

Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice



Hormuzd A Katki, Walter K Kinney, Barbara Fetterman, Thomas Lorey, Nancy E Poitras, Li Cheung, Franklin Demuth, Mark Schiffman, Shalom Wacholder, Philip E Castle

Summary

Background Concurrent testing for human papillomavirus (HPV) and cervical cytology (co-testing) is an approved alternative to cytology alone in women aged 30 years and older. We aimed to assess the safety in routine clinical practice of 3-year screening intervals for women testing negative for HPV with normal cytology and to assess if co-testing can identify women at high risk of cervical cancer or cervical intraepithelial neoplasia grade 3 (CIN3) or worse over 5 years.

Methods We assessed the 5-year cumulative incidence, starting in 2003–05, of cervical cancer and CIN3 or worse for 331 818 women aged 30 years and older who enrolled in co-testing at Kaiser Permanente Northern California (Berkeley, CA, USA) and had adequate enrolment co-test results. Follow-up continued until Dec 31, 2009. We defined cumulative incidence to include prevalence at enrolment and incidence after enrolment. Prevalence at enrolment was defined as the ratio of women diagnosed with each outcome on the biopsy visit immediately after their enrolment screening visit to the total enrolled women. At screening visits only HPV test and Pap smear samples were collected, and at biopsy visits colposcopically directed biopsies were taken. To estimate post-enrolment incidence, we used Weibull survival models.

Findings In 315 061 women negative by HPV testing, the 5-year cumulative incidence of cancer was 3·8 per 100 000 women per year, slightly higher than for the 306 969 who were both negative by HPV and Pap testing (3·2 per 100 000), and half the cancer risk of the 319 177 who were negative by Pap testing (7·5 per 100 000). 313 465 (99·5%) women negative by HPV testing had either normal cytology or equivocal abnormalities. Abnormal cytology greatly increased cumulative incidence of CIN3 or worse over 5 years for the 16 757 positive by HPV testing (12·1% vs 5·9%; $p < 0·0001$). By contrast, although statistically significant, abnormal cytology did not increase 5-year risk of CIN3 or worse for women negative by HPV testing to a substantial level (0·86% vs 0·16%; $p = 0·004$). 12 208 (73%) of the women positive by HPV testing had no cytological abnormality, and these women had 258 (35%) of 747 CIN3 or worse, 25 (29%) of 87 cancers, and 17 (63%) of 27 adenocarcinomas.

Interpretation For women aged 30 years and older in routine clinical practice who are negative by co-testing (both HPV and cytology), 3-year screening intervals were safe because a single negative test for HPV was sufficient to reassure against cervical cancer over 5 years. Incorporating HPV testing with cytology also resulted in earlier identification of women at high risk of cervical cancer, especially adenocarcinoma. Testing for HPV without adjunctive cytology might be sufficiently sensitive for primary screening for cervical cancer.

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Introduction

The findings of long-term prospective cohort studies and randomised clinical trials have shown that DNA testing for human papillomavirus (HPV) is substantially more sensitive than cervical cytology for the detection of cervical intraepithelial neoplasia grade 2 (CIN2) and grade 3 (CIN3) and cancer.^{1–9} Incorporation of testing for HPV into programmes screening for cervical cancer could reduce the incidence of this cancer in women aged 30 years and older^{4–5,8,10} (particularly for adenocarcinoma, the precursors of which are often missed by cytological methods¹¹), and even mortality.¹² Cohort studies and trials suggest that women's risk of CIN3 or cancer after a negative test for HPV is very low for 5 years.^{3,10,13,14} These findings were the basis for regulatory and clinical guideline approval^{15,16} of

routine testing for HPV in conjunction with cervical cytology (co-testing) for cervical cancer screening of women aged 30 years and older. In particular, the guidelines discourage the screening of women with normal cytology (ie, negative by Pap testing) and negative for HPV before 3 years, to avoid the detection of new infections with HPV. New infections with HPV are associated with an extremely low risk of cancer because they usually resolve without the need for medical intervention.¹⁷

Although promising and approved, co-testing has not been widely adopted in the USA. In a recent survey,¹⁸ only 19% of US clinicians would recommend the 3-year screening interval for women with normal cytology testing who are negative for HPV, which suggests concern about the cancer risk accrued over 3 years. Studies in

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Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, DHHS, Bethesda, MD, USA (H A Katki PhD, Prof M Schiffman MD, Prof S Wacholder PhD); Division of Gynecologic Oncology, Kaiser Permanente Medical Care Program, Oakland, CA, USA (Prof W K Kinney MD); Regional Laboratory, Kaiser Permanente Northern California, Berkeley, CA, USA (B Fetterman SCT[ASCP], T Lorey MD, N E Poitras ACS); Information Management Services Inc, Silver Spring, MD, USA (L Cheung MS, F Demuth BS); and American Society for Clinical Pathology, Washington, DC, USA (P E Castle PhD)

Correspondence to:

Dr Hormuzd Katki, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd Room 8014, EPS MSC 7244, Bethesda, MD 20882, USA
katkih@mail.nih.gov

routine clinical practice are needed to estimate the feasibility and safety of co-testing guidelines.¹⁹ Although clinical trials and research cohorts in specially selected populations can show efficacy in specific, tightly controlled, idealised circumstances, the final proof of the value of medical interventions is their effectiveness in general clinical practice, with all its attendant complexity, such as non-standard protocols, potential non-adherence by clinicians and patients to protocols, or screening tests done in non-ideal circumstances. Furthermore, very large samples are needed to establish actual cancer risks for each possible cytological abnormality and HPV test result, especially for women who are negative by HPV and cytology testing, for whom reassurance against cancer is the crucial factor for deciding their screening interval. Finally, each successive screen, if effective,^{20,21} should lower the subsequent population risk because those with previously evident and clinically relevant disease have already been identified and treated, and therefore do not contribute to the overall risk. Therefore, extended follow-up of very large numbers of women negative for HPV or by Pap provides an opportunity to assess how much further their cancer risks decrease after their return in 3 years for their second visit, which has not been previously assessed.

In 2003, Kaiser Permanente Northern California (KPNC, Berkeley, CA, USA), a large health-maintenance organisation, adopted a screening programme for cervical cancer based on co-testing, with extended screening intervals for women with normal cytology who test negative for HPV. The KPNC experience serves as a large-scale demonstration project of what could realistically be achieved in routine clinical practice, where providers receive no special training and do not need any special qualifications to participate and that no provider, provider group, patient, or group of patients is excluded. We established the risk of cervical cancer for women aged 30 years and older who enrolled in co-testing at KPNC between 2003 and 2005. We also assessed the risk of cervical cancer after the second screening visit in women who were negative by both HPV and Pap testing at enrolment, to establish whether the second co-test provided additional reassurance against cancer. Our principal aims were to establish the safety of 3-year screening intervals for women negative by both HPV and Pap testing and the value of adding testing for HPV to cytology screening to earlier identify women at high risk of CIN3 or worse or cervical cancer over 3–5 years.

Methods

Participants

KPNC membership is demographically similar to the US Census-enumerated population in the Bay Area Metropolitan Statistical Area, except for lacking representation of extremes in income.^{22,23} In a study of the KPNC population including the women in this study, of the 49% of women who self-reported their race or

ethnicity in KPNC, 62% were white, 12% were Asian/Pacific islander, 12% were Hispanic, and 8% were African-American.²⁴ The KPNC population is thought a well-screened population, and their risk of cervical cancer has historically been lower than the national average²⁵ (most cancer cases in the USA are in regions where screening services are unavailable²⁶). Over 90% of eligible women enrolled in co-testing.²⁴

Procedure

Conventional Pap tests were reported according to the 2001 Bethesda System²⁷ (in order of increasing severity): no intraepithelial lesion or malignancy (negative or normal by Pap test); atypical squamous-cells of undetermined significance (ASC-US); low-grade squamous intraepithelial lesion (LSIL); atypical glandular cells of undetermined significance or not otherwise specified (AGUS/NOS); atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H); high-grade squamous intraepithelial lesion (HSIL); or squamous-cell carcinoma (SCC). A positive Pap test means ASC-US or more severe cytology. We grouped ASC-H, HSIL, and SCC into a single high-risk Pap category because each is individually uncommon and their CIN3 or worse risks were similarly increased. Conventional Pap slides were manually reviewed after processing by the BD FocalPoint Slide Profiler (BD Diagnostics, Burlington, NC, USA) primary screening and directed quality control system, in accordance with protocols approved by the US Food and Drug Administration. As an important methodological point, we note that a meta-analysis²⁸ and two large randomised trials^{29,30} have not shown any clinical performance advantage of liquid-based cytology over conventional Pap smears for detection of CIN3 or worse. Hybrid Capture 2 (HC2; Qiagen, Germantown, MD, USA) was used to test for the pool of carcinogenic HPV types according to manufacturer's instructions. HC2 has high inter-operator reliability.³¹ All cytology and testing for HPV was done at the Regional Laboratory of the Northern California Kaiser Permanente Medical Care Program by a stable staff of about 30 cytotechnicians, laboratory technicians, clinical scientists, and pathologists.

Histological findings were classified (in order of increasing severity) as no lesion found, CIN1, CIN2, CIN3, adenocarcinoma in situ, SCC, or adenocarcinoma. CIN2 or CIN3/adenocarcinoma in situ (AIS) histology was sufficient to refer a woman for treatment by a loop electrosurgical excision procedure. All reported cervical cancers were verified by chart review by WKK and J Thomas Cox (University of California Santa Barbara, CA, USA). Abnormal biopsies were reviewed and signed out by a stable team of about 60 pathologists in the 12 pathology departments of KPNC. We primarily focused on CIN3 or cancer rather than CIN2 or worse, because CIN2 is unreliably identified by pathologists,^{32,33} often

regresses,^{34,35} and might simply relate to uncertainty between acute infection with HPV (CIN1) and CIN3.³⁶ However, our findings did not appreciably change when we used CIN2 or worse as our endpoint (the webappendix pp 3–6 includes all risks for CIN2 or worse).

KPNC has its own management guidelines for patients, which are fairly consistent with the 2004 interim guidelines for HPV testing¹⁵ and 2006 American Society for Colposcopy and Cervical Pathology guidelines,¹⁶ and KPNC clinicians are asked to adhere to their guidelines. Women with LSIL or more severe cytology, irrespective of the HPV test result, were sent for colposcopy. Women with ASC-US who tested positive for HPV were sent to colposcopy, whereas those who tested negative for HPV were asked to return for a 1-year follow-up. Women who were negative by both HPV and Pap testing were asked to return for screening in 3 years. Until 2005, women positive for HPV but negative by Pap testing were generally monitored annually for cytological evidence of disease. After 2005, women with consecutive positive HPV results were offered colposcopy. We had access to information about past history of an abnormal Pap test or abnormal biopsy predating the co-testing era at KPNC, but we could not independently verify the completeness of the information.

KPNC uses a computerised patient follow-up system, which reviews laboratory results daily and sets alarm flags for appropriate follow-up intervals for each abnormal result. If, for example, a biopsy has not been

recorded after an abnormal Pap test within a given period, an alarm is sent to the personnel responsible for the screening system at the appropriate facility. If this condition is not reset by receipt of a biopsy within a given period, escalating alarms follow to the practitioner, then the Chief of Obstetrics and Gynaecology, and finally the Physician-in-Chief of the facility. If a woman loses her job, leaves northern California, or changes her health insurance from KPNC, her future records are unknown to KPNC.

Statistical analysis

We estimated the cumulative incidence of the outcomes CIN2 or worse, CIN3 or worse, or cervical cancer for each possible combination of HPV test and Pap smear result with SAS version 9.0. We estimated cumulative incidence from enrolment for all women, and after the first return visit for women who co-tested negative by HPV test and Pap smear at enrolment. We defined cumulative incidence to include prevalence at enrolment and the incidence after enrolment. At screening visits only HPV test and Pap smear samples were collected. At biopsy visits, colposcopically directed biopsies were taken. The prevalence at enrolment was defined as the ratio of the number of women diagnosed with each outcome on the biopsy visit immediately after their enrolment screening visit to the total number of enrolled women. We used Weibull survival models³⁷ to estimate post-enrolment

See Online for webappendix

	All women	No biopsy or <CIN2	CIN2	CIN3/AIS	AIS	Squamous carcinoma	Adenocarcinoma	Total cancers
Total	331 818 (100%)	329 508 (100%)	1476 (100%)	747 (100%)	70 (100%)	49 (100%)	27 (100%)	87 (100%)
Baseline HPV								
Negative	315 061 (94.9%)	314 589 (95.5%)	322 (22%)	123 (16%)	14 (20%)	18 (37%)	6 (22%)	27 (31%)
Positive	16 757 (5.1%)	14 919 (4.5%)	1154 (78%)	624 (84%)	56 (80%)	31 (63%)	21 (78%)	60 (69%)
Baseline Pap								
Pap negative	319 177 (96.2%)	318 093 (96.5%)	687 (47%)	354 (47%)	42 (60%)	15 (31%)	23 (85%)	43 (49%)
Total Pap positive	12 641 (3.8%)	11 415 (3.5%)	789 (53%)	393 (53%)	28 (40%)	34 (69%)	4 (15%)	44 (51%)
ASC-US	8517 (2.6%)	8106 (2.5%)	283 (19%)	123 (16%)	12 (17%)	4 (8%)	1 (4%)	5 (6%)
LSIL	2527 (0.76%)	2208 (0.67%)	253 (17%)	61 (8%)	1 (1%)	4 (8%)	0 (0%)	5 (6%)
AGUS/NOS	764 (0.23%)	705 (0.21%)	26 (2%)	27 (4%)	7 (10%)	1 (2%)	2 (7%)	6 (7%)
ASC-H/HSIL/SCC	833 (0.25%)	396 (0.12%)	227 (15%)	182 (24%)	8 (11%)	25 (51%)	1 (4%)	28 (32%)
Baseline HPV/Pap								
HPV negative/Pap negative	306 969 (92.5%)	306 597 (93.0%)	258 (17%)	96 (13%)	11 (16%)	10 (20%)	6 (22%)	18 (21%)
HPV negative/Pap positive	8092 (2.4%)	7992 (2.4%)	64 (4%)	27 (4%)	3 (4%)	8 (16%)	0 (0%)	9 (10%)
HPV positive/Pap negative	12 208 (3.7%)	11 496 (3.5%)	429 (29%)	258 (35%)	31 (44%)	5 (10%)	17 (63%)	25 (29%)
HPV positive/Pap positive	4549 (1.4%)	3423 (1.0%)	725 (49%)	366 (49%)	25 (36%)	26 (53%)	4 (15%)	35 (40%)
Baseline HPV/ASC-US								
HPV negative/ASC-US	6496 (2.0%)	6455 (2.0%)	25 (2%)	14 (2%)	1 (1%)	2 (4%)	0 (0%)	2 (2%)
HPV positive/ASC-US	2021 (0.6%)	1651 (0.5%)	258 (17%)	109 (15%)	11 (16%)	2 (4%)	1 (4%)	3 (3%)

Total cancers includes squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and cervical cancer of unknown histology. CIN3/AIS includes 13 histologies that were either CIN3 or AIS, but precisely which is unknown. Pap positive is ASC-US or worse cytology. CIN2=cervical intraepithelial neoplasia grade 2. CIN3/AIS=cervical intraepithelial neoplasia grade 3 or adenocarcinoma in situ. HPV=human papillomavirus test. ASC-US=atypical squamous-cells of undetermined significance. LSIL=low-grade squamous intraepithelial lesion. AGNUS/NOS=atypical glandular cells of undetermined significance or not otherwise specified. ASC-H=atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion. HSIL=high-grade squamous intraepithelial lesion. SCC=squamous cell carcinoma.

Table 1: Distribution of worst histological diagnosis by enrolment HPV test and Pap smear

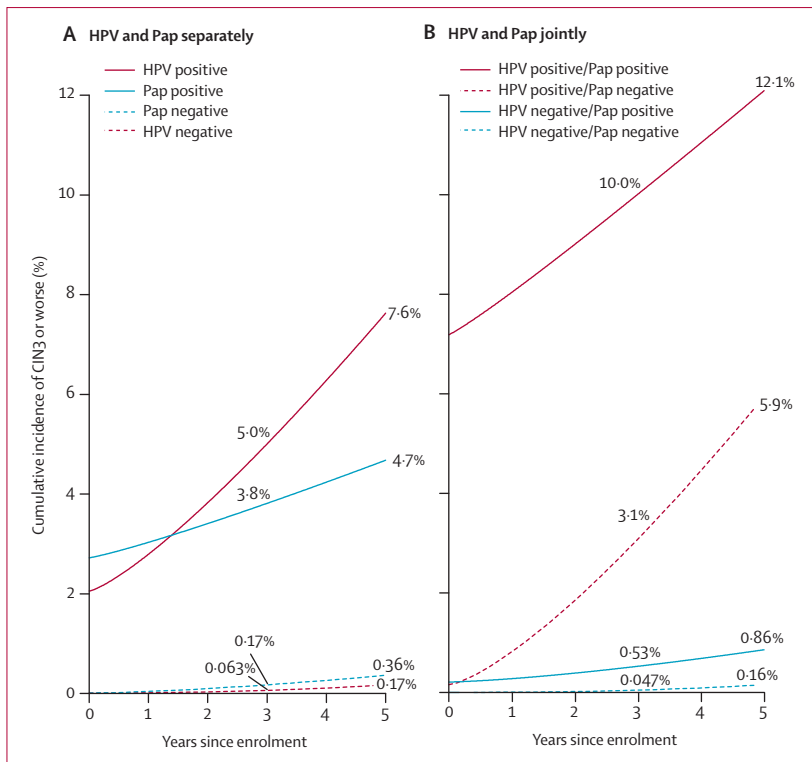


Figure 1: Value of HPV testing versus Pap smears (A) and value added by Pap smears to HPV testing (B)

Data are shown on cumulative incidence of CIN3 or worse by enrolment HPV test vs Pap smear. Prevalent CIN3 or worse is plotted at time 0 (enrolment) and incident CIN3 or worse is plotted from that point. Pap positive is atypical squamous cells of undetermined significance or worse. (A) The HPV test more clearly separated high-risk women from low-risk women than the Pap smear because both women positive for HPV at enrolment had higher risk of CIN3 or worse than the women positive by Pap after 3 years (5.0% vs 3.8%, $p=0.046$) and 5 years (7.6% vs 4.7%, $p=0.001$), and women negative for HPV at enrolment had lower risk of CIN3 or worse than women negative by Pap at enrolment after 3 years (0.063% vs 0.17%, $p=0.001$) and after 5 years (0.17% vs 0.36%, $p=0.02$). (B) A positive Pap test strongly modified risks for positive HPV at 3 years (10.0% vs 3.1%, $p<0.0001$) and 5 years (12.1% vs 5.9%, $p<0.0001$), but not for negative HPV either at 3 years (0.53% vs 0.047%, $p<0.0001$) or 5 years (0.86% vs 0.16%, $p=0.004$), although risks are statistically distinguishable. Comparing with A, also being negative by Pap did not reduce risk of CIN3 or worse from just being negative for HPV either at 3 years (0.047% vs 0.063%, $p=0.6$) or 5 years (0.16% vs 0.17%, $p=0.8$). The webappendix (pp 3–6) lists the 95% CIs for all risk estimates. HPV=human papillomavirus. CIN3=cervical intraepithelial neoplasia grade 3.

incidence, which make smoother and more accurate estimates, when the Weibull model holds, than non-parametric methods analogous to Kaplan-Meier.³⁸ We fitted separate Weibull models to each co-test result. We also used Weibull regression models to assess if age and history of abnormal Pap smears or CIN2 or worse biopsies affect cumulative incidence from enrolment and, in women who tested negative by both HPV and Pap testing at enrolment, from the second screening visit. The webappendix (pp 1–2) gives additional details of our statistical methods.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Intramural Research Program of the US National Institutes of Health and National Cancer Institute reviewed the final manuscript for

publication. The KPNC institutional review board (IRB) approved use of the data, and the National Institutes of Health Office of Human Subjects Research deemed this study exempt from IRB review. HAK and PEC had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We established the risk of CIN3 or cervical cancer for 331818 women aged 30 and older who enrolled in co-testing at KPNC (table 1). At enrolment, testing positive for HPV was slightly more common than having abnormal cytology (ASC-US or worse; 5.1% vs 3.8%, $p<0.0001$) and testing for HPV had slightly less specificity than cytology (95.5% vs 96.5%, $p<0.0001$; table 1). However, higher percentages of disease outcomes (sensitivities) were identified in the women positive for HPV at enrolment than in women positive by Pap for CIN2 (78% vs 53%, $p<0.0001$), CIN3 or adenocarcinoma in situ (84% vs 53%, $p<0.0001$), adenocarcinoma in situ (80% vs 40%, $p<0.0001$), total cancers (69% vs 51%, $p=0.02$), and adenocarcinoma (78% vs 15%, $p<0.0001$; table 1). Our comparison of discordant co-test results (negative HPV and positive Pap vs positive HPV and negative Pap) shows the relative importance of testing for HPV versus cytology. We identified higher percentages of disease outcomes in women who were positive by HPV and negative by Pap than women who were negative by HPV and positive by Pap for CIN2 (29% vs 4%, $p<0.0001$), CIN3 or adenocarcinoma in situ (35% vs 4%, $p<0.0001$), adenocarcinoma in situ (44% vs 4%, $p<0.0001$), total cancers (29% vs 10%, $p=0.004$), and especially adenocarcinoma (63% vs 0%, $p<0.0001$; table 1).

Figure 1A and the webappendix (pp 3–6) show that prevalent CIN3 or worse risks at enrolment were similar in women who were positive by HPV and Pap testing (2.1% vs 2.7%). However, enrolment HPV testing distinguished future risk of CIN3 or worse and cancer more clearly than enrolment cytology. Risk of CIN3 or worse was less in all women negative for HPV than in all women negative by Pap, not only for 3 years (0.063% vs 0.17%, $p=0.001$), but also for 5 years (0.17% vs 0.36%, $p=0.02$; figure 1). Women negative for HPV also had half the 5-year risk of invasive cervical cancer of women negative by Pap (3.8 vs 7.5 per 100000 women per year; $p=0.3$). Conversely, risk of CIN3 or worse was greater in women positive for HPV than women positive by Pap, not only for 3 years (5.0% vs 3.8%, $p=0.046$), but also for 5 years (7.6% vs 4.7%, $p=0.001$; figure 1).

Figure 1B and the webappendix (pp 3–6) shows that Pap smears further distinguished risk of CIN3 and cancer in women positive for HPV, but not women negative for HPV. Abnormal cytology substantially increased the risk of CIN3 or worse for all women positive for HPV not only for 3 years (10.0% vs 3.1%, $p<0.0001$) but also for 5 years (12.1% vs 5.9%, $p<0.0001$; figure 1).

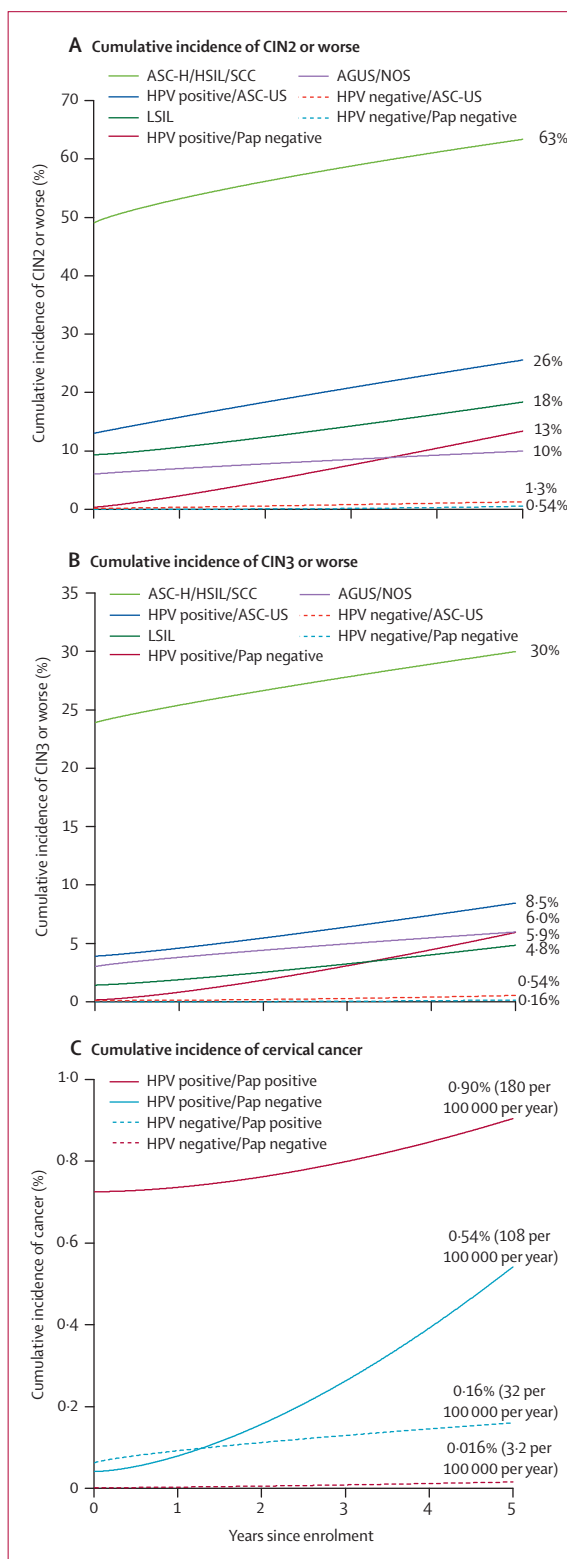
By contrast, although statistically significant, abnormal cytology did not increase the risk of CIN3 or worse for women negative for HPV to a substantial level, neither for 3 years (0.53% vs 0.047%, $p < 0.0001$) nor for 5 years (0.86% vs 0.16%, $p = 0.004$; figure 1). Furthermore, normal cytology did not further reduce the already low cancer risk of women negative for HPV (3.8 and 3.2 per 100 000 women per year, $p = 0.8$).

Figure 2 and the webappendix (pp 3–6) combine finely categorised enrolment co-test results to estimate risk of CIN2 or worse, CIN3 or worse, and cancer. The risks conferred by co-testing results clustered into high, low, and medium risk categories. The highest 3-year risks of CIN2 or worse (59%), CIN3 or worse (28%), and cancer (4%) were in the small group of 833 (0.25%) women with ASC-H, HSIL, or SCC Pap smears, 672 (81%) of whom were positive for HPV. Conversely, the 313 465 women (94.5%) with HPV negative/ASC-US or negative by HPV and Pap co-tests had the lowest 5-year risks of CIN2 or worse, CIN3 or worse, and cancer. The remaining 18 353 co-test results (5.25%) had intermediate risk: LSIL or AGUS/NOS (irrespective of HPV results), negative for HPV and positive by Pap, HPV positive/ASC-US. Women who were positive for HPV but negative by Pap testing, despite having the lowest prevalent disease risks at enrolment of any group positive for HPV, accrued 5-year risk of CIN2 or worse, CIN3 or worse, and cancer post-enrolment as quickly as women with ASC-H/HSIL/SCC (figure 2).

Figure 2: 5-year cumulative risks of CIN2 or worse (A), CIN3 or worse (B), and cervical cancer (C) by enrolment HPV test and finely-categorised enrolment Pap smears

Prevalent disease is plotted at time 0 (enrolment) and incident disease is plotted from that point. Although only 0.25% of women, women with ASC-H/HSIL/SCC Pap smears had by far the highest 3-year risks of CIN2 or worse (59%), CIN3 or worse (28%), and cancer (4.1%). Versus women with LSIL, women with HPV positive/ASC-US had higher 3-year risk of CIN2 or worse (21% vs 14%, $p = 0.01$) and CIN3 or worse (6.4% vs 3.2%, $p = 0.03$). Most women positive for HPV at enrolment (73%) were negative by Pap, and although they had by far the lowest disease risks at enrolment of any of the group positive for HPV (CIN2 or worse 0.36%, CIN3 or worse 0.16%, cancer 0.04%), they accrued 5-year risks post enrolment (CIN2 or worse 13%, CIN3 or worse 5.8%, cancer 0.5%) akin to women with ASC-H/HSIL/SCC (CIN2 or worse 14%, CIN3 or worse 6.1%, cancer 1.7%) or with HPV positive/ASC-US (CIN2 or worse 13%, CIN3 or worse 3.9%, cancer 0%; $p = 0.8$ vs ASC-H/HSIL/SCC, $p = 0.7$ vs HPV positive/ASC-US). Women positive for HPV but negative by Pap had higher 5-year cancer risk than women negative for HPV but positive by Pap (0.54% vs 0.16%, $p = 0.2$). HPV negative/ASC-US and women negative by both HPV and Pap testing had the smallest, and nearly indistinguishable, 5-year risks of CIN2 or worse (1.3% vs 0.54%, $p = 0.07$) and CIN3 or worse (0.54% vs 0.16%, $p = 0.08$). The 5-year cervical cancer risk in women who were negative by both HPV and Pap testing at enrolment was 0.016% (3.2 per 100 000 women per year), only slightly smaller than the 0.019% (3.8 per 100 000 per year) risk in the group negative for HPV at enrolment overall ($p = 0.8$). By comparison, the 5-year cervical cancer risk in women negative by Pap was 0.037% (7.5 per 100 000 per year; $p = 0.3$ vs cancer risk in women negative for HPV). On the per 100 000 women per year scale, the 5-year cancer risks are 3.2 for women negative by both HPV and Pap; 3.2 for women negative for HPV by positive by Pap; 108 for women positive for HPV but negative by Pap; 180 for women positive by both HPV and Pap testing. The webappendix (pp 3–6) lists the 95% CIs for all risk estimates. HPV=test for human papillomavirus. CIN2=cervical intraepithelial neoplasia grade 2. CIN3=cervical intraepithelial neoplasia grade 3.

The webappendix (p 7) shows that some risks of CIN3 or worse were strongly modified by previous history of abnormalities and by age. Most notably, the already low



	Total women	No biopsy or <CIN2	CIN2	CIN3/AIS	AIS	Squamous carcinoma	Adenocarcinoma	Total cancers
Total	195 975 (100%)	195 629 (100%)	244 (100%)	89 (100%)	10 (100%)	7 (100%)	5 (100%)	13 (100%)
Second visit HPV								
Negative	190 594 (97.3%)	190 523 (97.4%)	49 (20%)	16 (18%)	4 (40%)	2 (29%)	3 (60%)	6 (46%)
Positive	5381 (2.8%)	5106 (2.6%)	195 (80%)	73 (82%)	6 (60%)	5 (71%)	2 (40%)	7 (54%)
Second visit Pap								
Pap negative	187 522 (95.7%)	187 423 (95.8%)	67 (27%)	27 (30%)	7 (70%)	1 (14%)	3 (60%)	5 (38%)
Total Pap positive	8453 (4.3%)	8206 (4.2%)	177 (73%)	62 (70%)	3 (30%)	6 (86%)	2 (40%)	8 (62%)
ASC-US	6698 (3.4%)	6586 (3.4%)	92 (38%)	19 (21%)	1 (10%)	1 (14%)	0 (0%)	1 (8%)
LSIL	933 (0.48%)	864 (0.44%)	55 (23%)	13 (15%)	0 (0%)	1 (14%)	0 (0%)	1 (8%)
AGUS/NOS	531 (0.27%)	517 (0.26%)	4 (2%)	7 (8%)	1 (10%)	1 (14%)	2 (40%)	3 (23%)
ASC-H/HSIL/SCC	291 (0.15%)	239 (0.12%)	26 (11%)	23 (26%)	1 (10%)	3 (43%)	0 (0%)	3 (23%)
Second visit HPV/Pap								
HPV negative/Pap negative	184 197 (94.0%)	184 157 (94.1%)	24 (10%)	12 (13%)	4 (40%)	1 (14%)	2 (40%)	4 (31%)
HPV negative/Pap positive	6397 (3.3%)	6366 (3.3%)	25 (10%)	4 (4%)	0 (0%)	1 (14%)	1 (20%)	2 (15%)
HPV positive/Pap negative	3325 (1.7%)	3266 (1.7%)	43 (18%)	15 (17%)	3 (30%)	0 (0%)	1 (20%)	1 (8%)
HPV positive/Pap positive	2056 (1.0%)	1840 (0.94%)	152 (62%)	58 (65%)	3 (30%)	5 (71%)	1 (20%)	6 (46%)
Second visit HPV/ASC-US								
HPV negative/ASC-US	5428 (2.8%)	5416 (2.8%)	10 (4%)	1 (1%)	0 (0%)	1 (14%)	0 (0%)	1 (8%)
HPV positive/ASC-US	1270 (0.7%)	1170 (0.6%)	82 (34%)	18 (20%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)

Total cancers includes squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and cervical cancer of unknown histology. CIN3/AIS includes one histology that were either CIN3 or AIS, but precisely which is unknown. Pap positive is ASC-US or worse cytology. CIN2=cervical intraepithelial neoplasia grade 2. CIN3/AIS=cervical intraepithelial neoplasia grade 3 or adenocarcinoma in situ. HPV=human papillomavirus test. ASC-US=atypical squamous-cells of undetermined significance. LSIL=low-grade squamous intraepithelial lesion. AGUS/NOS=atypical glandular cells of undetermined significance or not otherwise specified. ASC-H=atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion. HSIL=high-grade squamous intraepithelial lesion. SCC=squamous cell carcinoma.

Table 2: Distribution of worst histological diagnosis since the second HPV test and Pap smear in women negative for both HPV and cytology at enrolment

risk of CIN3 or worse in women negative by HPV and Pap testing at enrolment was further reduced for women with no history of a previous abnormal Pap smear (hazard ratio [HR] 0.21, 95% CI 0.13–0.49; p=0.0002) and for women aged 50 years and older compared with women aged 30–34 years (HR 0.22, 0.11–0.32; p<0.0001).

Screening for cervical cancer is a repetitive process, in which women that tested normal in previous screens are re-screened after a specified interval. We also assessed the risk of CIN3 or cervical cancer after the second screening visit in 195 975 women who were negative by both HPV and Pap testing at enrolment (table 2), to establish whether the second co-test provided additional reassurance against cancer. Table 2, figure 3, and the webappendix (pp 8–11) show raw data and disease risks for the 195 975 women who were negative by both HPV and Pap testing at enrolment and returned for a second co-test (median of 2.9 years to return, IQR 2.3–3.2). The proportion of positive tests for HPV at the second screen was half the proportion at enrolment (2.8% vs 5.1%, p<0.0001; table 1 and table 2). The increase in the fraction of women positive by Pap (4.3% vs 3.8%, p<0.0001) was almost entirely due to an increase in HPV negative/ASC-US (2.8% vs 2.0%, p<0.0001), the lowest-risk Pap-positive co-test. Most importantly, the distribution of the second co-test results was down-staged in severity from the distribution of enrolment co-tests: the total fraction of women negative by both HPV and Pap testing

or HPV negative/ASC-US increased (96.8% vs 94.5%, p<0.0001), the fraction of women positive by HPV but negative by Pap was halved (1.7% vs 3.7%, p<0.0001), and the fraction HPV positive/ASC-US, LSIL, AGUS/NOS, or ASC-H/HSIL/SCC decreased (1.5% vs 1.9%, p<0.0001).

Moreover, the risks of CIN3 or worse in the years after positive screening tests at the second visit were lower than after positive screening tests at enrolment (figure 1A and figure 3A). Women who were positive for HPV at the second co-test after negative tests by both HPV and Pap at enrolment had notably reduced 3-year risk of CIN3 or worse than women who were positive for HPV at enrolment (3.0% vs 5.0%, p=0.09); we noted a similar pattern in women positive by Pap (1.3% vs 3.8%, p=0.04). The 3-year risks were also lower for women positive by both HPV and Pap (5.1% vs 10.0%, p=0.3), positive for HPV but negative by Pap (1.8% vs 3.1%, p=0.3), and negative by HPV and positive by Pap co-tests (0.19% vs 0.53%, p=0.4) at second co-test after being negative by both HPV and Pap at enrolment than the respective risk of the same screening combinations at enrolment.

However, at the return visit after an enrolment negative co-test (figure 1B and figure 3B), 3-year risks of CIN3 or worse were not lower for women testing negative for HPV again (0.082% vs 0.063%, p=0.6), negative by Pap again (0.15% vs 0.17%, p=0.8), or negative by both HPV and Pap again (0.079% vs 0.047%, p=0.5). The risk of cancer in the

subsequent 3 years was also no lower in women who re-tested negative by both HPV and Pap (2.7 vs 3.0 per 100 000 women per year, $p=0.9$; webappendix pp 8–11).

Discussion

The KPNC co-testing programme provided us with an opportunity to assess the real-world clinical effectiveness of concurrent testing for HPV with cytology in a large and diverse US screening population followed up for several years. Women testing negative for HPV had low risk of CIN3 or cancer over 5 years, irrespective of normal cytology or minor abnormalities. Although women positive for HPV with cytological abnormalities had the highest risks, women positive for HPV with normal cytology accrued substantial risk of CIN3 or cancer over 5 years. Co-testing was less able to identify women at high risk at their second visit at about 3 years after an enrolment test negative by both HPV and Pap. Their co-test results were down-staged to safer co-tests and the risk of CIN3 or worse for each possible second co-test result was generally diminished from the risk associated with that co-test result at enrolment.

Women negative by both HPV and Pap at enrolment had a low risk of cervical cancer of 3.2 per 100 000 women per year over 5 years. This risk is similar to the risk of vulvar cancer in northern California in women aged 30 years and older (3.1 per 100 000 women per year; SEER 2003–07), another potentially preventable cancer that is too rare to justify organised prevention. This finding supports lengthening the screening interval for women negative by both HPV and Pap to 5 years, as has already been done in many European countries.³⁹ The substantially lower risks of CIN3 or worse in women negative by both HPV and Pap aged 50 years or older (vs women aged 30–34 years), or for women negative by both HPV and Pap with no history of previous abnormal cytology, raise the possibility that some subgroups of these women could be safely screened at even longer intervals.

A single negative test for HPV was sufficient to reassure a woman of extremely low risk of CIN3 or cancer for 5 years. We identified that negative cytology provided no extra reassurance against cancer beyond that conferred by a negative HPV test result. The practically equal 5-year risks of CIN3 or cancer for women negative by both HPV and Pap and for women that are HPV negative/ASC-US suggests that women in this latter group could be safely screened in routine clinical practice at the same extended interval as women negative by both HPV and Pap.^{40–42} Our findings are consonant with the biological fact that carcinogenic HPV is involved in almost every cervical cancer. Finally, the low yields of LSIL or worse Pap smear (0.5%) and ASC-H/HSIL/SCC Pap smears (0.05%) in women negative for HPV might not be sufficient to justify Pap smear tests for women negative for HPV. Our findings strongly suggest that primary HPV testing, with a

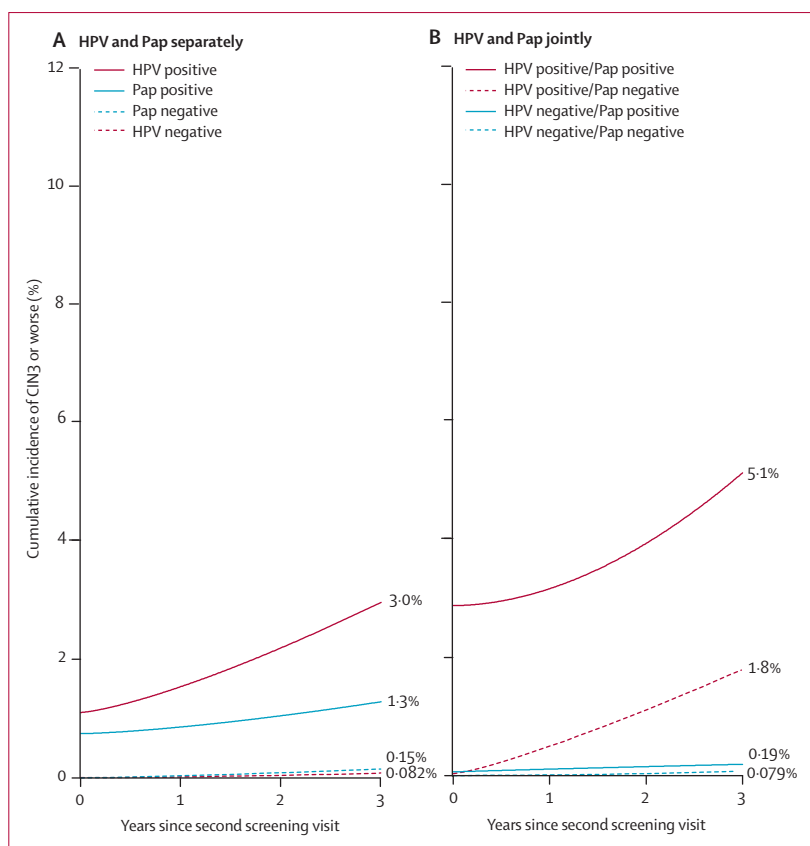


Figure 3: Cumulative incidence of CIN3 or worse after second visit in women negative by HPV and Pap co-test at enrolment, by second HPV test and second Pap test separately (A) and jointly (B)

Data are shown on cumulative incidence of CIN3 or worse based on HPV test and Pap smear at the second screen, in women negative for both tests at enrolment, for HPV test and Pap smear separately (A), and jointly (B). Prevalent CIN3 or worse is plotted at time 0 (second visit) and incident CIN3 or worse is plotted from that point. The y-axis goes to 12%, the maximum risk recorded based on enrolment co-tests (figure 1), to emphasise that CIN3 or worse risks for women positive for either test after negative tests at enrolment are decreased from women positive for either test at enrolment. Pap positive is atypical squamous cells of undetermined significance or worse. (A) Women positive for HPV at their return visit had higher 3-year risk of CIN3 or worse than women positive by Pap at their return visit (3.0% vs 1.3%, $p=0.2$) and women negative for HPV at their return visit had half the 3-year risk of CIN3 or worse of women negative by Pap at their return visit (0.082% vs 0.15%, $p=0.3$). Neither is statistically significant since there were only 102 CIN3 or worse at or after the second screening visit in women negative for both tests at enrolment. (B) Being positive by Pap modified risks more for the women positive for HPV (5.1% vs 1.8%, $p=0.4$) than for women negative for HPV (0.19% vs 0.079%, $p=0.4$), although neither is statistically significant. The webappendix (pp 8–11) lists the 95% CIs for all risk estimates. CIN2=cervical intraepithelial neoplasia grade 2. CIN3=cervical intraepithelial neoplasia grade 3. HPV=human papillomavirus.

positive test for HPV triaged by cytology (or other tests with high specificity), a strategy that might preserve nearly all the safety of co-testing while reducing the number of Pap tests by 95% in our population, could be more efficient than co-testing—as has been suggested by others.^{5,13,39,43}

Although cytological abnormalities suggested prevalent disease, testing for HPV predicted future disease much better than cytology. For example, women positive for HPV but negative by Pap, who were the majority of women positive for HPV (73%), had low prevalent disease risk at enrolment (in part because these women were rarely sent for immediate colposcopy), yet accrued substantial incident disease risks over 5 years that were similar to

For the SEER database see <http://seer.cancer.gov/>

Panel: Research in context**Systematic review**

Evidence from long-term prospective cohorts and randomised clinical trials shows that incorporating testing for HPV into cervical cancer screening programmes could reduce cervical cancer incidence in women 30 years and older^{4-5,8,10} and even cervical cancer mortality.¹² Cohorts and trials suggest that women's risk of cervical intraepithelial neoplasia grade 3 (CIN3) or cancer after a negative HPV test is very low for 5 years,^{3,10,13,14} although these studies were too small to reliably estimate risk of cancer itself. This evidence was the basis for regulatory and clinical guideline approval^{15,16} of routine HPV testing in conjunction with cervical cytology (co-testing) for cervical cancer screening of women 30 years and older. In particular, the guidelines discourage screening of women testing negative for HPV with normal cytology before 3 years to avoid detection of new infections with HPV because their cancer risk is probably low. Although very promising, co-testing has not been widely adopted in the USA. In a recent survey, less than a fifth of US clinicians would recommend the 3-year screening interval for women testing negative for HPV with normal cytology.¹⁸ These facts suggested to us that there remains serious concern about the safety of co-testing guidelines against cancer in real-life clinical practice. To address this issue, we collaborated with a health-care provider that serves a population large enough to directly estimate cancer risks for even the lowest-risk women undergoing co-testing.

Interpretation

Previous clinical trials and research cohorts showed that HPV testing can prevent CIN3 or worse in tightly-controlled, research settings, but it remained unclear if HPV testing could prevent cancer in a real-life clinical setting. Our findings showed that women testing negative for HPV had extremely low risk of developing cervical cancer over 5 years, so low that it was similar to their risk of developing vulvar cancer, a cancer that is thought too rare to justify screening. Therefore, the 3-year screening interval for women testing negative for HPV with a normal Pap test is safe in routine clinical practice. Furthermore, testing positive for HPV identified more women at enrolment who developed cervical cancer, and especially cervical adenocarcinoma, an uncommon but particularly lethal form of cervical cancer whose precursors are poorly-identified by Pap tests. The earlier identification of these women by HPV testing can facilitate earlier treatment and closer monitoring of high-risk women. In particular, women positive for HPV with normal cytology accrued substantial risk of cancer over 5 years and require stringent follow-up.

women with ASC-H/HSIL/SCC cytology. Furthermore, 17 of the 27 adenocarcinomas were identified in women positive for HPV but negative by Pap. Follow-up of women positive for HPV but negative by Pap must strike the difficult balance of being stringent yet avoiding excessive early intervention.^{44,45} Thus, it is imperative to develop new biomarkers (such as HPV genotyping^{42,46} or P16-INK4a/Ki67 immunocytochemistry⁴⁷) to better triage women positive for HPV but negative by Pap into finer risk categories,⁴⁸ especially risk of adenocarcinoma, whose incidence is on the rise in the USA.⁴⁹

We expected that the ability of co-testing to separate high-risk women from low-risk women would diminish at the second co-test in women negative by both HPV and Pap co-test at enrolment because there are always fewer high-risk women with prevalent CIN3 or worse at the second and subsequent screens. Therefore, women positive for HPV at the second co-test, about 3 years after a negative HPV and Pap test at enrolment, had lower risk of CIN3 or worse and cancer risks than women testing

positive for HPV at enrolment. By contrast, a second consecutive negative test for HPV and Pap (at a median of 2·9 years after the first) seemed to offer no detectable additional reassurance against CIN3 or cervical cancer than the first negative co-test for HPV and Pap. Our findings suggest that the common practice of yearly HPV testing for women negative for HPV just to be sure¹⁸ is unnecessary and support guidelines¹⁵⁻¹⁶ that women negative for both HPV and Pap testing should not be re-screened before at least 3 years.

Although KPNC practices are not typical in many ways, because KPNC serves a large and diverse population involving providers without special qualifications or special training to participate, we believe that the KPNC experience serves as a large-scale demonstration project of what could realistically be achieved in real-life clinical practice. Consequently, non-compliance by clinicians or patients to protocols, imperfect diagnostic testing, and other imperfections are real-life complexities that should be incorporated into our risk estimates. The value of our study is to reassure women, clinicians, and screening guidelines committees that the benefits of testing for HPV identified in clinical trials and research cohorts, which might not represent routine clinical practice in a general population, are indeed noted in general practice with all its attendant complexities.

However, the KPNC experience might be difficult to apply to substantially different screening programmes. For example, most women at KPNC with abnormal cytology were referred for colposcopy at enrolment, but almost three-quarters of women positive for HPV had normal cytology and few of them were referred for colposcopy at enrolment. Thus, an alternate screening programme that sends more women positive for HPV but negative by Pap to colposcopy would probably report greater risk of CIN3 or worse at enrolment than we did in KPNC. For another example, although we recorded low 5-year cancer rates in women negative by both HPV and Pap testing, a programme that actually implements a 5-year screening interval for these women would record a slightly higher cancer rate than that noted in KPNC because the second co-test at 3 years, although having little effect, still excised the few CIN2 or CIN3 lesions that could have progressed to cancer in 2 years. For a final example, the cancer risk for all women negative for HPV in a primary HPV testing programme, where all women negative for HPV have extended screening intervals, should be somewhat higher than the cancer risk in all women negative for HPV in KPNC, because KPNC referred the 0·5% of women negative for HPV with LSIL or worse abnormalities for colposcopy where excision of CIN2 or CIN3 lesions might have prevented a few future cancers.

In summary, our findings show that adding HPV testing to cytology screening promoted earlier identification of the women at high risk of cervical cancer (especially adenocarcinoma) and allowed safe 3-year screening intervals for women negative by both HPV and

Pap testing that reduced the burden of screening on patients and clinicians. Furthermore, our findings suggest that 5-year screening intervals for women negative by both HPV and Pap testing might be safe and that HPV testing without adjunctive cytology might be sufficiently sensitive for primary cervical cancer screening. The results of co-testing in 330 000 women over 5 years at KPNC definitively shows that concurrent HPV testing and cytology can be feasibly implemented in routine clinical practice to provide powerful prevention of cervical cancer (panel).

Contributors

HAK, WKK, and PEC contributed to study design. HAK, WKK, BF, MS, SW, and PEC contributed to drafting. BF, TL, NEP, and FD collected, arranged, cleaned, and managed the data. HAK, LC, and PEC contributed to the statistical analysis. All authors contributed to revisions of the manuscript.

Conflicts of interest

MS and PEC have worked with Qiagen Inc on independent evaluations of non-commercial uses of CareHPV (a low-cost HPV test for low-resource regions) for which they have received research reagents and technical aid from Qiagen for free. The other authors declare no conflicts of interest.

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