thus, a total of 4,481 women were considered eligible to enroll in the study. [1]Of these 4,481 women, 52 were excluded for missing either HC2 or AHPV test; [2]Total number of women having both tests and cytology. These women constitute the analysis data set unless noted otherwise; [3]Original cytology, with abnormal diagnosis based on ASC-US cut-off; [4]Manufacturer recommended cut-off; [5]Colposcopy was obtained on a simple random sample of women with normal cytology who were HC2 negative and AHPV negative. Also the few vaginal lesions with no CIN were excluded from the analysis. Abn: abnormal; norm: normal. CIN: cervical intraepithelial neoplasia; ADC: adenocarcinoma; IC: invasive cancer. *Patients failed to return to the clinic despite three written notices.

The AHPV RNA assay is based on the qualitative detection of E6/E7 viral mRNA of 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) from cervical specimens collected into ThinPrep LBC vials.[27] AHPV utilizes the technologies of target capture, transcription-mediated amplification and dual kinetic assay.[21–23,27] All AHPV assays were performed at CDL Pharma (Marseille, France) according to the manufacturer's instructions.[27] Samples were considered positive when the signal to cut-off (S/CO) was $\geq$1.0.[27]

Colposcopy and biopsies

Women positive for any one of these three screening tests were referred for colposcopy. The criteria for referral included ASC-US or greater, or at least one positive HPV test. To control for verification bias, 14% of women with negative results for the three tests were selected by simple random sampling to undergo colposcopy. Colposcopy was performed at each clinic according to standard operating procedures, using the international nomenclature.[28]

Per protocol, all women with abnormal colposcopy were to receive at least one biopsy from the most severe area and a minimum of one biopsy from each quadrant of atypical transformation zone (TZ). For women with normal colposcopy (and not in the random control group), two biopsies were performed at 12 and 6 o'clock of TZ. No biopsy was performed in women from the control group with normal colposcopy. All acetowhite areas of the cervix were to be subjected to at least one biopsy within the most severe area of the TZ and iodine-negative regions of the vagina during the colposcopic examination. LEEP cone biopsy or cold knife conization with endocervical curettage was to be performed in cases with: (*i*) PAP test showing high-grade squamous intraepithelial lesion (HSIL) and atypical TZ on colposcopy, (*ii*) large abnormal TZ ($\geq$50% of TZ), regardless of Pap test result, (*iii*) endocervical lesion and unsatisfactory colposcopy or (*iv*) abnormal TZ and squamo-columnar junction within $>$3 mm of the endocervix. The lesion was considered endocervical in all cases where atypical TZ extended to the cervical canal. No LEEP or conization was to be performed exclusively on the basis of HPV test results, *i.e.*, the decision for treatment in HPV+ women was based on biopsy results.

All biopsies were examined at a central laboratory by pathologists who were blinded to HPV test results. Histopathologists were not blinded to cytology results for safety reasons. The three-tier CIN nomenclature was used for biopsy classification, and the most severe abnormality selected for final histological diagnosis. An independent (international) reviewer (KS) re-examined all biopsies. In all discrepant cases, the final diagnosis was the consensus reached by a panel of three pathologists: the original pathologist, the reviewing pathologist and a third additional laboratory pathologist.

Statistical methods

Differences in demographic data and screening test results by age were assessed by the Wilcoxon rank sum test for quantitative variables and Fisher's exact test for categorical variables. The kappa statistic was used to measure agreement between the AHPV and HC2 tests. McNemar's test was used to compare the prevalence of AHPV and HC2 positivity. To estimate the operating characteristics of AHPV, HC2 and cytology, an "uncorrected" analysis using data only from women with histology results can lead to biased inference.[29] Therefore, sensitivity, specificity and predictive values were estimated using maximum likelihood adjusted for verification bias.[30,31] In particular, the verification-bias adjusted estimators proposed by Zhou et al.[30] and Roldan-Nofuentes et al.[31] for two screening tests were extended to allow for three screening tests (here AHPV, HC2 and cytology). The verification adjustment accounted for women with cytology but missing colposcopy and for women with colposcopy but missing histology. The maximum likelihood methods employed to adjust for verification bias are considered valid, provided that the data are missing at random.[30] In this setting, the missing-at-random assumption is that, among women with the same HPV and cytology results, women with missing colposcopy results are similar to women with colposcopy results.

Differences in sensitivity, specificity and predictive values of the screening tests were assessed using Wald-type tests (*e.g.*, as given by the last equation in Section 3 of Zhou et al.[30]). All statistical analyses were performed using SAS® 9.1.3. Two-sided *p*-values less than 0.05 were considered statistically significant. No adjustments were made for multiple comparisons.

Results

Subject characteristics

Overall, 5,006 consented women (20–65 years of age) were enrolled. A total of 577 women were excluded from the analyses (three did not provide consent; 53 were not aged 20–65; 469 had missing cytology results; and 52 were missing at least one HPV test results). Women with cytology results were similar in age ($p = 0.74$) and had similar AHPV positivity ($p = 0.49$) compared to women without cytology results. However, women with cytology results were more likely to be HC2 positive compared to women without cytology results (15.6% *vs.* 11.6%, $p = 0.02$). The 4,429 women with both HPV tests and cytological results constituted the study cohort (Fig. 1).

Women of age $\geq$30 years reported a higher number of previous pregnancies, a later onset of sexual activity and lower number of recent sexual partners, oral contraceptive use and current smoking ($p < 0.001$) than women of age 20–29 years (Table 1). Prevalence of HPV infection varied notably, with an overall 15.7% positivity in HC2 and 10.3% positivity in AHPV. For both tests, HPV positivity was lower among women $\geq$30 years than those 20–29 ($p < 0.001$). Prevalence of abnormal cytology was 9.6% in the whole cohort and 8.3% for women $\geq$30. Women aged 20–29 also had a higher prevalence of ASC-US and low-grade cytological abnormalities (low-grade squamous intraepithelial lesion [LSIL]) ($p < 0.001$), but a similar prevalence of ASC-H (atypical squamous cells, cannot rule out a high-grade lesion), HSIL, and atypical glandular lesion (AGC). Women of ages $\geq$30 and 20–29 years had overall similar histological results ($p = 0.43$).

Cytological screening

There were 723 women with normal LBC cytology who had a histology result; 278 were in the random sample and 445

Table 1. Demographic data and screening test results

	Overall (N = 4,429)[1]	Age 20–29 years (N = 1,109)	Age 30–65 years (N = 3,320)	p*
Age				–
20–29	1,109 (25.0%)	1,109 (100%)	–	
30–39	1,250 (28.2%)	–	1,250 (37.4%)	
40–49	1,190 (26.9%)	–	1,190 (36.5%)	
50–65	880 (19.9%)	–	880 (26.1%)	
Median (range) of previous pregnancies	1 (0–12)	0 (0–4)	2 (0–12)	<0.001
First sexual relation				<0.001
≤16 years old	410 (9.4%)	176 (16.0%)	234 (7.2%)	
>16 years old	3,951 (90.6%)	926 (84.0%)	3,025 (92.8%)	
Number of sexual partners within last 12 months				<0.001
0	269 (6.2%)	29 (2.7%)	240 (7.4%)	
1	3,715 (85.3%)	881 (80.7%)	2,834 (86.8%)	
2+	372 (8.5%)	182 (16.7%)	190 (5.8%)	
Current use of oral contraceptive and hormones				<0.001
Yes	2,394 (54.2%)	870 (78.7%)	1,524 (46.0%)	
No	2,023 (45.8%)	235 (21.3%)	1,788 (54.0%)	
Current smoking status				<0.001
Smoker	1,080 (24.5%)	361 (32.7%)	719 (21.8%)	
NonSmoker	3,325 (75.5%)	743 (67.3%)	2,582 (78.2%)	
HC2 (HPV DNA)				<0.001
Positive	693 (15.7%)	261 (23.5%)	432 (13.0%)	
Negative	3,736 (84.4%)	848 (76.5%)	2,888 (87.0%)	
AHPV (HPV RNA)				<0.001
Positive	456 (10.3%)	173 (15.6%)	283 (8.5%)	
Negative	3,973 (89.7%)	936 (84.4%)	3,037 (91.5%)	
LBC[2]				<0.001
Normal	4,004 (90.4%)	959 (86.5%)	3,045 (91.7%)	
ASC-US	130 (2.9%)	39 (3.5%)	91 (2.7%)	
ASC-H	17 (0.4%)	3 (0.3%)	14 (0.4%)	
LSIL	226 (5.1%)	94 (8.5%)	132 (4.0%)	
HSIL	47 (1.1%)	12 (1.1%)	35 (1.1%)	
AGC	5 (0.1%)	2 (0.2%)	3 (0.1%)	
Histology[3]				0.43
Normal	496 (54.2%)	160 (53.3%)	336 (54.7%)	
CIN1	516 (43.5%)	196 (44.3%)	320 (43.1%)	
CIN2	74 (1.7%)	29 (2.1%)	45 (1.5%)	
CIN3	22 (0.5%)	6 (0.4%)	16 (0.6%)	
ADC *in situ*	2 (0.1%)	0 (0.0%)	2 (0.1%)	
Invasive cancer	3 (0.1%)	0 (0.0%)	3 (0.1%)	
HC2 positive given LBC normal	505 (11.4%)	200 (18.0%)	309 (9.3%)	<0.001
AHPV positive given LBC normal	301 (6.8%)	123 (11.1%)	179 (5.4%)	<0.001

Values are N (%) unless otherwise noted.
[1]Women age 20–65 who consented and had valid HPV DNA, HPV RNA, and cytology test results. [2]LBC was performed using ThinPrep in PreservCyt.
[3]Histology for n = 1,113 women overall; n = 391 age 20–29; n = 722 age 30–65; percentages are adjusted for verification bias by weighting by the inverse of the sampling probability (i.e., the probability that the woman was selected for verification); p-value is for a weighted χ^2 test.
*p-value for age 20–29 versus 30-65; for quantitative variables P-value is for Wilcoxon rank sum test; for categorical variables p-value corresponds to Fisher's exact test, unless otherwise noted.
Abbreviations: ADC: adenocarcinoma; AGC: atypical glandular lesion; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells cannot exclude HSIL; CIN: cervical intraepithelial neoplasia; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion.

were cytology negative with an HPV positive result. Of these 723 women, 24 had CIN2, six had CIN3 and one had adenocarcinoma (ADC) *in situ*. These 31 women were all HPV positive by HC2 or AHPV, *i.e.*, none of them were in the random sample.

There were 385 women with abnormal cytology who had colposcopy results. Of these, four were missing histology. Of the remaining 381, 64 were CIN2+ and 18 were CIN3+ (16 were CIN3, one had ADC *in situ* and one had ICC). Therefore, in women with abnormal cytology, approximately six (381/64) colposcopies would need to be performed to find one CIN2+ woman and approximately 21 (381/18) colposcopies would need to be performed to find one CIN3+.

AHPV and HC2 test results *versus* cytology and histology

AHPV and HC2 test results are presented in relation with cytology and histology results in Table 2 and Figure 2. The overall agreement between AHPV and HC2 tests was substantial (kappa statistic = 0.69; 95% CI: 0.66–0.72). The proportion of HPV positive results was significantly higher with HC2 than with AHPV for normal cytology (11.4% *vs.* 6.8%; Table 1; $p < 0.001$), minor and undetermined cytological abnormalities (LSIL: $p < 0.001$ and ASC-US: $p = 0.003$) but was not significantly different for ASC-H, HSIL and AGC cytology. For histology findings, the rate of HPV positivity was significantly higher for HC2 compared with AHPV in normal and low-grade histological lesions (CIN1 and lower) and was similar for high-grade lesions (Fig. 2 and Table 2). These results were similar in both age groups (data not shown). One CIN3 case was HC2+/AHPV− and one CIN3 case was HC2-/AHPV+, both of which were in women ≥30 years.

HPV test performance (as stand-alone tests)

The performance of AHPV or HC2 as stand alone tests is presented in Table 3. Both HC2 and AHPV showed high sensitivity in detecting CIN2+ (96.7% and 92.0%, respectively) and CIN3+ (95.3 and 95.7%) overall and were more sensitive ($p < 0.006$) than LBC (69.1 and 73.3% for CIN2+ and CIN3+ detection, respectively). HC2 and AHPV were 99.7% sensitive at detecting CIN2+ and 98.2% for CIN3+ in women aged 20–29, while the sensitivity of LBC was 67.7 and 81.4%, respectively. Overall, both HC2 and AHPV showed specificity over 84% in detecting CIN2+ (86.4 and 91.8%, respectively) and CIN3+ (84.9 and 90.3%). AHPV was significantly more specific than HC2 (by 5.4 to 8.3 percentage points [%Pt] for all categories; $p < 0.001$), with the largest difference in specificity (7−8%Pt) observed for women aged 20−29 (AHPV: 87.4% and 84.9% for CIN2+ and CIN3+, respectively; HC2: 79.1% and 76.9%, respectively; $p < 0.001$). In this age group and in all categories, AHPV and LBC had similar specificity.

Among women with normal LBC, for CIN3+ detection, AHPV has a similar sensitivity to that of HC2 (84.3% *vs.*

Table 2. HPV test results stratified by cytology and histology results among 4,429 women with a valid HC2 test, AHPV test and cytology

		Histology								
	Not performed[2]	Normal	CIN1	CIN2	CIN3	ADC	ICC	Total	
LBC[1]	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)	*N* (APHV+, HC2+)[3]
Normal	3,281 (26, 41)	378 (108, 197)	314 (109, 190)	24 (22, 24)	6 (5, 5)	1 (1, 1)	0 (0, 0)	4,004 (6.8%, 11.4%)
ASC-US	13 (3, 3)	54 (7, 10)	61 (14, 20)	1 (1, 1)	1 (1, 1)	0 (0, 0)	0 (0, 0)	130 (20.0%, 26.9%)
ASC-H	1 (1, 1)	5 (0, 0)	6 (1, 2)	3 (3 , 3)	2 (2, 2)	0 (0, 0)	0 (0, 0)	17 (41.2%, 47.1%)
LSIL	19 (9, 11)	56 (20, 25)	127 (57, 85)	23 (19, 21)	1 (1, 1)	0 (0, 0)	0 (0, 0)	226 (46.9%, 63.3%)
HSIL	1 (1, 1)	2 (1,1)	5 (2, 4)	23 (22, 23)	12 (12, 12)	1 (1, 1)	3 (3, 3)	47 (89.4%, 95.7%)
AGC	1 (1, 1)	1 (0, 0)	3 (3, 3)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	5 (80.0%, 80.0%)
Total	3,316 (41, 58)	496 (136, 233)	516 (186, 304)	74 (67, 72)	22 (21, 21)	2 (2, 2)	3 (3, 3)	4,429 (10.3%, 15.6%)

Numbers in parentheses indicate the number of women who were APHV+ and HC2+. For example, of the 3,281 women with normal cytology and missing histology, 26 were APHV+ and 41 were HC2+.
[1]LBC was performed using ThinPrep in PreservCyt. [2]Not performed per protocol or missing. [3]Numbers in parenthesis are percentages AHPV+ or HC2+ within the row.

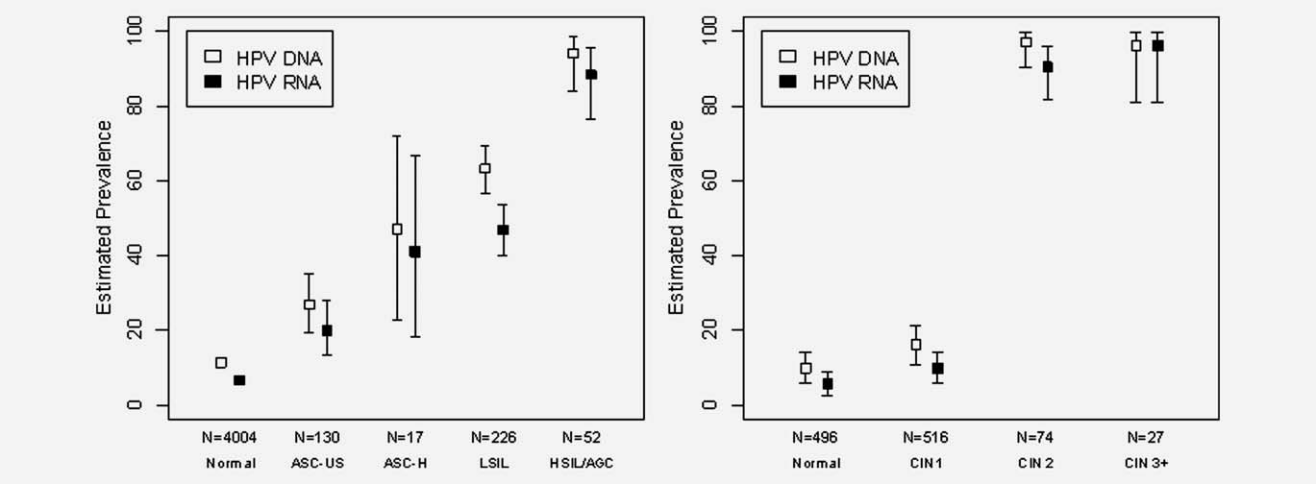

Figure 2. Estimated prevalence of HPV infection by HPV DNA and RNA tests stratified by LBC (left panel) and histology (right panel). In the left panel, the estimated prevalence equals the observed proportion of women who tested HC2 positive or AHPV positive given a particular adjudicated cytology result. In the right panel, the estimated prevalence equals a weighted proportion where each observation is weighted by the inverse of the sampling probability (*i.e.*, the probability that the woman was selected for verification). Vertical bars denote approximately 95% CIs.

82.5%; $p = 0.93$) but a significantly higher specificity (93.4% *vs.* 88.7%; $p < 0.001$). Again, the differences were most marked among women aged 20–29.

NPV was 98.8–100% for all three tests in all categories, while the PPV of all three tests was low (15–22% for CIN2+ and 4–6% for CIN3+) (NPV and PPV estimates are not presented).

Of the 278 women with normal cytology, HC2 and AHPV negative results, and normal histology, none (0/278) were CIN2+ (Fig. 1). To assess the instability of the verification-bias adjusted estimates, we considered how the estimated sensitivity and specificity would have changed had, contrary to fact, one (1/278) of these women had CIN2+ histology. In this case, the estimated specificities for AHPV and HC2 for CIN2+ were unchanged; however, the estimated sensitivities decreased to 86.8% (95% CI: 68.9–100) for HC2 and 82.5% (95% CI: 65.1–100) for AHPV.

Combining LBC with either HPV test

We compared the performance of combining LBC plus AHPV or LBC plus HC2 to that the best of either test alone (estimates are presented in Table 3). Combining LBC with either of the two HPV tests slightly (2–5%Pt; overall population) increased test sensitivity for CIN2+ but not CIN3+ detection. Either LBC/HPV test combination slightly decreased (by 6–10%Pt) the overall detection specificity. The LBC/AHPV test combination had a slightly (4–6%Pt) higher specificity than the LBC/HC2 combination for all categories, but the LBC/HC2 combination had a slightly (2–3%Pt) higher sensitivity for CIN2+ detection (but not CIN3+), overall and in women $\geq$30 years.

Discussion

The present study, to our knowledge, is the first to compare AHPV with HC2 and LBC in a population-based screening setting. The study demonstrates that AHPV has a high sensitivity (similar to that of HC2) for CIN2+ and CIN3+ detection and a high specificity (similar to that of LBC).

The prevalence of HPV positivity with the HC2 assay (15.7%) in our population is higher than in other settings[32,33] but is similar to that reported in France.[34,35] This can be explained by the fact that our population contained a substantial proportion (25%) of young women. The overall prevalence of HPV positivity was lower with AHPV than with HC2 (10.3% *vs.* 15.7%), reflecting the higher specificity of AHPV.

The CIN2/CIN3 prevalence ratio observed in our study (3.4) is higher than reported in two other studies (1.5 and 1.03), but is similar to that reported in another study (3.0 in the conventional arm).[32] The high CIN2/CIN3 prevalence ratio in our study is due to the high prevalence of CIN2 histology and may be attributed to the following parameters in our study: (*i*) a high proportion (25%) of women in 20–29 years, an age group that has a higher prevalence of CIN2[36]; (*ii*) a higher prevalence of HPV DNA infection (15.7%) compared with other studies (8–10%)[36,37]; (*iii*) shorter screening intervals (typically every 1.5–2 years), that may lead to increased detection of transient CIN2 lesions compared with longer screening intervals (every 3–5 years) and (*iv*) overestimation of CIN2 due to the low reproducibility of CIN2 diagnosis.[7]

Both HPV tests were more sensitive than LBC (by >20%Pt), in agreement with earlier studies.[11,12,24,38–41] AHPV and HC2 were highly sensitive ($\geq$92%) for both CIN2 and CIN3 detection, with HC2 detecting a few more CIN2

Table 3. Screening test performance: estimated corrected[1] sensitivity and specificity

		CIN2+		CIN3+	
Screening Test	Age	Corrected[1] sensitivity (95% CI)	Corrected[1] specificity (95% CI)	Corrected[1] sensitivity (95% CI)	Corrected[1] specificity (95% CI)
STAND-ALONE					
HC2	Overall	96.7% (92.6–100)	86.4% (85.4–87.4)	95.3% (83.9–100)	84.9% (83.8–86.0)
(CO $\geq$ 1 pg/mL)	20–29	99.7% (95.0–100)	79.1% (76.7–81.6)	98.2% (71.7–100)	76.9% (74.4–79.4)
	30–65	95.0% (88.5–100)	88.8% (87.7–89.9)	93.8% (78.5–100)	87.6% (86.4–88.7)
AHPV	Overall	92.0% (86.4–97.6)	91.8% (91.0–92.6)	95.7% (85.0–100)	90.3% (89.4–91.2)
(S/CO $\geq$ 1.0)	20–29	99.7% (95.0–100)	87.4% (85.4–89.4)	98.2% (71.7–100)	84.9% (82.8–87.0)
	30–65	87.7% (79.2–96.2)	93.2% (92.4–94.1)	94.5% (79.9–100)	92.1% (91.2–93.0)
LBC[2]	Overall	69.1% (60.0–78.1)	91.9% (91.1–92.7)	73.3% (55.6–91.0)	90.8% (90.0–91.7)
(ASC-US+)	20–29	67.7% (52.0–83.4)	88.4% (86.5–90.3)	81.4% (44.5–100)	86.9% (84.9–88.9)
	30–65	69.7% (58.4–81.0)	93.1% (92.2–94.0)	70.7% (49.7–91.7)	92.2% (91.2–93.1)
Combined					
LBC or HC2	Overall	98.7% (95.6–100)	82.1% (80.9–83.2)	95.2% (83.9–100)	80.6% (79.4–81.7)
	20–29	99.7% (95.0–100)	73.4% (70.7–76.0)	98.1% (71.8–100)	71.3% (68.6–74.0)
	30–65	98.0% (92.8–100)	84.9% (83.7–86.2)	93.7% (78.3–100)	83.7% (82.4–84.9)
LBC or AHPV	Overall	96.8% (92.8–100)	86.3% (85.3–87.4)	95.7% (84.8–100)	84.8% (83.8–85.9)
	20–29	99.7% (94.9–100)	79.6% (77.2–82.0)	98.0% (71.5–100)	77.4% (74.9–79.8)
	30–65	95.1% (88.8–100)	88.6% (87.5–89.7)	94.4% (79.6–100)	87.3% (86.2–88.5)
Women with normal LBC					
HC2	Overall	95.9% (86.0–100)	89.3% (88.3–90.3)	82.5% (44.6–100)	88.7% (87.8–89.7)
(CO $\geq$ 1 pg/mL)	20–29	99.2% (85.0–100)	83.0% (80.6–85.4)	91.8% (0–100)*[3]	82.1% (79.8–84.3)
	30–65	93.5% (77.3–100)	91.3% (90.2–92.3)	79.2% (33.6–100)	90.8% (89.8–91.8)
AHPV	Overall	89.8% (77.5–100)	93.9% (93.2–94.7)	84.3% (47.8–100)	93.4% (92.6–94.1)
(S/CO $\geq$ 1.0)	20–29	99.2% (85.0–100)	90.1% (88.1–92.0)	91.9% (0–100)*[3]	89.0% (87.2–90.9)
	30–65	84.2% (65.1–100)	95.2% (94.3–96.0)	81.6% (37.2–100)	94.8% (94.0–95.5)

[1]Using maximum likelihood to adjust for verification bias. [2]LBC was performed using ThinPrep in PreservCyt. [3]Approximate 95% CIs throughout table were calculated as point estimate $\pm$ 1.96 $\times$ SE, where SE is the estimated standard error. For the two instances marked with * the SE equalled 67.5% and 67.4%; these values were particularly high since only six women age 20–29 with histology were CIN3+ (see Table 1).
Abbreviations: CO: cut-off; CI: confidence interval; S/CO: signal to cut-off ratio.

cases than AHPV in agreement with previous studies.[22,24] HPV RNA assays would be expected to detect fewer CIN2 cases than DNA assays since some CIN2 lesions are more likely to be transient and to regress as compared to CIN3 lesions.

AHPV had a higher specificity than HC2 for CIN2+ and CIN3+ detection, concurring with two referral studies comparing the two assays for the detection of CIN2+/3+.[22,24] In this study, the estimated probability of a false positive result for CIN2+ was 13.6% for HC2 compared to only 8.2% for AHPV. This could be explained in part by HC2's propensity to crossreact with some low-risk HPV genotypes,[26] and/or the ability of AHPV to identify clinically significant cervical precancer. Thus, using HC2 alone for screening would lead to a high rate of false positives, resulting in unnecessary referrals to colposcopy with unwanted patient burden and health care costs. AHPV, however, is relatively more specific at detecting clinically significant disease.[22–24] Further analyses are warranted to compare the cost-effectiveness of these HPV tests in clinical practice.

The overall prevalence of cytological abnormalities in both first reading and adjudicated cytology is 9.6%, which is in agreement with other screening studies performed in the United States[13] and Europe,[37] including France.[34] However, this prevalence is slightly higher than in other settings,[38] probably due in part to the high proportion (25%) of young women in our cohort; also, technical difficulties with Thinprep could have contributed to the higher rate of ASC-US and LSIL in the first and adjudicated cytology, respectively.[8] The HPV DNA assay was much more sensitive (by >25%Pt) than LBC but slightly less specific [by ~5%Pt], in agreement to that reported in other trials.[11–14,38,39] AHPV, however, was much more sensitive than LBC (by >25%Pt) but had a similar specificity. Given the higher sensitivity of AHPV, longitudinal validation trials should be conducted to evaluate the feasibility of replacing LBC with AHPV as a stand alone

screening test. Moreover, we found that combining LBC with either of the two HPV tests slightly increased test sensitivity but substantially decreased test specificity, suggesting that combining LBC with an HPV test provides no added value for screening.

We also evaluated test performance in older and younger women (age 30 years cut-off). HC2 performed better in older women than in younger women (similar sensitivity, higher specificity), as shown in another study.[13] The most important age group for screening is women $\geq$30 years. In this age group, AHPV had a much higher sensitivity than LBC, and similar sensitivity but higher specificity compared to HC2. Although most ICCs are found in older women, the prevalence of ICC in young women is not negligible: 7.4 % of all ICCs in the United States occur in women 20–29 years,[42] and the incidence of ICC among women 25–29 in France is 3.8 per 100,000 women.[43] Thus, accurate screening of women aged 20–30 may be important due to the relatively high rates of transient cytological abnormalities.[13,14] In women 20–29, AHPV had a higher specificity than HC2, and a higher sensitivity and similar specificity compared to LBC for CIN2+ detection, suggesting that AHPV may be an attractive option for the screening of women 20–29. However, similar to HPV DNA testing,[15] AHPV testing in young women may overdetect CIN2 lesions which are likely to regress spontaneously.

This study has several advantages that allow the valid comparison between the three tests. First, we used biopsy as the gold standard. Biopsies were also taken from women referred to colposcopy, even when no lesions were present, to detect any underlying occult disease. Second, we corrected for verification bias to characterize the performance of all three tests by conducting colposcopy and biopsy in a high proportion of women with negative results in all three tests. Failure to correct for verification bias generally overestimates test sensitivity and underestimates test specificity.[29,30,44] Third, abnormal cytology and all histology findings were reviewed by an independent expert, followed by a consensus panel for discrepant cases.

This study also has some limitations. First, it is a cross-sectional study, not a prospective, randomized controlled trial, precluding the determination of the tests' longitudinal NPV to substantiate an increase of screening intervals. Based on the cross-sectional sensitivity equivalence of AHPV to HC2 in this and other studies, it would be expected that AHPV screening intervals would likely be the same as HC2. Nevertheless, longitudinal trials should be conducted to confirm that AHPV can safely offer screening intervals comparable to those used with HPV DNA assays. Second, our study did not include HPV genotyping data, which may partly explain the difference in clinical specificity between AHPV and HC2. Third, histological results were obtained for all women who were HPV positive or had abnormal cytology, but only for a small proportion (14%) of women who were HPV negative and had normal cytology. Thus, the estimates of sensitivity and specificity are less precise than had histological results been obtained from all women in the study.

This is the first study comparing AHPV with HC2 and LBC in a screening setting. The greater performance of the AHPV assay compared with cytology and HC2 provides data in favour of its use for cervical cancer screening, either as an adjunct test to cytology or as an ASC-US triage test in women 20–65. We recommend that longitudinal studies be conducted to assess the performance of AHPV as a stand alone test for primary screening.

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