Results at Recruitment From a Randomized Controlled Trial Comparing Human Papillomavirus Testing Alone With Conventional Cytology as the Primary Cervical Cancer Screening Test

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Background In the first recruitment phase of a randomized trial of cervical cancer screening methods (New Technologies for Cervical Cancer Screening [NTCC] study), we compared screening with conventional cytology with screening by human papillomavirus (HPV) testing in combination with liquid-based cytology. HPV-positive women were directly referred to colposcopy if aged 35 or older; if younger, they were retested after 1 year.

Methods In the second recruitment phase of NTCC, we randomly assigned women to conventional cytology (n = 24661) with referral to colposcopy if cytology indicated atypical squamous cells of undetermined significance or more severe abnormality or to testing for high-risk HPV DNA alone by Hybrid Capture 2 (n = 24535) with referral to colposcopy if the test was positive at a concentration of HPV DNA 1 pg/mL or greater. For the main endpoint of the study, histologic detection of cervical intraepithelial neoplasia of grade 2 or more (CIN2+), we calculated and compared sensitivity and positive predictive value (PPV) of the two screening methods using HPV DNA cutoffs of 1 pg/mL and 2 pg/mL. All statistical tests were two-sided.

Results For women aged 35–60 years, the relative sensitivity of HPV testing for detection of CIN2+ at a cutoff of 1 pg/mL vs conventional cytology was 1.92 (95% CI = 1.28 to 2.87) and the relative PPV was 0.80 (95% CI = 0.55 to 1.18). At a cutoff of 2 pg/mL HPV DNA, the relative sensitivity was 1.81 (95% CI = 1.20 to 2.72) and the relative PPV was 0.99 (95% CI = 0.67 to 1.46). In this age group, there was no evidence of heterogeneity between study phases. Among women aged 25–34 years, the relative sensitivity for detection of CIN2+ of HPV testing at a cutoff of 1 pg/mL vs cytology was 3.50 (95% CI = 2.11 to 5.82), statistically significantly larger (P = .019) than that observed in phase 1 at this age (1.58; 95% CI = 1.03 to 2.44).

Conclusions For women aged 35–60 years, HPV testing with a cutoff of 2 pg/mL achieves a substantial gain in sensitivity over cytology with only a small reduction in PPV. Among women aged 25–34 years, the large relative sensitivity of HPV testing compared with conventional cytology and the difference between relative sensitivity during phases 1 and 2 suggests that there is frequent regression of CIN2+ that are detected by direct referral of younger HPV-positive women to colposcopy. Thus, triage test or repeat testing is needed if HPV is to be used for primary testing in this context.

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Several studies based on double testing of all study women have shown that, compared with cytology, human papillomavirus (HPV) testing has greater sensitivity but lower specificity in detecting high-grade cervical intraepithelial neoplasia (CIN) (1–14). A design based on double testing, although suitable for examining

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the cross-sectional sensitivity and false-positive rate of different tests, does not permit long-term evaluation of different management strategies because women abnormal on either test are subjected to further follow-up.

We have conducted a population-based randomized controlled trial, the New Technologies for Cervical Cancer (NTCC) screening study, in which women were randomly assigned to conventional cytology or to an experimental arm that followed two phases depending on the period of recruitment. Thus, each woman was tested by a single approach and managed accordingly. Such a design will permit direct comparison of long-term disease rates (CIN and cancer) associated with each of the two experimental approaches with disease rates associated with the conventional approach.

During phase 1 of recruitment, 22 466 women were randomly assigned to the conventional arm for screening by conventional cytology and 22 708 women to the experimental arm, which used both HPV DNA testing and liquid-based cytology. HPV-positive women were managed differently according to age. Among women aged 35–60 years, those who had a cytologic abnormality or were HPV positive were referred to colposcopy. Among women aged 25–34 years, all women with abnormal cytology were referred to colposcopy but those who were HPV positive with normal cytology were retested after 1 year and referred to colposcopy only if HPV positivity persisted or if cytology became atypical squamous cells of undetermined significance (ASCUS) or higher according to the Bethesda 2001 system (Figure 1) (15). Results on cross-sectional sensitivity and positive predictive value (PPV) at recruitment for detection of high-grade cervical intraepithelial neoplasia during phase 1 have been previously reported (16,17).

We now present data on cross-sectional test accuracy for the detection of CIN2+ among women recruited during phase 2, in which women randomly assigned to the experimental arm were tested only for HPV and, if positive, were referred immediately to colposcopy, regardless of age. We also compared the two phases to study the effect of different management protocols for younger women.

CONTEXT AND CAVEATS

Prior knowledge

The optimal strategy for screening women for cervical cancer precursors to ensure high sensitivity without overtreatment was not known.

Study design

Randomized trial of screening methods (conventional cytology vs human papillomavirus [HPV] testing) for which the main endpoint was histologic detection of cervical intraepithelial neoplasia of grade 2 or more (CIN2+).

Contribution

Screening by HPV testing alone achieved a substantial gain in sensitivity compared with conventional cytology with limited loss of positive predictive value. Among younger women there was frequent regression of CIN2+ lesions.

Implications

HPV testing with a somewhat higher cutoff value than the one conventionally used can be applied as a sole primary screening test. HPV-positive women aged 25–34 should be referred to colposcopy only if either cytology is also abnormal or HPV infection persists for 1 year.

Limitations

The percentage of women whose lesions were destined to regress could not be directly estimated in this study.

Methods

Subjects and Trial Design

A controlled randomized trial, the NTCC, with two recruitment phases was conducted in nine organized cervical screening programs in Italy. Recruitment periods and numbers of women recruited in each center for phase 2 are shown in Table 1. Women aged 25–60 years who were not pregnant, had never undergone hysterectomy, had not been treated for CIN in the last 5 years, and were attending for a new routine cervical screening episode were

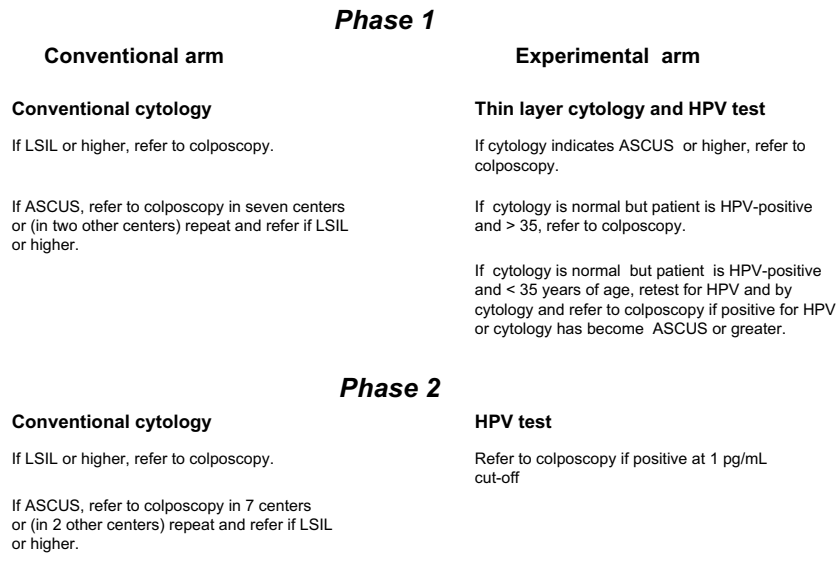

Figure 1. Testing and intended management by arm and phase in the New Technologies for Cervical Cancer study. HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

Table 1. Features of participating centers in phase 2 of recruitment of the New Technologies for Cervical Cancer study*

Center	No. of eligible women	Period of random assignment	Management of ASCUS in conventional arm
Turin	14686	July 2003–October 2004	Colposcopy
Trento	3056	June 2003–December 2004	Repeat cytology
Padua	6565	September 2003–November 2004	Colposcopy
Verona (Soave)	4796	October 2003–June 2004	Colposcopy
Bologna	1882	October 2003–December 2004	Colposcopy
Imola	3042	September 2003–July 2004	Repeat cytology
Ravenna	4389	September 2003–April 2004	Colposcopy
Florence	7790	July 2003–December 2004	Colposcopy
Viterbo	3017	October 2003–December 2004	Colposcopy

* ASCUS = atypical squamous cells of undetermined significance.

eligible. Written informed consent was obtained from all participants. The women were randomly assigned to the conventional or the experimental arm in a 1:1 ratio. Random assignment was performed by computer in two centers without blocking and by sequentially opening sealed numbered envelopes in the seven other centers (using blocks of eight in three centers and without blocking in the four others). The results of assignment were communicated to consenting women by the person taking the smear. The study was approved by the local ethical committees of participating centers. This trial was registered as an International Standard Randomized Controlled Trial, number ISRCTN81678807.

Screening

Conventional Arm. During phase 2 of recruitment, women assigned to the conventional arm were screened by the same procedures used in phase 1. A cervical sample was taken with an Ayre's spatula and cytobrush, prepared for conventional cytology, interpreted by cytoscreeners in 14 local laboratories participating in regular screening programs, and classified according to the Bethesda 1991 system (15). Slides judged to be abnormal were reviewed by a local supervisor or a panel of cytologists in Florence. The diagnosis was communicated to women and used for their management and also in data analysis. All centers regularly applied quality assurance measures (including monitoring of the distribution of diagnoses and of PPV and the circulation and discussion of sets of slides within and between laboratories) that were continued during both recruitment phases. Women in the conventional arm were managed according to the standard protocol of each center. They were always referred to colposcopy if cytology showed a low-grade squamous intraepithelial lesion (LSIL) or higher. In seven centers (Table 1), women with ASCUS cytology were directly referred to colposcopy, whereas two centers recommended that the cytologic examination be repeated and the women referred for colposcopy if the new examination indicated LSIL or a more advanced lesion.

Experimental Arm. During phase 2, women assigned to the experimental arm had a sample of cervical cells taken by a broom-like device ("Cervical sampler," Digene Corporation, Gaithersburg, MD). The cells were put in standard transport medium (Digene Corporation) and used only for HPV DNA testing. HPV DNA testing was done in seven laboratories, using the Hybrid Capture 2 hybridization assay (Digene Corporation). Only the "high-risk" cocktail of probes, which is designed to detect HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, was used. Results of the Hybrid Capture 2 tests for HPV DNA were expressed as relative light units (RLU), specifically, the ratio of the specimen's light emission to the average of three concurrently tested HPV DNA controls that consisted of HPV DNA at a concentration of 1 pg/mL. Quality assurance procedures were previously described (18). These included co-testing of commercially available (Digene Corporation) panels with known HPV DNA concentration and the circulation of clinical samples between laboratories to assess reproducibility, which was found to be very high (kappa coefficient = 0.93 for positive vs negative with standard transport medium) (18). During phase 2, all women were immediately referred for colposcopy if the HPV test was positive at 1 pg/mL cutoff. The women's management by phase and age and screening arm is summarized in Figure 1.

Colposcopy and Histology

The same colposcopists examined women in the experimental and conventional arms. They had access to the notes in the patients, medical records both for cytologic examination and HPV testing. All suspicious areas were biopsied.

In this report, we consider only the screening tests performed during the recruitment phase, which included any repeats of cytology performed until the woman was either advised to return for a new screening after 3 years or referred to colposcopy. The primary endpoint of the study was histologically confirmed CIN2+ detected as a result of such tests. We included all histology taken within 1 year of referral to colposcopy.

Histologic preparations were first read by local pathologists, who were not blinded to results from cytology or HPV testing. For women in both phases of recruitment whose biopsy was locally determined to be CIN, all histologic specimens from the relevant period were reviewed independently (19). In phase 2, specimens were reviewed blindly and independently by two of nine pathologists (one per center). If either of them did not agree with the original diagnosis regarding the presence of CIN2+, the slides were discussed by the full group of nine pathologists and a consensus diagnosis was reached. Only the consensus diagnosis was used in the analysis. For 30 women, the relevant specimens could not be retrieved for review of the local diagnosis and the most severe local diagnosis was used. On review, 23 of 351 cases of CIN1 (6.7%)

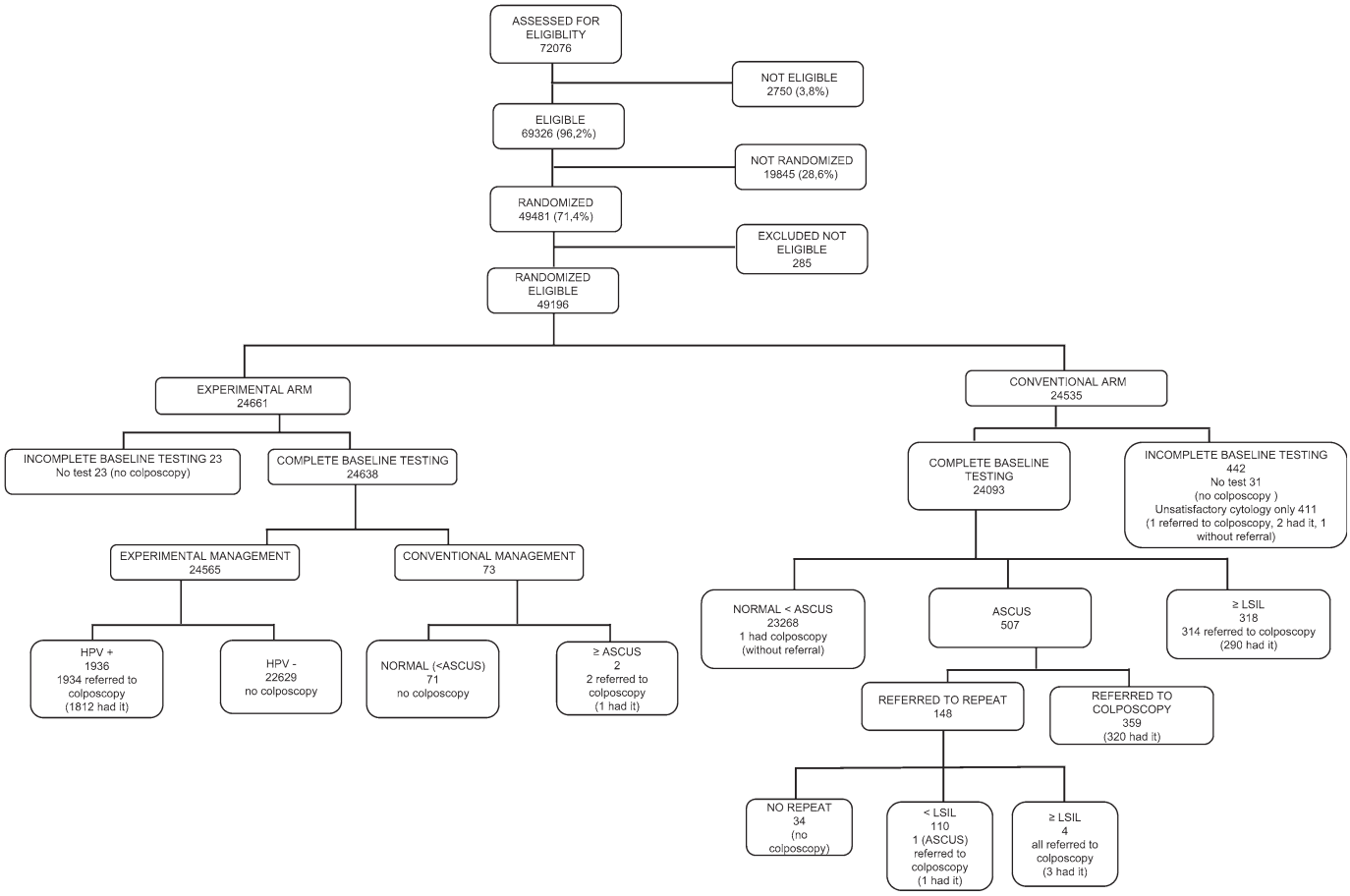

Figure 2. Trial profile. HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

were upgraded to CIN2 and one to CIN3, and 34 of 182 (19%) cases of CIN2 or 3 were downgraded to CIN1 or no CIN.

Statistical Analysis

The relative sensitivity of HPV testing by Hybrid Capture 2 hybridization assay (at 1 and 2 pg/mL cutoffs) vs conventional cytology was calculated as the ratio of detection rates of CIN2+ in the experimental and conventional arms. All eligible women randomly assigned were included in the analysis (intention to screen). However, the relative PPV of HPV testing vs conventional cytology was computed only for women who actually received colposcopy. Both histologically confirmed CIN2+ and CIN3+ were considered as endpoints. Confidence intervals for relative sensitivity and relative PPV were calculated on the basis of the normal approximation of the log (relative frequency) distribution using the Taylor series–derived variance of log (relative frequency) (20). Homogeneity in relative sensitivity and relative PPV between different groups was tested by the Breslow–Day test (21).

In assessing sensitivity and PPV of screening methods, we analyzed women according to age (25–34 vs 35–60 years). This was done because the prevalence of HPV infection is higher in younger women and because HPV-positive women of these two age groups had a different management in the two phases of recruitment. We also compared relative sensitivity and relative PPV across trial phases within each age group. For phase 1, we considered only the CIN detected in HPV-positive women. When there was no evidence of heterogeneity, pooled estimates from the two phases were computed.

The association between relative sensitivity and age was tested using an unconditional logistic regression analysis with CIN2+ as the outcome and the recruiting center, the trial arm, the age, and the interaction between age and trial arm as the covariates. All *P* values were from two-sided tests.

Results

During phase 2 of recruitment to NTCC, we randomly assigned 49481 women (Figure 2) to the two screening arms. Of these, 285 were excluded because they were not eligible. Therefore, a total of 24661 eligible women were in the conventional arm and 24535 in the experimental arm.

The age distribution was very similar in the two arms ($P = .24$ by two-sided median score test), and the median age was 42 years in both arms. A total of 12774 (52%) women in the conventional arm and 12873 (52%) in the experimental arm had had a screening test registered in an organized screening program within the 4 years before enrollment.

Women were managed according to the prescribed protocol, with the few exceptions due to local clinical decisions (Figure 2).

Table 2. Compliance with colposcopy, mean number of colposcopies, and biopsy rate by phase, arm, and age group in recruitment phase 2 of the New Technologies for Cervical Cancer study*

	Conventional arm (conventional cytology)		Experimental arm (HPV testing)	
Variable	**Women aged 25–34**	**Women aged 35–60**	**Women aged 25–34**	**Women aged 35–60**
Compliance to colposcopy*	86.5% (211/244)	92.9% (404/435)	93.7% (850/907)	93.6% (963/1029)
Colposcopies per patient, mean No. (SD)†	1.37 (0.63)	1.33 (0.55)	1.23 (0.47)	1.20 (0.47)
Biopsy rate†	59% (126/213)	49% (197/404)	51% (436/850)	37% (352/963)
Biopsies per patient, mean No. (SD)†	0.81 (0.85)	0.64 (0.86)	0.66 (0.77)	0.45 (0.68)

* HPV = human papillomavirus. Women who have had colposcopy were among those referred.

† Among women who had at least one colposcopy. Two women aged 25–34 in the conventional arm had colposcopy without referral and are included in the denominator for the biopsy rate calculation.

In the experimental arm, 73 women had conventional cytology instead of HPV testing. Of 2615 women referred to colposcopy, 2428 (93%) had at least one. Among women 25–34 years old, compliance with colposcopy was slightly lower in the conventional arm than in the experimental arm. Conversely, among all women, the mean number of colposcopies and the biopsy rate was slightly higher in the conventional than in the experimental arm. However, the differences between arms were always small (Table 2).

Cytology, HPV, and Histology Results in Phase 2

Among women recruited in phase 2 in the conventional arm, 4.0% of those aged 25–34 (270/6788) and 3.1% (555/17747) of those aged 35–60 had ASCUS or more severe cytology. The proportion of women with LSIL or more severe cytology was 2.0% (136/6788) and 1.0% (182/17747) in the age groups 25–34 and 35–60, respectively. In the conventional arm, 55 women were found to have CIN2+, 19 (14 with cytology LSIL or more) of which were among women aged 25–34 years and 36 (24 with cytology LSIL or more) of which were among women aged 35–60 years (Table 3). Rates of HPV positivity (>1 pg/mL) were 13.1% for women aged 25–34 (907/6937) and 5.8% (1029/17724) for women aged 35–60 (Table 4). The proportion of women with RLU ≥2.0 was 11.5% (796/6937) among women aged 25–34 and 4.5% (789/17724) among women aged 35–60. The proportions of women with abnormal cytology results (ASCUS or more severe) and women who were HPV positive decreased with age in the conventional and experimental arms, respectively (Figure 3).

Relative Accuracy of HPV Testing in Women Aged 35–60: Results During Phase 2, Comparison With Phase 1, and Combined Estimates

In phase 2, among women 35–60 years of age using a threshold of 1 pg/mL HPV DNA, sensitivity was statistically significantly

Table 3. Cytology and histology results in the conventional arm for phase 2 of the New Technologies for Cervical Cancer study*

	Histology					
Cytology result	**No colposcopy**	**No CIN†**	**CIN1**	**CIN2**	**CIN3+**	**Total No. of patients (%)**
All ages						
Unsatisfactory‡	440	1	1	0	0	442 (1.8%)
Normal or benign change	23267	1	0	0	0	23268 (94.8%)
ASCUS or AGCUS§	183 (39)	257 (256)	50 (47)	8 (8)	9 (9)	507 (359) (2.1% [1.5%])
LSIL	25	185	46	11	6	273 (1.1%)
HSIL+	3	8	13	10	11	45 (0.2%)
Total	23918	452	110	29	26	24535 (100%)
Women aged 25–34						
Unsatisfactory‡	189	0	1	0	0	190 (2.8%)
Normal or benign change	6327	1	0	0	0	6328 (93.2%)
ASCUS or AGCUS§	45 (19)	69 (69)	15 (14)	1 (1)	4 (4)	134 (107) (2.0% [1.6%])
LSIL	14	79	26	4	2	125 (1.8%)
HSIL+	0	2	1	5	3	11 (0.2%)
Total	6575	151	43	10	9	6788 (100%)
Women aged 35–60						
Unsatisfactory only	251	1	0	0	0	252 (1.4%)
Normal or benign change	16940	0	0	0	0	16940 (95.5%)
ASCUS or AGCUS§	138 (20)	188 (187)	35 (33)	7 (7)	5 (5)	373 (252) (2.1% [1.4%])
LSIL	11	106	20	7	4	148 (0.8%)
HSIL+	3	6	12	5	8	34 (0.2%)
Total	17343	301	67	19	17	17747 (100%)

* CIN = cervical intraepithelial neoplasia; ASCUS = atypical squamous cells of undetermined significance; AGCUS = atypical glandular cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

† Includes women who had colposcopy but not histology.

‡ Women were advised to repeat cytology, but no satisfactory smear became available.

§ Number of women directly referred to colposcopy is shown in parentheses.

Table 4. Distribution of women according to human papillomavirus DNA and histology results in the experimental arm of phase 2*

	Histology					
Hybrid Capture 2 result, RLU	**No colposcopy**	**No CIN†**	**CIN1**	**CIN2**	**CIN3+**	**Total No. of patients (%)**
All ages						
No valid test	95	1	0	0	0	96 (0.4)
<0.30	17116	0	0	0	0	17116 (69.4)
0.30–0.99	5513	0	0	0	0	5513 (22.4)
1.00–1.99	23	290	33	5	0	351 (1.4)
2.00–3.99	12	194	23	3	3	235 (1.0)
4.00–9.99	13	150	28	7	4	202 (0.8)
≥10	76	805	152	63	52	1148 (4.7)
Total	22848	1440	236	78	59	24661 (100)
Women aged 25–34						
No valid test	22	0	0	0	0	22 (0.3)
<0.30	4488	0	0	0	0	4488 (64.7)
0.30–0.99	1520	0	0	0	0	1520 (21.9)
1.00–1.99	9	89	12	1	0	111 (1.6)
2.00–3.99	5	78	9	0	1	93 (1.3)
4.00–9.99	7	65	14	2	0	88 (1.3)
≥10	36	417	98	41	23	615 (8.9)
Total	6087	649	133	44	24	6937 (100)
Women aged 35–60						
No valid test	73	1	0	0	0	74 (0.4)
<0.30	12628	0	0	0	0	12628 (71.3)
0.30–0.99	3993	0	0	0	0	3993 (22.5)
1.00–1.99	14	201	21	4	0	240 (1.4)
2.00–3.99	7	116	14	3	2	142 (0.8)
4.00–9.99	6	85	14	5	4	114 (0.6)
≥10	40	388	54	22	29	533 (3.0)
Total	16761	791	103	34	35	17724 (100)

* CIN = cervical intraepithelial neoplasia; RLU = relative light units (the ratio of the specimen's light emission to the average light emission of three concurrently tested 1 pg/mL HPV DNA controls).

† Includes women who had colposcopy but not histology.

greater with HPV testing than with conventional cytology, both for CIN2+ (relative sensitivity = 1.92; 95% CI = 1.28 to 2.87) and CIN3+ (relative sensitivity = 2.06; 95% CI = 1.16 to 3.68) as endpoints (Table 5). Relative PPVs were only slightly reduced (0.80, 95% CI = 0.55 to 1.18, for CIN2+ and 0.86, 95% CI = 0.49 to 1.52, for CIN3+), and the difference was not statistically significant. In this age group, there was no evidence of heterogeneity between the estimates of relative sensitivity and relative PPV of HPV testing alone vs cytology obtained in phase 2 and those previously obtained in phase 1 (15) (Table 5). With CIN2+ as the endpoint and a cutoff of signal intensity corresponding to 1 pg/mL HPV DNA, the estimates obtained by combining the two phases for relative sensitivity and relative PPV were 1.63 (95% CI = 1.25 to 2.12) and 0.67 (95% CI = 0.52 to 0.7), respectively. At a 2 pg/mL cutoff, the corresponding values were 1.57 (95% CI = 1.20 to 2.06) and 0.85 (95% CI = 0.66 to 1.09), respectively. With CIN3+ as the endpoint, and using a 1 pg/mL cutoff, the combined estimates were 1.52 (95% CI = 1.06 to 2.19) for relative sensitivity and 0.63 (95% CI = 0.44 to 0.89) for

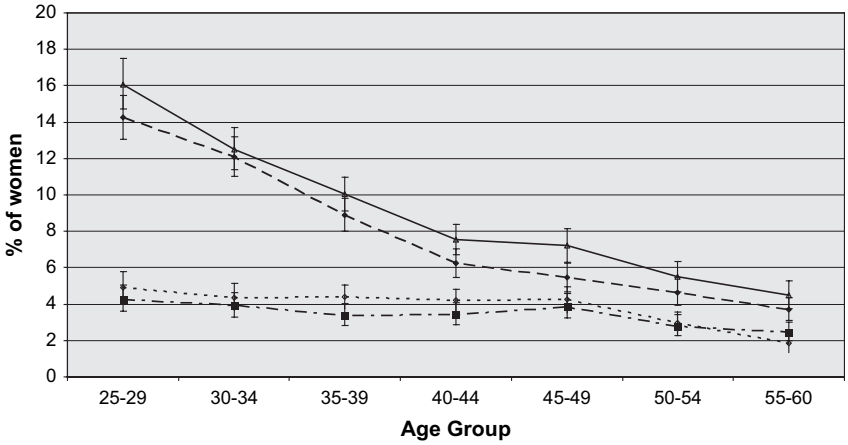

Figure 3. Proportion of women positive for human papillomavirus (HPV+) with abnormal cytology by age. **Filled diamonds**: HPV+ experimental arm phase 2; **filled squares** = atypical squamous cells of undetermined significance or more (ASCUS+) conventional arm phase 2; **open triangles** = HPV+ experimental arm phase 1; **open diamonds** = ASCUS+ conventional arm phase 1.

Table 5. Relative sensitivity and relative positive predictive value of human papillomavirus testing vs conventional cytology for women aged 35–60*

Screening	Phase 2		Phase 1	Both phases combined	$P_{heterogeneity}$ between phases‡
	Detection rate per 1000	Relative sensitivity (95% CI)	Relative sensitivity (95% CI)†	Relative sensitivity (95% CI)	
Endpoint CIN2+					
Experimental arm, HPV ≥ 1 pg/mL	3.89	1.92 (1.28 to 2.87)	1.43 (1.00 to 2.04)	1.63 (1.25 to 2.12)	.280
Experimental arm, HPV ≥ 2 pg/mL	3.67	1.81 (1.20 to 2.72)	1.41 (0.98 to 2.01)	1.57 (1.20 to 2.06)	.366
Conventional arm, cytology ≥ ASCUS	2.03	1.00	1.00	1.00	
Endpoint CIN3+					
Experimental arm, HPV ≥ 1 pg/mL	1.97	2.06 (1.16 to 3.68)	1.22 (0.76 to 1.96)	1.52 (1.06 to 2.19)	.170
Experimental arm, HPV ≥ 2 pg/mL	1.97	2.06 (1.16 to 3.68)	1.19 (0.74 to 1.92)	1.50 (1.04 to 2.16)	.150
Conventional arm, cytology ≥ ASCUS	0.96	1.00	1.00	1.00	

	Phase 2		Phase1	Both phases Combined	$P_{heterogeneity}$ between phases‡
	PPV %	Relative PPV (95% CI)	Relative PPV (95% CI)*	Relative PPV (95% CI)	
Endpoint CIN2+					
Experimental arm, HPV ≥ 1 pg/mL	7.2	0.80 (0.55 to 1.18)	0.58 (0.33 to 0.98)	0.67 (0.52 to 0.87)	.216
Experimental arm, HPV ≥ 2 pg/mL	8.8	0.99 (0.67 to 1.46)	0.75 (0.45 to 1.27)	0.85 (0.66 to 1.09)	.284
Conventional arm, cytology ≥ ASCUS	8.9	1.00	1.00	1.00	
Endpoint CIN3+					
Experimental arm, HPV ≥ 1 pg/mL	3.6	0.86 (0.49 to 1.52)	0.50 (0.32 to 0.79)	0.63 (0.44 to 0.89)	.137
Experimental arm, HPV ≥ 2 pg/mL	4.8	1.22 (0.64 to 1.99)	0.63 (0.40 to 1.00)	0.81 (0.56 to 1.15)	.118
Conventional arm, cytology ≥ ASCUS	4.2	1.00	1.00	1.00	

* CI = confidence interval; CIN2+ = histology-confirmed cervical intraepithelial neoplasia grade 2 or more severe; HPV= human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; CIN3+ = histology-confirmed cervical intraepithelial neoplasia grade 3 or more severe; PPV = positive predictive value. All eligible women randomly assigned in the relevant phase were considered for detection rate and relative sensitivity. Only women who actually had colposcopy were included in PPV and relative PPV calculations.

† Comparing experimental arm phase 1 (taking into account only lesions detected in HPV-positive women) with conventional arm phase 1 (15).

‡ By Breslow–Day test.

relative PPV. At 2 pg/mL cutoff the corresponding values were 1.50 (95% CI = 1.04 to 2.16) and 0.81 (95% CI = 0.56 to 1.15), respectively.

Relative Accuracy of HPV Testing in Women Aged 25–34: Results During Phase 2 and Comparison With Phase 1

In the age group 25–34, during phase 2 we observed a much higher sensitivity with HPV testing than with conventional cytology, both for histologically confirmed CIN2+ and CIN3+ (Table 6). The relative sensitivity at a 1 pg/mL cutoff was 3.50 (95% CI = 2.11 to 5.82) for CIN2+ and 2.61 (95% CI = 1.21 to 5.61) for CIN3+. The corresponding relative PPVs were 0.89 (95% CI = 0.55 to 1.44) and 0.66 (95% CI = 0.31 to 1.40).

The increase in sensitivity with HPV testing was much larger among these younger women than among older women. Upon pooling all phases and age groups in which HPV-positive women were directly referred to colposcopy (phase 2, all ages, and phase 1, ages 35–60) relative sensitivity decreased linearly with age (P = .044 after adjustment for phase and center by unconditional logistic regression).

Much smaller increases in sensitivity for HPV testing relative to conventional cytology had been observed in the same age group during phase 1 (where HPV-positive women had been managed differently), and there was a statistically significant increase in relative sensitivity of HPV from phase 1 to 2 (P values ranged from .006 for CIN3 or greater to .019 for CIN2 or greater with a 1 pg/mL cutoff), although relative PPVs were not statistically significantly different between phases (P values ranged between .114 and .665; Table 6).

Discussion

Here we have presented results from the baseline screen from the second phase of a large individually randomized controlled trial that compared HPV testing alone with conventional cytology. Other randomized controlled trials conducted in developed countries have compared cytology with the combined use of cytology and HPV testing and have shown a substantially increased sensitivity with the latter approach (22–23). Another trial randomly assigned the sequence of HPV and Papanicolau testing (24).

Among women aged 35–60 years, who, if HPV positive, were managed in the same way in both phases of recruitment, the relative sensitivity of HPV testing vs conventional cytology did not change to a statistically significant extent between phases, and our results indicate that HPV testing provides higher cross-sectional sensitivity than conventional cytology. The gain in sensitivity was virtually unchanged when the cutoff was increased from 1 to 2 pg/mL, and the latter threshold provided a much better PPV and is therefore preferable. Based on the combined

Table 6. Relative sensitivity and relative positive predictive value of human papillomavirus testing vs conventional cytology for women aged 25–34*

	Phase 2		Phase 1	$P_{heterogeneity}$
	Detection rate per 1000	Relative sensitivity (95% CI)	Relative sensitivity (95% CI)†	between phases‡
Endpoint CIN2+				
Experimental arm, HPV ≥ 1 pg/mL	9.80	3.50 (2.11 to 5.82)	1.58 (1.03 to 2.44)	0.019
Experimental arm, HPV ≥ 2 pg/mL	9.66	3.45 (2.08 to 5.74)	1.58 (1.03 to 2.44)	0.021
Conventional arm, cytology ≥ ASCUS	2.80	1.00	1.00	
Endpoint CIN3+				
Experimental arm HPV ≥ 1 pg/mL	3.46	2.61 (1.21 to 5.61)	0.66 (0.34 to 1.27)	0.006
Experimental arm, HPV ≥ 2 pg/mL	3.46	2.61 (1.21 to 5.61)	0.66 (0.34 to 1.27)	0.006
Conventional arm, cytology ≥ ASCUS	1.33	1.00	1.00	
	Phase 2		Phase 1	$P_{heterogeneity}$
	PPV %	Relative PPV (95% CI)	Relative PPV (95% CI)*	between phases‡
Endpoint CIN2+				
Experimental arm, HPV ≥ 1 pg/mL	8.0	0.89 (0.55 to 1.44)	0.78 (0.52 to 1.16)	0.665
Experimental arm, HPV ≥2 pg/mL	9.0	0.99 (0.62 to 1.62)	0.84 (0.56 to 1.25)	0.580
Conventional arm, cytology ≥ ASCUS	9.0	1.00	1.00	
Endpoint CIN3+				
Experimental arm, HPV ≥ 1 pg/mL	2.8	0.66 (0.31 to 1.40)	0.33 (0.17 to 0.61)	0.143
Experimental arm, HPV ≥ 2 pg/mL	3.2	0.75 (0.36 to 1.59)	0.35 (0.19 to 0.66)	0.114
Conventional arm, cytology ≥ ASCUS	4.3	1.00	1.00	

* CI = confidence interval; CIN2+ = histology-confirmed cervical intraepithelial neoplasia grade 2 or more severe; HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; CIN3+ = histology-confirmed cervical intraepithelial neoplasia grade 3 or more severe; PPV = positive predictive value. All eligible women randomly assigned in the relevant phase were considered for detection rate and relative sensitivity (relative to that of the conventional arm). Only women who actually had colposcopy were included in PPV and relative PPV calculations.

† Comparing experimental arm phase 1 (taking into account only lesions detected in HPV-positive women) with conventional arm phase 1 (16).

‡ By Breslow–Day test.

data from both phases of testing, representing nearly 70 000 randomly assigned women, we estimate that, compared with conventional cytology, HPV testing with a 2 pg/mL cutoff achieves a statistically significant gain of about 50% in sensitivity for detection of CIN2+ and CIN3+ and leads to a non–statistically significant reduction in PPV of only about 15%–20%. A pooled analysis of pair-sample studies (25) showed a small reduction of specificity of HPV testing compared with conventional cytology at this age. The statistically significant increase in sensitivity for CIN3+ is particularly relevant because these lesions are more persistent.

The relative sensitivity of HPV testing compared with conventional cytology was much larger among women 25–34 years of age than among older women. In addition, there was a striking increase in relative sensitivity of HPV testing vs conventional cytology from phase 1 to 2 that cannot be explained by random variation or bias and is due at least in part to the different management strategies employed for younger HPV-positive women during the two phases. During phase 1, younger HPV-positive women were immediately referred to colposcopy only if cytology was abnormal (ASCUS+), whereas those with normal cytology were retested after 1 year and referred to colposcopy only if Hybrid Capture 2 persisted positive or if cytology became abnormal. During phase 2, all HPV-positive women were directly referred to colposcopy (Figure 1).

The high relative sensitivity of HPV testing vs conventional cytology in phase 2 among women aged 25–34 indicates that a large number of high-grade lesions are normal by conventional cytology in this age group. Greater gains in sensitivity of Hybrid Capture 2 hybridization assay compared with conventional cytology among younger women were also found in a pooled analysis of studies on HPV for primary cervical screening (25). Furthermore, a very high incidence of high-grade lesions was observed shortly after HPV infection in teenagers or women in their early twenties who were screened intensively (26–28). The difference in high-grade lesion rates in young women in our two phases combined with these observations suggests that most abnormalities in young women, including CIN2 and CIN3, regress spontaneously. Thus, the larger increase in sensitivity relative to cytology obtained by direct referral to colposcopy than by triaging of HPV-positive women may represent overtreatment of lesions that are destined to regress and is not likely to be an advantage, especially because excisional treatment of cervical lesions is associated with increased risk of pregnancy-related morbidity (29). Consequently, HPV-positive women aged 25–34 should be referred to colposcopy only if cytology is also abnormal or if infection persists after 1 year. High regression rates seem, however, to be specific to younger women. The HPV in Addition to Routine Testing study (11) was conducted only among older women (age = 30–60, mean age = 42 years) who tested positive for HPV according to the Hybrid Capture 2 assay. These women were randomly assigned either to immediate colposcopy or to triage by cytology. The rate of detection of high-grade lesions was similar in the two groups.

Our study was conducted within organized screening programs in a situation very similar to routine application. More than 70% of eligible women were enrolled, suggesting that results are applicable to routine practice.

A limitation of our study is that colposcopy and the local interpretation of histology could not be blinded. However, histology from both arms was reviewed blindly. In addition, compliance to colposcopy was high in both study arms, and the rates of repeat colposcopy and of biopsy were similar in the two arms or higher in the conventional arm. Therefore, bias in endpoint assessment is not likely to explain the increased sensitivity with HPV testing.

Another limitation of our study is that, given its cross-sectional nature, the data do not exclude the possibility of regression of the additional lesions detected by HPV testing even among older women. However, recently published longitudinal results of two other randomized controlled trials that included only women at least 30 (23) or 35 (22) years of age—and that employed conservative strategies for referral to colposcopy of HPV-positive women—showed that the additional lesions detected at baseline are persistent and suggest that prolonged screening intervals can be applied with HPV testing (30). The persistence of the additional lesions detected and the possibility of applying prolonged screening intervals are crucial factors to be considered before routinely introducing HPV testing for primary screening (30).

The longitudinal studies showing the persistence of the additional lesions detected by HPV testing (22,23) applied HPV testing in addition to cytology. To our knowledge, phase 2 of our study is the only randomized comparison of a strategy of HPV testing alone vs cytology alone. Our results show increased cross-sectional sensitivity and limited loss of PPV with HPV testing, suggesting that HPV testing can better be applied as the sole primary screening test and that a cutoff for the Hybrid Capture 2 hybridization assay of 2 pg/mL HPV DNA is clearly preferable to the conventional 1 pg/mL threshold. Comparison of the two phases show that, for women aged less than 35 years, triage by cytology and retesting after 1 year in HPV-positive women with normal cytology are needed. For this age group, referring HPV-positive women who are cytologically negative to colposcopy will lead to substantial overtreatment and should be avoided. For older women, some triage is probably also appropriate (11), but this is less crucial and a much larger proportion of lesions found in this group are likely to be persistent and potentially progressive. Data from the follow-up phase of this study, which is currently underway, will allow a direct estimate of regression rates at different ages with the different strategies that we applied and permit us to examine the feasibility of extending screening intervals. Newer tests based on HPV typing or different molecular markers are likely to help refine the indications for referral to colposcopy, but it seems clear that an HPV DNA–based approach to primary screening is a very attractive option that should be actively developed and evaluated.

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