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Human Papillomavirus DNA versus Papanicolaou Screening Tests for Cervical Cancer

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ABSTRACT

BACKGROUND

To determine whether testing for DNA of oncogenic human papillomaviruses (HPV) is superior to the Papanicolaou (Pap) test for cervical-cancer screening, we conducted a randomized trial comparing the two methods.

METHODS

We compared HPV testing, using an assay approved by the Food and Drug Administration, with conventional Pap testing as a screening method to identify high-grade cervical intraepithelial neoplasia in women ages 30 to 69 years in Montreal and St. John's, Canada. Women with abnormal Pap test results or a positive HPV test (at least 1 pg of high-risk HPV DNA per milliliter) underwent colposcopy and biopsy, as did a random sample of women with negative tests. Sensitivity and specificity estimates were corrected for verification bias.

RESULTS

A total of 10,154 women were randomly assigned to testing. Both tests were performed on all women in a randomly assigned sequence at the same session. The sensitivity of HPV testing for cervical intraepithelial neoplasia of grade 2 or 3 was 94.6% (95% confidence interval [CI], 84.2 to 100), whereas the sensitivity of Pap testing was 55.4% (95% CI, 33.6 to 77.2; P=0.01). The specificity was 94.1% (95% CI, 93.4 to 94.8) for HPV testing and 96.8% (95% CI, 96.3 to 97.3; P<0.001) for Pap testing. Performance was unaffected by the sequence of the tests. The sensitivity of both tests used together was 100%, and the specificity was 92.5%. Triage procedures for Pap or HPV testing resulted in fewer referrals for colposcopy than did either test alone but were less sensitive. No adverse events were reported.

CONCLUSIONS

As compared with Pap testing, HPV testing has greater sensitivity for the detection of cervical intraepithelial neoplasia. (Current Controlled Trials number, ISRCTN57612064.)

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Cervical cancer remains the second most common cancer in women worldwide,[1] even though screening with cervical cytologic testing (the Papanicolaou [Pap] test) has been available for over 50 years. In resource-rich countries, there was a decrease in the incidence of cervical cancer after the introduction of Pap testing. This decrease has recently leveled off, and frequent retesting is required to achieve an acceptable sensitivity because of the low sensitivity of the Pap test.[2]

Testing cervical specimens for DNA of oncogenic (high-risk) types of human papillomavirus (HPV), the causal agents of cervical cancer, has entered clinical practice, but this test is used mainly to triage for colposcopy those women with Pap smears labeled as "atypical squamous cells of undetermined significance" (ASCUS).[3-5] Nonrandomized studies and reviews indicate that HPV testing is more sensitive than Pap testing for identifying cervical cancer and its precursors in population screening.[5-19] HPV testing has received only limited approval as an adjunct to Pap cytologic testing, however, and only in the United States,[2,20] even though no published, randomized, controlled trials have compared Pap testing alone with cotesting. Cotesting substantially increases the cost of screening by doubling the number of tests. Moreover, the simultaneous performance of two tests complicates clinical follow-up procedures.

For these reasons, we investigated how HPV testing performs as a stand-alone screening test. Evidence from randomized, controlled trials is needed before HPV testing can be incorporated in screening, and there is concern that HPV testing is less specific than Pap testing.[21,22] Most data are from cross-sectional studies, many of which were conducted in infrequently screened populations with limited access to health care.[6-8,12,16,17] To our knowledge, a randomized, controlled trial of HPV testing as a stand-alone screening test for cervical-cancer precursors in a North American population with access to quality care has not previously been conducted.

We report here the first screening round of the Canadian Cervical Cancer Screening Trial (CCCaST). The trial is designed to compare HPV testing and Pap testing in parallel as stand-alone screening tests to identify cervical cancers and their high-grade precursors among women ages 30 to 69 years who present for routine screening.

METHODS

DESIGN

The design of the trial has been described previously.[23] We randomly assigned participants at a 1:1 ratio to a "focus on Pap" or a "focus on HPV" screening group. Ethical considerations led us to include both tests in each group but to randomize the sampling order. In the "focus on Pap" group, the women received a Pap test first, whereas in the "focus on HPV" group, the women received an HPV test first; the tests were performed sequentially at the same visit. In each group, the first test is referred to as the index test. This strategy enabled us to analyze each index test as if it had been done alone. The sponsors had no role in the design of the study, data accrual, data interpretation, or manuscript preparation.

PARTICIPANTS

The sample consisted of women ages 30 to 69 years who sought screening tests for cervical cancer in any of 30 clinics in Montreal and St. John's, Canada. Women who were currently being followed up for a cervical lesion, lacked a cervix, were pregnant, had a history of cervical cancer, had undergone Pap testing in the previous year, or were unable to provide consent were excluded. Written informed consent was obtained from all participants. Information on demographics and risk factors was obtained by a self-administered questionnaire.

RANDOMIZATION AND BLINDING

Assignment of the tests was done at the coordination center by computer-assisted block randomization stratified according to clinic, with randomly variable block sizes. A clinic-stratified, random subsample of 10% of the women in St. John's and 20% of the women in Montreal with a negative index test in each group were invited to undergo colposcopy. The participants were unaware of the test assignment. The Pap tests were read at the participating sites by cytotechnologists and cytopathologists without knowledge of the patient's status as a participant or her HPV test result. The colposcopists and pathologists evaluating the biopsy specimens were unaware of the screening-test results.

SCREENING TESTS

Conventional Pap tests were used, with results reported or reclassified according to the 2001

Bethesda System terminology.[24] According to the Bethesda system, squamous-cell abnormalities are classified as atypical (i.e., ASCUS; or atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion [known as ASC-H]), low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or carcinoma. Glandular-cell abnormalities are classified as atypical glandular cells (AGC), adenocarcinoma in situ, or adenocarcinoma. In this study, a result of ASCUS, AGC, or worse was considered positive. The Hybrid Capture 2 test (HC2 probe B, Digene) was used for HPV testing. The manufacturer of the test had no role in this study; all supplies and reagents were purchased at the regular cost. Specimens were considered positive if the ratio of relative light units (RLUs) of the specimen to the mean RLU of positive control triplicates was at least 1 (equivalent to 1 pg of HPV DNA per milliliter).

DIAGNOSTIC PROCEDURE

Participants were referred for colposcopy if they had a positive Pap or HPV screening test or if they were randomly selected from among women with a negative index test. Colposcopists at the participating sites followed a standardized protocol that included endocervical curettage, ectocervical biopsies of all abnormal-appearing cervical regions, and at least one biopsy of normal-appearing ectocervical epithelium aiming at an aceto-white transformation zone, if present. Patients underwent a biopsy first and, if warranted, were treated thereafter (except for two patients who had a loop electrosurgical excision procedure [LEEP] without having undergone a previous biopsy). LEEP or cold-knife conization was also performed in cases of significant discrepancy between cytologic and histologic results, as recommended.[25] Precolposcopy cytologic testing was not required. Pathologists at each center where the colposcopies were performed provided histologic diagnoses for all biopsy specimens. Most high-grade cervical intraepithelial neoplasia lesions were treated by LEEP. When ablative treatment was performed, confirmatory biopsies were performed at the treatment visit.

CASE DEFINITIONS

High-grade (grade 2 or higher) cervical intraepithelial neoplasia is the accepted end point for cervical screening and an actionable finding for clinical management of the disease. We used two case definitions: conservative and liberal. The liberal definition included all cases of grade 2 or 3 cervical intraepithelial neoplasia, adenocarcinoma in situ, or cervical cancers that were histologically confirmed on the basis of any of the histologic specimens. Conservatively defined cases were those that met the liberal criteria and that in addition were confirmed in the LEEP specimen (including the two participants who were treated by LEEP on the first colposcopy visit and found to have grade 3 cervical intraepithelial neoplasia) or by a confirmatory biopsy in the case of ablative treatment. Ablative treatment was used for 10 cases (liberal definition); 6 of 10 were also identified on confirmatory biopsy, as compared with 34 of 45 confirmed lesions in women who received excisional treatment ($P=0.43$).

The ethics review boards of all participating hospitals, clinics, and universities (McGill and Memorial Universities) approved the study.

STATISTICAL ANALYSIS

Differences in categorical data were assessed by Fisher's test and the chi-square test, and differences in continuous data by the Kruskal–Wallis test. All tests were two-sided. Group-specific estimates of sensitivity, specificity, and predictive values (and the respective 95% confidence intervals) for the conservative and liberal case definitions included data from the index tests for all participants who were assigned to that group. Crude estimates included only participants who underwent colposcopy.

Verification bias, caused by verification of the lesion only in participants with a positive result, can result in overestimates of sensitivity. Obtaining a biopsy specimen for histologic verification from all participants with negative tests is not feasible in cervical-cancer screening because of the associated discomfort and costs. Verification in a random sample of participants with negative screening tests is, however, feasible, and the results can be used to calculate the likely number of cases that would have been found if all participants with negative screening tests had been fully investigated.[13,15] We used this strategy.

In brief, the data were divided into strata of combined Pap and HPV test results. Disease prevalence in each stratum was assumed to be independent of whether the women underwent biopsy. Stratum-specific probabilities were then applied to the remainder of the women who had not undergone biopsy, which permitted an estimate of

the number of cases that would have been found if all study participants had undergone histologic verification. Corrected sensitivity and specificity estimates were then calculated, and 95% confidence intervals were computed by the method described by Zhou.[26] A z-test was performed on the differences between the sensitivity and specificity of the HPV and Pap tests.

RESULTS

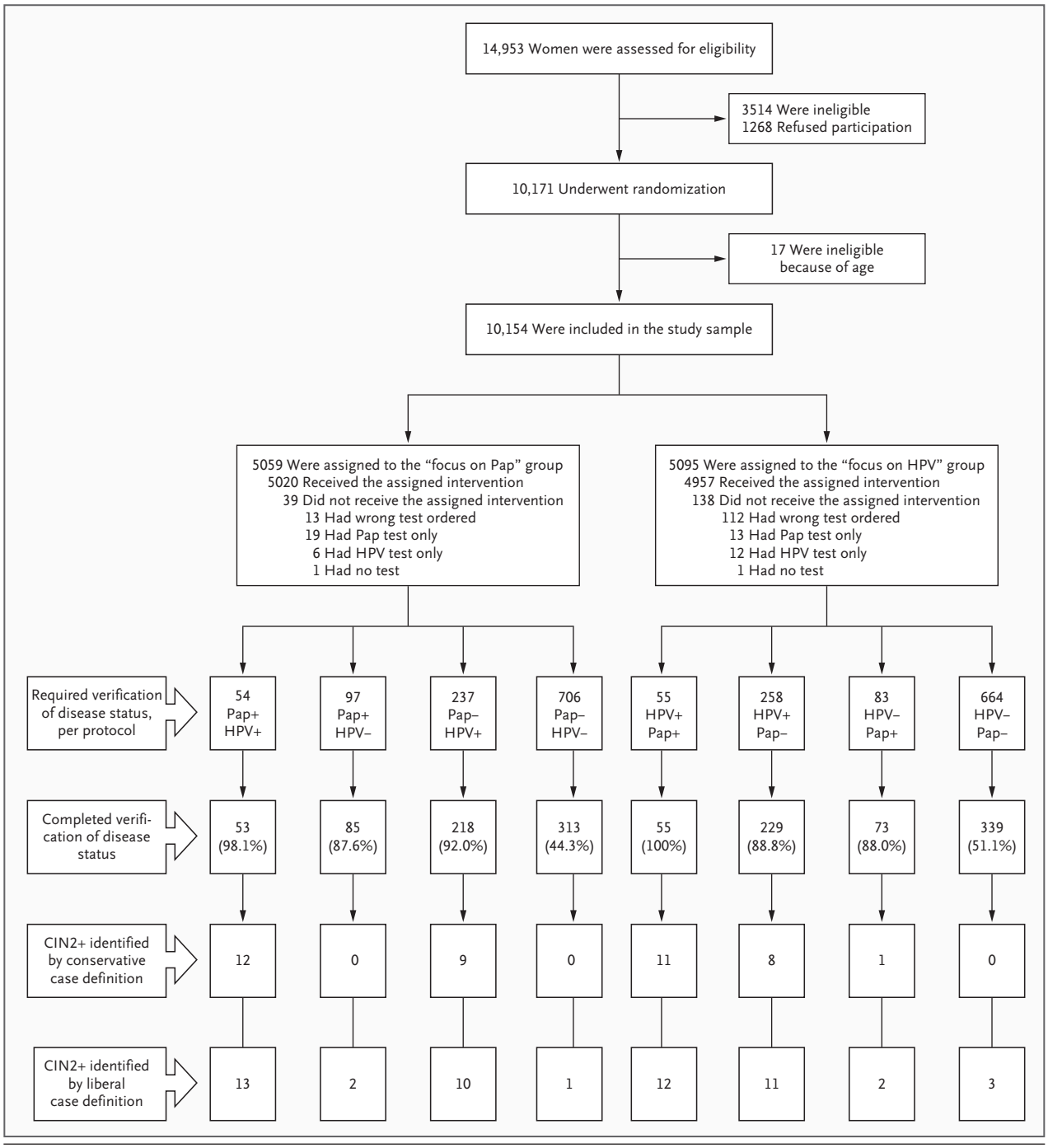
Figure 1 shows the test results and outcomes in the two groups. Between September 26, 2002, and February 3, 2005, 14,953 women were assessed for eligibility and invited to participate. Of these, 10,154 women were randomly assigned to screening. In the "focus on Pap" group, 99.2% of the women received the assigned intervention, as did 97.3% of those in the "focus on HPV" group. More than 90% of participants with at least one positive test (723 of 795) and 7.1% of those with negative tests (665 of 9359) underwent colposcopy. The 665 women with negative tests represent 47.4% of the 1402 women invited to undergo colposcopy by random assignment. Among women in whom both tests were negative, there were no significant differences in most characteristics between those who underwent colposcopy and those who did not (data not shown). The only exception was for women assigned to HPV testing in St. John's; participants who underwent colposcopy were older than those who did not (median age, 46 vs. 43 years; P<0.001). No adverse events were reported.

Table 1 shows the characteristics of the participants. Randomization produced similar groups. However, in addition to the expected preponderance of French Canadians, the participants in Montreal were slightly younger and less likely to be married than those in St. John's. At least 95% of the participants had previously had a Pap smear.

Table 2 shows group-specific estimates of screening-test results with 95% confidence intervals. With the use of the conservative definition, and after correction for verification bias, the sensitivity of the Pap test (55.4%) was significantly lower than the sensitivity of the HPV test (94.6%, P=0.01). After correction for verification bias, the specificity of the Pap test (96.8%) was slightly higher than the specificity of the HPV test (94.1%, P<0.001), with the use of the conservative definition. The sensitivity and specificity of the Pap test and the HPV test with the use of the liberal definition were similar to those with the use of the conservative definition. However, the sensitivity of the HPV test fell to 45.9% after correction for verification bias. Negative predictive values were higher than 99% for both tests, regardless of definition.

Figure 1 (facing page). Enrollment and Outcomes.
Positive Pap tests (Pap+) are defined as atypical squamous cells of undetermined significance (ASCUS) or worse; positive human papillomavirus (HPV) tests (HPV+) are defined as at least 1 pg of HPV DNA per milliliter. Random selection for colposcopy for those with both negative tests was performed as follows: 706 of 4575 Pap−/HPV− participants and 664 of 4600 HPV−/Pap− participants were assigned to the "focus on Pap" group and the "focus on HPV" group, respectively. Participants who underwent only one test or who had a Pap test deemed unsatisfactory for interpretation are not included in the figure but were included in efficacy analyses. In the "focus on Pap" group, 95 participants had only one screening test that could be evaluated: 19 had a negative Pap test, 72 had a negative HPV test, and 4 had a positive HPV test. The latter 4 participants underwent colposcopy, and none were found to have disease by either case definition. Among the 91 participants with a negative screening test, 13 were randomly selected to undergo colposcopy; 3 complied, and none were found to have disease by either definition. In the "focus on HPV group," 98 participants had only one screening test that could be evaluated: 79 had a negative HPV test, 6 had a positive HPV test, 12 had a negative Pap test, and 1 had a positive Pap test. Among the 7 participants who had a positive test, 6 underwent colposcopy and 1 (HPV+) was found to have disease by the liberal case definition only. Among the 91 participants with a negative test, 18 were randomly selected to undergo colposcopy; 10 complied and none were found to have disease by either definition. Both groups were balanced with respect to the required disease verification (P=0.43). CIN2 denotes grade 2 cervical intraepithelial neoplasia.

According to the liberal definition, there were four cases of high-grade cervical intraepithelial neoplasia among participants in whom both screening tests were negative, as compared with no cases according to the conservative definition. These four cases were influential in the extrapolation of the occurrence of high-grade cervical intraepithelial neoplasia lesions among all women with negative tests, hence the difference in corrected sensitivity between the liberal and conservative definitions (Table 2). Only 12% of the high-grade cervical intraepithelial neoplasia lesions (one of eight) that were found on biopsy in HPV-negative women were also found in the excisional specimens,



whereas 68% of the biopsy-proven high-grade cervical intraepithelial neoplasia lesions (17 of 25) were found in the excisional specimens from women with negative Pap tests (P=0.01). Similar proportions of high-grade lesions in women with positive HPV tests (40 of 46 [87.0%]) and women with positive Pap tests (24 of 29 [82.8%]) were confirmed in the excisional specimens (P=0.74).

Table 3 shows test performance according to sampling order. The performances of the tests were not influenced by the order in which specimens were collected (i.e., first or second), as judged by test positivity, unsatisfactory smears or those showing ASCUS, RLU distribution, and sensitivity or specificity.

Since sampling order did not affect perfor-

Table 1. Characteristics of Participants According to Study Group and Center.*

Characteristic	Montreal (N=4400)		St. John's (N=5754)	
	Pap Group (N=2191)	HPV Group (N=2209)	Pap Group (N=2868)	HPV Group (N=2886)
	no./total no. (%)			
Age				
30–39 yr	732/2191 (33.4)	746/2209 (33.8)	1213/2868 (42.3)	1215/2886 (42.1)
40–49 yr	766/2191 (35.0)	772/2209 (34.9)	1028/2868 (35.8)	992/2886 (34.4)
50–59 yr	519/2191 (23.7)	511/2209 (23.1)	486/2868 (16.9)	556/2886 (19.3)
60–69 yr	174/2191 (7.9)	180/2209 (8.1)	141/2868 (4.9)	123/2886 (4.3)
Marital status				
Single	393/2159 (18.2)	380/2179 (17.4)	220/2849 (7.7)	269/2869 (9.4)
Married or living with a partner	1388/2159 (64.3)	1442/2179 (66.2)	2287/2849 (80.3)	2324/2869 (81.0)
Divorced, separated, or widowed	378/2159 (17.5)	357/2179 (16.4)	342/2849 (12.0)	276/2869 (9.6)
Ethnic group				
French Canadian	1808/2138 (84.6)	1831/2165 (84.6)	17/2855 (0.6)	18/2861 (0.6)
English Canadian	53/2138 (2.5)	50/2165 (2.3)	2792/2855 (97.8)	2807/2861 (98.1)
Other	277/2138 (13.0)	284/2165 (13.1)	46/2855 (1.6)	36/2861 (1.3)
Schooling				
Elementary school	243/2157 (11.3)	221/2177 (10.2)	287/2857 (10.0)	289/2873 (10.0)
High school	491/2157 (22.8)	508/2177 (23.3)	629/2857 (22.0)	656/2873 (22.8)
Junior college	588/2157 (27.3)	577/2177 (26.5)	878/2857 (30.7)	872/2873 (30.4)
University	835/2157 (38.7)	871/2177 (40.0)	1063/2857 (37.2)	1056/2873 (36.8)
Ever had a Pap smear				
Yes	2006/2102 (95.4)	2020/2127 (95.0)	2853/2859 (99.8)	2869/2872 (99.9)
No	96/2102 (4.6)	107/2127 (5.0)	6/2859 (0.2)	3/2872 (0.1)
Ever had an abnormal Pap smear				
Yes	474/1916 (24.7)	442/1913 (23.1)	798/2609 (30.6)	847/2607 (32.5)
No	1442/1916 (75.3)	1471/1913 (76.9)	1811/2609 (69.4)	1760/2607 (67.5)

* HPV denotes human papillomavirus.

mance, we pooled the two groups to investigate different screening algorithms (Table 4). As expected, increasing the positivity threshold for Pap and HPV testing resulted in a decrease in sensitivity and colposcopy referrals. Triaging Pap results of ASCUS with HPV testing resulted in somewhat reduced sensitivity as compared with Pap testing alone but yielded fewer referrals (1.6% vs. 2.9%, P<0.001). Triaging women with positive HPV tests with Pap tests resulted in estimates similar to those obtained with the inverse triage strategy. Cotesting achieved 100% sensitivity and resulted in a 7.9% referral rate.

DISCUSSION

We report here the results of a randomized, controlled trial designed to compare Pap testing with HPV testing as stand-alone screening tests for cervical-cancer precursors. HPV testing was more sensitive (39.2% difference) and only 2.7% less specific than Pap testing (according to the conservative definition). Correcting for verification bias provided absolute rather than relative estimates. Absolute estimates probably reflect the community-level screening performance that can inform cost-effectiveness studies.

Table 2. Group-Specific Comparison of Pap and HPV Testing to Identify High-Grade Cervical Intraepithelial Neoplasia and Cancer.

Case Definition*	Screening Test†	Crude Estimate	Corrected Estimate‡
		% (95% CI)	
Conservative			
Sensitivity	Pap	57.1 (34.0–78.2)	55.4 (33.6–77.2)
	HPV	95.0 (75.1–99.9)	94.6 (84.2–100.0)
Specificity	Pap	80.6 (77.4–83.6)	96.8 (96.3–97.3)
	HPV	60.9 (57.2–64.6)	94.1 (93.4–94.8)
Positive predictive value	Pap	8.7 (4.6–14.7)	7.1 (4.8–10.3)
	HPV	6.6 (4.0–10.1)	6.4 (5.0–8.0)
Negative predictive value	Pap	98.3 (96.8–99.2)	99.8 (99.7–99.9)
	HPV	99.8 (98.7–100.0)	100.0 (98.6–100.0)
Liberal			
Sensitivity	Pap	57.7 (36.9–76.6)	43.4 (13.2–73.6)
	HPV	82.8 (64.2–94.2)	45.9 (18.9–72.9)
Specificity	Pap	80.9 (77.7–83.9)	96.9 (96.4–97.4)
	HPV	61.1 (57.4–64.8)	94.2 (93.5–94.9)
Positive predictive value	Pap	10.9 (6.2–17.3)	9.1 (4.7–16.7)
	HPV	8.3 (5.4–12.1)	8.0 (5.6–11.3)
Negative predictive value	Pap	97.9 (96.3–99.0)	99.6 (99.3–99.8)
	HPV	98.8 (97.3–99.6)	99.4 (99.1–99.5)

* According to the conservative definition, cases were considered only if confirmed on the loop electrosurgical excision procedure (LEEP) specimen or in the confirmatory biopsy when ablative treatment was used. The liberal definition includes all cases of grade 2 or 3 cervical intraepithelial neoplasia, adenocarcinoma in situ, or cervical cancers confirmed by histologic examination of any of the ectocervical or endocervical biopsy specimens.

† Positivity was defined as a result of atypical squamous cells of undetermined significance (ASCUS) or worse for Pap testing and at least 1 pg of HPV DNA per milliliter for the HPV DNA test.

‡ The estimates are corrected for verification bias.

Our protocol, which mandated endocervical curettage and biopsies in all participants who were undergoing colposcopy, reduced the possibility of missing cervical intraepithelial neoplasia lesions. However, this approach may lead to the discovery of more incipient or indolent lesions than would be revealed by routine colposcopies. Liberal inclusion of all such lesions, although informative, yielded detection rates of cervical intraepithelial neoplasia that are unlikely to reflect real-world community screening. Our conservative definition, based on confirmation of cervical intraepithelial neoplasia in an excisional specimen, reduced overdiagnosis bias. We believe that misclassification of squamous metaplasia as cervical intraepithelial neoplasia can explain most of the cervical intraepithelial neoplasia–negative specimens obtained in the LEEP procedure.[27] We found that most grade 2 cervical intraepithelial neoplasia–positive lesions that were found in biopsies from women with negative HPV tests were not confirmed by examination of the excisional specimens, which suggests that HPV testing could improve the accuracy of the pathological diagnosis.[28] More important, it is reassuring that the sensitivity of HPV testing is unlikely to result in length bias (due to identification of indolent lesions), since among women whose biopsy specimens showed high-grade cervical intraepithelial neoplasia, these lesions were confirmed slightly but not significantly more often in those with positive HPV tests than in those with positive Pap tests.

Table 3. Analysis of Test Performance According to Sampling Order.*

Screening Test†	Sampling Order		P Value‡
	Pap Followed by HPV	HPV Followed by Pap	
	percent		
Pap			
Overall positivity	3.0	2.7	0.44
Unsatisfactory smears	1.4	1.4	0.83
Smears showing ASCUS	1.9	1.8	0.79
Crude sensitivity	57.1	60.0	1.00
Crude specificity	80.6	82.7	0.32
HPV			
Overall positivity	5.8	6.3	0.37
Distribution of viral load§			0.81
<0.75 RLU	91.8	91.1	
0.75–0.99 RLU	2.0	2.3	
1.00–1.99 RLU	1.1	1.3	
2.00–3.99 RLU	0.8	0.9	
4.00–9.99 RLU	0.8	0.7	
10.00–39.99 RLU	1.2	1.2	
≥40.00 RLU	2.0	2.1	
Crude sensitivity	100.0	95.0	0.49
Crude specificity	61.1	60.9	0.95

* HPV denotes human papillomavirus, and ASCUS atypical squamous cells of undetermined significance.
† Positivity is defined as a result of ASCUS or worse for the Pap test and at least 1 pg per milliliter for the HPV DNA test; screening indexes are based on the conservative case definition.
‡ P values are for the difference between distributions of results for the two sampling schemes.
§ Viral load was measured by means of the chemiluminescence signal of the HPV assay expressed as relative light units (RLU), where 1 RLU is approximately equal to 1 pg of HPV DNA per milliliter.

It has been suggested that the performance of Pap tests can be influenced by the order of specimen collection when multiple cervical samples are obtained,[29] such as in the case of Pap and HPV cotesting. Our findings, which do not support this claim, permitted us to pool data from both groups to obtain insights concerning different screening algorithms. Raising the threshold for HPV positivity from 1 to 2 pg of DNA per milliliter reduced referrals for colposcopy while keeping the sensitivity greater than that of Pap testing at a threshold for ASCUS. Our results support the proposal that HPV triage of smears that show ASCUS is nearly as sensitive as immediate colposcopy, and referrals for colposcopy remain low.[3,4] Another option, using HPV for screening followed by triage of patients with positive HPV tests with Pap testing, is supported by other work.[30,31] Our post hoc assessment of this strategy indicated a much lower sensitivity than stand-alone HPV testing (53.8% vs. 97.4%). However, the cytotechnologists in this trial were unaware of the HPV results, which leads us to speculate that in a true triage situation the cytotechnologists would be made aware that the slides to be read were from women with positive HPV tests, and this would probably lead to more meticulous assessment of smears and reduced case load.[30] This hypothesis remains to be tested. Cotesting, an acceptable option for cervical screening in the United States,[2,20] reached 100% sensitivity in our trial. However, as Ronco et al.[32] have found, this approach only marginally improved sensitivity as compared with HPV testing alone, while doubling the number of tests and increasing referrals. The cost-effectiveness of cotesting will need further evaluation.

The success of HPV vaccines[33,34] opens a new era of cervical-cancer prevention. Vaccination will not, however, eliminate screening. Not all women will be vaccinated, and women who have already been exposed to HPV type 16 or 18 may not benefit.[35,36] That the present vaccines target only two of the cancer-causing HPV types makes it mandatory to continue screening. For vaccinated women, continued HPV screening provides the added benefit of HPV surveillance.[30]

Our findings concur with those of previous split-sample and randomized studies showing that HPV testing is more sensitive than Pap testing for screening cervical-cancer precursors.[6-18,31,32] The higher sensitivity and the more "upstream" focus on cervical carcinogenesis conferred by HPV testing, relative to Pap testing, should safely permit prolongation of screening intervals, thus offsetting the waiting times, physical and psychological complications, and costs incurred by an increased number of colposcopy referrals after the initial screening. Triage algorithms that identify women with positive HPV tests who are at higher risk for cervical intraepithelial neoplasia, such as the "HPV followed by Pap" strategy, are essential and should be assessed in controlled trials.

In settings with quality assurance for screening, diagnostic, and therapeutic procedures, it is difficult to predict whether a change from Pap testing to HPV testing will further reduce the rates

Table 4. Comparison of Pap Testing and HPV DNA Testing Using Combined Study Groups According to Different Positivity Thresholds and Test Combinations.*

Screening Approach	Definition of Positivity	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	No. of Tests Needed for Screening	Referrals for Colposcopy
				%			%
Pap only	ASCUS or worse	56.4	97.3	8.5	99.8	9,959	2.9
	LSIL or worse	42.2	99.1	17.5	99.7	9,959	1.0
HPV only	≥1 pg HPV DNA/ml	97.4	94.3	7.0	100.0	9,959	6.1
	≥2 pg HPV DNA/ml	81.1	95.5	9.1	99.9	9,959	4.8
Pap screening followed by HPV triage	Triage of all results of ASCUS; ≥1 pg HPV DNA/ml	53.8	98.7	14.9	99.8	10,145	1.6
HPV screening followed by Pap triage	Triage of all with ≥1 pg HPV DNA/ml; Pap threshold of ASCUS or worse	53.8	99.1	21.4	99.8	10,563	1.1
Pap and HPV cotesting	Pap result of ASCUS or worse, or HPV result of ≥1 pg HPV DNA/ml	100.0	92.5	5.5	100.0	19,918	7.9

* Estimates are corrected for verification bias according to the conservative case definition and are based on pooled data from 9959 women in the two study groups who had available Pap and HPV results. HPV denotes human papillomavirus, ASCUS atypical squamous cells of undetermined significance, and LSIL low-grade squamous intraepithelial lesion.

of death from cervical cancer. For women who are screened less frequently than recommended, a more sensitive test, such as the HPV test, may prove important. The incorporation of HPV testing into primary screening will require the education of patients. Our understanding of the natural history of HPV infection and cervical intraepithelial neoplasia has evolved rapidly, making it difficult to provide clear and consistent information.[37] However, participants readily accept HPV testing when proper information is available.[38] We believe that a shift from cellular to viral tests, coupled with education and vaccination, will contribute to a more efficient control of cervical cancer.

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APPENDIX

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