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Neither One-Time Negative Screening Tests nor Negative Colposcopy Provides Absolute Reassurance against Cervical Cancer

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Abstract

A population sample of 10,049 women living in Guanacaste, Costa Rica was recruited into a natural history of human papillomavirus (HPV) and cervical neoplasia study in 1993–4. At the enrollment visit, we applied multiple state-of-the-art cervical cancer screening methods to detect prevalent cervical cancer and to prevent subsequent cervical cancers by the timely detection and treatment of precancerous lesions. Women were screened at enrollment with 3 kinds of cytology (often reviewed by more than one pathologist), visual inspection, and Cervicography. Any positive screening test led to colposcopic referral and biopsy and/or excisional treatment of CIN2 or worse. We retrospectively tested stored specimens with an early HPV test (Hybrid Capture Tube Test) and for >40 HPV genotypes using a research PCR assay. We followed women typically 5–7 years and some up to 11 years. Nonetheless, sixteen cases of invasive cervical cancer were diagnosed during follow-up. Six cancer cases were failures at enrollment to detect abnormalities by cytology screening; three of the six were also negative at enrollment by sensitive HPV DNA testing. Seven cancers represent failures of colposcopy to diagnose cancer or a precancerous lesion in screen-positive women. Finally, three cases arose despite attempted excisional treatment of precancerous lesions. Based on this evidence, we suggest that no current secondary cervical cancer prevention technologies applied once in a previously under-screened population is likely to be 100% efficacious in preventing incident diagnoses of invasive cervical cancer.

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Introduction

It is well-recognized that cytology-based programs reduce the burden of cervical cancer in developed countries. The incidence of cervical cancer has fallen by 50% or more since introduction of Pap smear screening for cervical cancer detection in developed countries (1;2). However, a single cervical cytology test is insensitive for the detection of precancer and cancer of the cervix (3;4), and cytology testing must be repeated frequently to achieve programmatic effectiveness (1). A more efficient and accurate screen could extend the reach of screening to many regions in great need of cervical cancer prevention programs (5).

Based on the central role of persistent, carcinogenic human papillomavirus (HPV) in cervical carcinogenesis, testing for carcinogenic HPV has been introduced recently into cervical cancer screening. Compared with cytology, carcinogenic HPV testing has proven to be more reliable than cytology (6;7) and to have greater sensitivity for detection of cervical precancer (cervical intraepithelial neoplasia grade 3 [CIN3]) and cancer (4;8–11). In the U.S., carcinogenic HPV testing with cytology is approved for primary screening of women aged 30 years and older (12), who are past the peak of self-limited infections (13). Women aged 30 years and older who test negative for carcinogenic HPV and are cytologically normal are at an extremely low risk for incipient precancer and cancer for the subsequent 10 years or more (14;15), permitting an extension of the screening interval to at least 3 years. A recent publication by the International Agency for Research on Cancer, based on a meeting of experts, concluded that HPV testing is an acceptable alternative to Pap smears/cervical cytology for cervical cancer screening (2).

The success of cytology screening and the advent of a more sensitive and reliable molecular testing for HPV could lead to the perception that cervical cancer is entirely preventable by detecting and treating all cases of CIN3 and CIN2 (equivocal precancer), especially where resources are available to combine tests. Even if combinations of tests that are 100% sensitive for identifying ≥CIN3 are not perfectly specific. Because many women test positive but do not have ≥CIN3, diagnosis by colposcopy and biopsy still has an important role in distinguishing those women with precancerous lesions from those with benign HPV infections. However, the sensitivity of colposcopy and colposcopically-directed biopsies for detection of precancerous and cancerous lesions, especially small precancerous lesions, has recently been questioned (16–18).

With the advent of HPV testing and liquid-based cytology, it is uncertain how sensitive we can make a single round of screening and colposcopy referral especially for women without good prior screening coverage. In the beginning of our decade-long cohort study in Guanacaste, Costa Rica, we hoped that we could provide nearly absolute reassurance against cervical cancer by combining multiple state-of-the-art screening tests, colposcopy, biopsies, and excisional treatment of all lesions diagnosed as CIN2 or worse. While the screening did detect and lead to treatment of many precancerous lesions at baseline and during follow-up and did identify women with early cancers at enrollment (19;20), some women who did not screen positive developed cancer during follow-up. Here, we present clinical patterns of incidently-detected cancer diagnoses in the context of multi-modality screening and colposcopy.

Methods

Study Population

The Proyecto Guanacaste Epidemiólogico (PEG) is a population-based cohort study begun in 1993 to study the natural history of HPV and cervical neoplasia and the performance of alternative screening methods. Detailed methods of this NCI and local IRB-approved

population-based study in Guanacaste, Costa Rica have been reported elsewhere (21;22). From a random sample of census tracts of this mainly rural population (240,000 inhabitants), 11,742 potentially eligible subjects aged 18 years and older were identified, 10,738 women were eligible and invited to participate, and 10,049 women (94% of eligible women) agreed to visit one of our study clinics. After excluding 583 virgins, 291 women who either refused or could not have a pelvic exam, 630 hysterectomized women, and 290 women who were treated and censored for possible high-grade cervical neoplasia detected at enrollment, 8,255 women were included in the main analytic cohort and followed for up to 11 years. There were 7,450 (90% of 8,255) women who had at least one follow-up visit.

At enrollment (21;22), women underwent a pelvic examination by a small team of experienced nurses; the nurses noted any women with a visual diagnosis of possible cancer. Cervical cells were collected using a Cervex broom. After preparation of a conventional Pap smear, the residual cells were placed into PreservCyt for semi-automated liquid-based cytology (ThinPrep, Hologic Corporation (formerly Cytyc)), Boxborough, MA). The conventional Pap was interpreted by both a Costa Rican expert cytopathologist and by a U.S. pathologist using a Food and Drug Administration (FDA)-approved automated technology (PapNet) that located worst cells and cell clusters. ThinPrep slides were prepared initially and read in the U.S., but the task of preparing slides was transferred gradually to Costa Rica after which time two readings (Costa Rica and U.S.) were produced. The threshold for abnormality was atypical squamous cells of undetermined significance (ASC) or worse (≥ASC). The cervix was sampled again using a Dacron swab and the cells were placed into specimen transport medium (STM; Qiagen (formerly Digene), Gaithersburg, MD, USA) for HPV testing. The STM specimen was initially tested using then-current, FDA-approved technology (Hybrid Capture Tube Test [HCT], Qiagen Corporation (formerly Digene), with a detection threshold of 10 pg HPV DNA/mL). Finally, Cervigrams (National Testing Laboratories, Fenton, MO) were taken and interpreted in the U.S., with a referral threshold of P1 (low-grade) or worse diagnosis. A standardized questionnaire on demographics and cervical cancer risk factors was administered by an interviewer at each visit.

Women with any positive screening test except HCT were sent to colposcopy, where a single gynecologic oncologist (trained in colposcopy in Costa Rica and the U.S., and trained in LEEP in the U.S.) biopsied and/or treated for suspicious lesions. All women with histologic CIN2 or worse (≥CIN2) were treated by excision of the cervical lesion. Women with preliminary evidence suggesting ≥CIN2, but without confirmatory histology received individual care and referred for further follow-up by the national health care system. The remaining women without evidence of ≥CIN2 were assigned to follow-up groups based on their perceived risk of developing high-grade precursors or cancer as informed by their enrollment testing results or lifetime number of sexual partners, with more intensive follow-up (with screening every 6 or 12 months) assigned to those at the greatest risk, having 5 or more sexual partners, or because they were selected in 2% random sample of the lower-risk population as described in detail elsewhere (22).Otherwise, the lower-risk population was screened a second time during years 5–7 of follow-up, approximately 1/3 of this subgroup evaluated each year. Throughout the study, women with cytologic, visual, or Cervigram evidence of ≥CIN2 were referred to colposcopy. Follow-up was typically 5–7 years following enrollment but some were seen up to 11 years either for safety reasons and/or recruited to participate in ancillary studies of sub-populations.

At the last visit attended by 6,798 (82.4%) of the women in the cohort, the colposcopic referral criteria, relaxed for additional safety, were as follows: 1) any ≥ASC cytologic interpretation; 2) a positive Cervigram (P0 or P1) in either of the last two screening visits; or 3) persistent infection by a carcinogenic HPV genotype (HPV16, 18, 31, 33, 35, 39, 45, 51,

52, 56, 58, 59, 68) or 4) HPV 16 or HPV 18 at either of the last two screening visits. Finally, a 6.25% random sample of the cohort was referred for an exit colposcopy.

HPV DNA Detection

To genotype the stored enrollment STM specimens retrospectively, we used a MY09/M11 L1 degenerate primer PCR method for amplification of HPV DNA and PCR products were typed using dot blot hybridization for 48 types as previous described (23;24). Replicate testing was done on select specimens to adjudicate seemingly equivocal test results; a listing of results from HPV testing by PCR, HCT, and/or Hybrid Capture 2 (hc2; Qiagen, Corporation) by visit for each follow-up cancer case are presented in the Supplemental Online Table.

Pathology

Histologic specimens from biopsies, loop electrosurgical excision procedures (LEEPs), and hysterectomy underwent dual review by Costa Rican and U.S. pathologists; the final diagnosis was produced by an algorithm as previously described (22).

Statistics

Because we could not distinguish between cancers present but missed by the enrollment screen and truly incident cancer, we refer to the cases diagnosed after enrollment as “follow-up” cases to reflect our uncertainty regarding the origins of the cancer. We compared the follow-up cases of cancer to those cases diagnosed at enrollment and to the entire cohort of women not diagnosed with cancer for select enrollment characteristics, using a Kruskal-Wallis test (continuous) or Fisher's exact test (categorical) to test pair-wise for statistical significant ($p< 0.05$) differences. We primarily describe the enrollment characteristics of these three groups of participants because we hypothesized that it was the risk behaviors up to the time of enrollment that led to the development of cancer, and that the cancer or a precancerous lesion with invasive potential was already present at enrollment whether detected or not.

Results

Eighteen cervical cancers, all squamous cell carcinomas, were diagnosed during follow-up. Sixteen cases were newly diagnosed during follow-up and are the focus of this report; two other cases were excluded from this report because these cases appeared to be recurrent as chart review revealed that their original diagnosis of cancer occurred before enrollment. Thirteen cases were identified during follow-up in the cohort study and were confirmed histologically upon review; three were identified through linkage of patient social security number with the national cancer registry and were not confirmed histologically. Of the 16 cases, 10 cases were diagnosed among the sub-cohort of women in active follow-up (17,811 person-years or 5.6 cases per 10,000 person years) and 5 cases were diagnosed among the sub-cohort of women in passive follow-up (31,729 person-years or 1.6 cases per 10,000 person-years). The other case was diagnosed subsequent to a CIN3 diagnosis at enrollment (and therefore was not assigned to a follow-up sub-cohort) (6.3 person-years). Six of 15 cases for whom staging data were retrieved had stage 1A/AB, and five of 15 had died as of January of 2008.

Women who were diagnosed with cervical cancer during follow-up were similar in their enrollment age to women diagnosed with cancer at enrollment (n = 12) and women who were never diagnosed with cancer (n = 10,019) (Table 1). However, these follow-up cases tended to have earlier sexual debut, to have more sexual partners, and to be more parous than women diagnosed with cancer at enrollment and women without cancer at enrollment.

Cancer cases diagnosed during follow-up were less likely (non-significantly) than enrollment cases to test positive for carcinogenic HPV by HCT (37.5% vs. 63.6%, respectively) and retrospectively by PCR for HPV (62.5% vs. 90.9%, respectively). Follow-up cancers were less likely (non-significantly) than enrollment cases to have positive cytology by either cytologic method or review of the cytology. There was a non-significant difference in the age of diagnosis for follow-up cancer cases (median = 51.5 years, mean = 52.2 years) versus the enrollment cancer cases (median = 39.5 years; mean = 40.6 years) ($p = 0.06$). There were no significant differences between cancer groups for the use of oral contraceptives (ever/never) and smoking (ever/never). Interestingly, the age at sexual debut was correlated with the age of diagnosis for the follow-up cases (Spearman $\rho = 0.48$, $p = 0.05$), suggesting a relationship between the peak exposure to/prevalence of HPV that typically occur shortly after sexual debut (19;25) and the timing of cancer diagnoses.

Details about follow-up time and follow-up visits are shown in Table 2. Follow-up cancer cases and non-cases were followed for a similar duration and with similar number of visits.

The case histories of all 16 women (Patients A–P) diagnosed with cancer during follow-up are shown in Table 3; cases E, F, and M were identified through the national cancer registry. Five cases (excluding those who were treated during the enrollment phase) were passively followed (because there were no indicators of cervical abnormalities) and subsequently diagnosed with cancer at their exit visit 5–7 years later. Cases can be assigned to the following causes: I) six failures (Patients A–F) to detect abnormalities by enrollment cytology screening, three of which (Patients B, E, and F) also would have been completely missed by HPV DNA detection had HPV DNA detection been used for screening; II) seven failures of diagnosis by colposcopy (Patients G–M); and III) three recurrences of disease after excisional treatment for a diagnosis of a cervical precancerous lesions (Patients N–P); in two (Patient N and O) of the three patients , the LEEP was considered insufficient and margins were taken.

HPV16 infections caused six follow-up cases (37.5%). Interestingly, another five cases (31.3%) were caused by HPV18 (n = 4, 25.0%) or HPV45 (n = 1, 6.3%), the HPV genotype most closely related genetically to HPV18; 31.3% caused by HPV18 or HPV45 was non-significantly greater ($p = 0.09$, two-sided binomial test) than the expected percentage (17.4%) based on a recent meta-analysis of prevalent-detected cancers (26). By comparison, 50% of the enrollment cancer cases were HPV16 positive and 25% were HPV18 or HPV45 positive although the distribution was not significantly different from cases diagnosed during follow-up ($p = 0.9$). One case (6.3%) was due to HPV52 infection while another (Patient D) was probably due to HPV33 infection although the enrollment test was negative by PCR for this type. Three cases (Patient E, F, and J) tested HPV negative at all time points, despite repeated testing at each time point.

Discussion

Despite multiple screening modalities for detection, colposcopic evaluations, and treatment of ≥CIN2, more than 1 out of 1000 women followed in the cohort was still diagnosed with cervical cancer during follow-up. We observed that, for varied reasons described here, one round of even multi-modal screening (with rigorous follow-up of the screen positives and other high-risk participants) can not be used to take a region with previously poor screening to nearly zero risk of subsequent invasive cancer. We effectively detected and treated several hundred precancerous lesions (19;20), significantly lowering but not eliminating the cancer risk. In populations without a long history of widespread, effective screening, like Guanacaste at the start of our project, the CIN3 lesions were likely to be more advanced than those lesions found in well-screened populations and perhaps have a greater potential for

invasion (27;28) when missed. The median age of high-grade cervical neoplasia diagnosed at enrollment in Guanacaste was 34 years (29), approximately 5–10 years older than median age in the U.S., while the median age of sexual debut of 18 years in Guanacaste (Table 1) (when the population becomes "at risk") is similar to the estimated median age of 17–18 years in the U. S. (30).

In addition to describing the failures (cancers) of the screening program, it would be useful to estimate the effectiveness of the screening program by comparing the incidence rates of cancer in the cohort to the expected rates without screening, based on the rates of cancer in the other $5/6^{th}$ of the Guanacaste population not included in the cohort study. We are, unfortunately, not able to estimate the effectiveness of the screening program of cancer by comparing the incidence rates of cancer in the cohort to the expected rates without screening. We observed an approximate two-fold higher rate of cancers found within the cohort (12 in $1/6^{th}$ of the population) than in the remaining population (31 in $5/6^{th}$ of the population) based on the Guanacaste cancer registry during the enrollment phase. This paradoxical increase, rather than decrease, at enrollment within cohort is the consequence of improved screening and accelerated the detection of early cancers that would have been missed otherwise and only found later when they manifested symptoms. Thus, any calculation of the rate ratio (observed versus expected) is an underestimate of the effectiveness of the screening program: many cancers that could be prevented by screening the women not in the cohort would not be detected until after the end of cohort follow-up because their cancer diagnosis would be based on symptomatic manifestation, given the limitations of community-based screening in Guanacaste. We note, for example, that the median age of the screen-detected 12 cancer diagnoses within the cohort at enrollment was significantly younger than the median age of the 31 supplemental cases of cancer identified in the Guanacaste through the cancer registry(29) (39.5 years vs.59.0 years, respectively, $p = 0.004$ [Kruskal-Wallis]).

We have also observed a secular trend of reduced incidence of cervical cancer over the time period of the cohort (data not shown). This was presumably due to improved cytologic screening, as we introduced U.S.-quality liquid-based cytologic screening to the Guanacaste region, and timely management of the screen positives. We therefore were unable to estimate the expected number of cancers without intervention and the number of cancers averted within the cohort study.

Notably, there were no cases of adenocarcinoma (95% CI = 0.0%–20.6%), the precancerous lesion for which (adenocarcinoma *in situ*) generally is believed to be more commonly missed by screening programs than CIN3/CIS (31). It is unknown how common adenocarcinoma is relative to squamous cell carcinoma in general population of Costa Rica but presumably it is less so than in the U.S. and Europe, where its incidence is on the rise (32;33). Thus, it is uncertain whether finding no adenocarcinoma is unusual or not.

In retrospect, the most promising test for detection of cervical cancer would have been HPV PCR or an equivalently sensitive test, because in most but not all cancer cases, women were DNA positive for a carcinogenic HPV genotype years before cancer was detected. The next iteration of hybrid capture, hc2, resembles PCR testing in its clinical sensitivity (34) and is approximately 10–20% more sensitive for the detection of ≥CIN3 than HCT in use at the time of PEG enrollment (data not shown). As correlate of sensitivity, a negative HPV test therefore provides the most reassurance against cancer of all one-time screening methods. Yet, HPV testing using a well-validated research PCR assay failed to test positive repeatedly for two cases (Patient F and J) even at the time of diagnosis and two others (Patient B and D) tested positive for HPV sporadically.

Three limitations of our HPV screening bear mentioning. First, HCT, the predecessor and less sensitive version of the current FDA-approved test, hc2, was used to screen women at baseline. Use of a more sensitive test at baseline might have improved the risk stratification of the population, resulting in more women assigned to the active follow-up, and possibly detecting and censoring women with CIN3 prior to invasion. We used the retrospective testing by a well-validated research PCR assay to simulate how a very analytically sensitive test might have performed had it been available at the time of enrollment. Second, women were not routinely referred to colposcopy after testing positive for carcinogenic HPV by PCR, except during exit colposcopy where Patient J was referred to colposcopy due to the evidence of long-term HPV18 persistence that ultimately lead to cancer detection. Third, a Dacron swab, rather than the currently standard of cervical brush and Ayre's spatula or a cervical broom, was used to collect specimens for HPV testing. This choice may have led to less than optimal cervical sampling. As a consequence of the latter, the HPV testing used in this study may not have performed as sensitively as the current standards of performance.

Liquid-based cytology, read very sensitively (and non-specifically) by an U.S. expert reviewer (M.L.H.)(34), was negative repeatedly for one case (Patient L) and was negative until the time of cancer diagnosis for another (Patient D). Conventional cytology was less sensitive (and more specific) (34), albeit within the range observed in other studies (4;11;35;36), as were the nurse impression of possible cancer and Cervicography interpreted in the U.S. We have observed endemic levels of unexplained cervical inflammation and cervicitis in this population (37) (unpublished observations), which could have obfuscated precancerous lesions from detection.

It is particular noteworthy that half the incidently-detected cancers had a colposcopic evaluation called normal within two years of the diagnosis, suggesting that colposcopic evaluation missed either CIN3 about to invade or cancer itself. Given the insensitivity of colposcopy (16;38;39), we cannot determine with certainty whether these cases represented truly incident or prevalent cases of cancer missed at enrollment. Even when colposcopy found evidence of an abnormality, it did not always result in taking a biopsy of the most severe disease with resultant appropriate treatment and clinical management, as is the case of Patient G. In high-risk populations in which colposcopic evaluation is used for diagnosis, careful follow-up of colposcopically-evaluated women with <CIN2 is warranted if the resources permit. Without advances in colposcopy (17), it is unclear whether simply increasing the sensitivity of screening (e.g. including HPV testing) will lead to an equal decrease in the incidence of cancer.

There were also several examples of failed treatments. During the study, three patients were treated for precancerous lesions by LEEP, which has been shown to be 90–95% effective (40–42), prior to their cancer diagnosis. However, it is notable that these women were ages 39, 41, and 48 at the time of LEEP, perhaps indicating that they had larger, more advanced precancerous lesions in the cervical canal with a greater potential to invade (27) if the treatment was incomplete and the margins positive (n.b. two were). Based on recent research, careful post-treatment surveillance and follow-up using HPV DNA detection for sensitive identification of recurrence/failed treatment (40;43) might be warranted these high-risk, post-treatment women.

While there were no significant differences in the HPV genotype distribution of the cancers found at enrollment versus cancers found during follow-up, HPV18 and HPV45 together were somewhat more common in follow-up cases than what is expected based on a recent meta-analysis (26). Screening by cytology and diagnosis by colposcopy of HPV18-related precancerous lesions may not be as effective as for HPV16-related lesions. Patient L is a didactic example of elevated risk due to a long-term persistent HPV18 infection that was

largely missed by the classic screening methods for cervical cancer prevention, cytology and colposcopy.

It is important to recognize that cases of cervical cancer can arise as the consequences of the false negative test results and diagnostic procedures and not necessarily from malfeasance or even human error (44–49). Routine auditing of large cervical cancer programs will help provide the quality control to minimize these errors (44–49) but intrinsically there are inaccuracies in all medical procedures. Secondary cervical cancer prevention programs require 3 steps, screening, diagnosis, and treatment, each of which contributes to the error in timely detection and treatment of cervical precancerous lesions. Even with the possible advent of low-cost HPV testing for low-resource settings (5;50), cancers will occur because these inherent errors in each prevention step happen in the context of women with potentially invasive precancerous lesions, as predicted from Bayes' Theorem, which says the risk of having cancer given a negative test is a function of probability of a false-negative test and the prevalence of the disease. A broader recognition that the most effective cervical cancer prevention programs will have a very low but non-zero risk of cancer will help avoid the tendencies to over-screen and over-treat women in a misguided effort to achieve a 100% efficacious program.

In summary, it seems unlikely that any negative test or combination of negative tests, even including colposcopic evaluation of the cervix that has been considered the gold standard of cervical cancer screening and diagnosis, can provide absolute reassurance against cancer in this population or in any setting. A single good screening can vastly reduce the risk of subsequent cancer in previously unscreened women. Yet, the inherent errors of each step of the secondary prevention of cervical cancer in a population at an elevated risk will result in a “significant” residual risk of cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

A comparison of baseline characteristics of cancer cases diagnosed in follow-up (A),cancer cases diagnosed at enrollment (B), and women who never developed cancer (C) enrolled in a natural history study of human papillomavirus (HPV) and cervical neoplasia in Guanacaste, Costa Rica (1993–2003). Cancers diagnosed in follow-up were compared to the other groups in a pair-wise manner, using non-parametric test (Kruskal-Wallis) for continuous variables and Fisher's exact test for categorical variables, to test for differences between groups. Bolded p values indicates statistical differences (<0.05) between the follow-up cancers and the two other groups. N is the number of test results available, %Pos is the percentage of positive tests. Pap smears and cervical cytology were considered positive if atypical squamous cells of undetermined significance or worse. HCT, Hybrid Capture Tube Test

	A. Non-Cancers (N = 10,019)			B. Enrollment Cancer (N = 12)			C. Follow-Up Cancers (N = 16)	
Baseline Data	**Median**	**Mean**	**p***	**Median**	**Mean**	**p***	**Median**	**Mean**
Age (Years)	38.0	41.0	0.4	39.5	40.6	0.6	47.0	46.8
Age @ First Sex (Years)	18.0	18.6	**0.0008**	17.0	18.0	0.1	15.0	15.9
Lifetime Number of Sexual Partners	1.0	1.7	**0.0008**	2.0	1.9	0.2	2.5	3.1
Lifetime Number of Pregnancies	3.0	4.5	**0.003**	3.5	4.3	0.08	5.5	8.3

Baseline Test Data	**N**	**%Pos**	**p***	**N**	**%Pos**	**p***	**N**	**%Pos**
HCT	9,130	8.6%	**0.002**	11	63.6%	0.3	16	37.5%
PCR	9,123	12.3%	**<0.001**	11	90.9%	0.2	16	62.5%
Pap Smear (Costa Rica)	9,064	6.5%	**0.3**	11	90.9%	**<0.001**	16	12.5%
Pap Smear (Johns Hopkins)	7,348	4.1%	**<0.001**	11	90.9%	**0.005**	15	33.3%
Liquid-Based Cytology (U.S.)	8,667	12.5%	**<0.001**	11	100%	**0.008**	14	50.0%

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Table 2

Summary of the follow-up of 16 women diagnosed with invasive cervical cancer during follow-up and 10,019 women without cancer participating in the natural history study of human papillomavirus and cervical neoplasia in Guanacaste, Costa Rica (1993–2003).

	All	Follow-Up Cancers	Non-Cancers
	n = 10,035	**n = 13**	**n = 10,022**
Follow-up time (Months)			
Mean	71.3	88.9	71.3
Median	84.1	100.1	84.1
IQR	60.2–96.3	91.1–105.3	60.2–96.2
Range	0.0–141.7	21.1–131.0	0.0–141.7
Number of Follow-Up Visits			
Mean	3.1	3.1	6.7
Median	1	1	6
IQR	1–5	1–5	5–8
Range	0–20	0–20	3–12

Table 3

Medical histories of 16 women who were diagnosed with invasive cervical cancer during follow-up while participating in the natural history study of human papillomavirus (HPV) and cervical neoplasia in Guanacaste, Costa Rica (1993–2003). The cases history until the first histologic diagnosis of cancer is presented. Patients A, H, I and P reported respectively only 2, 4, 2, and 2 lifetime numbers of sexual partners in their follow-up questionnaires. Cancer patients E, F, and M were found through linkage with the cancer registry.
Colpo = colposcopic evaluation; Post-colpo = post-colposcopic evaluation; Pap = Papanicolaou smear; LBC = liquid-based cytology; CR = Costa Rica; JHU = Johns Hopkins Pathologist (M.E.S.); US = U.S. pathologist (M.H.L.); LEEP = loop electrosurgical excision procedure; Hyst = hysterectomy; CIN = cervical intraepithelial neoplasia; ASCUS = atypical cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; Unsat = unsatisfactory; #Partners = lifetime number of sexual partners at enrollment; HCT = hybrid capture tube test result; AFS = age at first sexual intercourse; #Partners = lifetime number of sexual partners at enrollment

Patient A (Age = 36; AFS = 15; #Partners = 4)										
Time (Months)	**Visit Type**	**Pap (CR)**	**Pap (JHU)**	**LBC (US)**	**LBC (CR)**	**Cervigram (Review)**	**HCT**	**HPV (PCR)**	**Colpo**	**Histology**
0.0	Screen	Normal	Normal	Normal		Normal (Normal)†	Negative	16		
0.0	Screen	ASCUS		Cancer		P3-Cancer (Cancer)*		16;45		
60.8	Colpo								Cancer	Biopsy = Cancer
Patient B (Age = 50; AFS = 15; #Partners = 3)										
Time (Months)	**Visit Type**	**Pap (CR)**	**Pap (JHU)**	**LBC (US)**	**LBC (CR)**	**Cervigram (Review)**	**HCT**	**HPV (PCR)**	**Colpo**	**Histology**
0.0	Screen	Normal	Normal	Normal		Normal (Normal)†	Negative	Negative		
72.2	Screen	ASCUS		CIN3#	Reactive	Normal (Normal)*		18		
84.1	Screen	CIN3			CIN3	Normal		Missing		
85.8	Colpo								HSIL	LEEP = Cancer
Patient C (Age = 29; AFS = 17; #Partners = 1)										
Time (Months)	**Visit Type**	**Pap (CR)**	**Pap (JHU)**	**LBC (US)**	**LBC (CR)**	**Cervigram (Review)**	**HCT**	**HPV (PCR)**	**Colpo**	**Histology**
0.0	Screen	Reactive	Normal	Inadequate		Normal	Negative	18		
60.3	Screen	Reactive		ASCUS		P2-HSIL*		18		
63.5	Colpo								HSIL	LEEP = Cancer
Patient D (Age = 63; AFS = 18; #Partners = 6)										
Time (Months)	**Visit Type**	**Pap (CR)**	**Pap (JHU)**	**LBC (US)**	**LBC (CR)**	**Cervigram (Review)**	**HCT**	**HPV (PCR)**	**Colpo**	**Histology**
0.0	Screen	Normal	Normal	Normal		Normal‡	Negative	51;61		
13.1	Screen	Normal		Normal		Normal‡		33		
31.4	Screen	Reactive		Normal		Normal‡		61		
44.3	Screen	Normal		Normal		Normal‡		Negative		

Patient D (Age = 63; AFS = 18; #Partners = 6)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
56.7	Screen	Normal		Normal		Normal‡		Negative		
60.4	Screen	Normal		Normal		Normal‡		Negative		
84.2	Screen	Cancer		CIN3	ASCUS	Normal‡		33;35;52;58		
91.4	Colpo								Cancer	Biopsy = Cancer

Patient E (Age = 75; AFS = 12; #Partners = 1)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	Normal	Normal		Normal	Negative	Negative		
132.3	Colpo									Biopsy = Cancer

Patient F (Age = 48; AFS = 23; #Partners = 1)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Reactive	Normal	Normal		Normal	Negative	Negative		
72.3	Screen	Reactive		Normal	Reactive	Normal		Negative		
73.6	Colpo									Biopsy = Cancer

Patient G (Age = 23; AFS = 14; #Partners = 2)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	ASCUS	ASCUS		P1-LSIL(HSIL)‡	Positive	16		
1.4	Colpo								LSIL	Biopsy = CIN1
18.4	Screen	CIN2	CIN2	Missing		P1-LSIL(HSIL)‡		16		
21.8	Colpo								HSIL	Biopsy = CIN1
23.9	Post-colpo								HSIL	LEEP = Cancer

Patient H (Age = 64; AFS = 15; #Partners = 5)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Reactive	Normal	ASCUS		Normal (Normal)‡	Positive	45;51;58;84		
5.2	Colpo							45; 58	Normal	
19.6	Screen	ASCUS	Cancer	Cancer		P0-Probably Normal*		45		
21.2	Colpo								Cancer	Biopsy = Cancer

Patient I (Age = 49; AFS = 15; #Partners = 3)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	ASCUS	Reactive	ASCUS		Normal (Normal)‡	Positive	16		
2.9	Colpo							16	Normal	
13.2	Screen	Reactive	ASCUS	Reactive		Normal (Normal)*		16		
26.4	Screen	Normal		CIN3		Normal (Normal)*		16		
34.4	Colpo$^{\Omega}$									Biopsy = Cancer

Patient J (Age = 61; AFS = 18; #Partners = 2)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	ASCUS	ASCUS		Normal (Normal)‡	Negative	Negative		
4.1	Colpo							Negative	Normal	
13.6	Screen	CIN3	CIN3	Cancer		P0-Probably Normal (HSIL)‡		Negative		
15.7	Colpo								HSIL	Biopsy = Cancer

Patient K (Age = 37; AFS = 14; #Partners = 2)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Reactive	ASCUS	ASCUS		Normal (HSIL)‡	Positive	18		
3.8	Colpo							18;61	Normal	
10.4	Screen	Reactive		Reactive		Normal (Metaplasia)				
13.4	Screen	Inadequate	Inadequate	ASCUS		Normal		18;85		
17.5	Colpo								Unsat	Biopsy = Cancer

Patient L (Age = 28; AFS = 14; #Partners = 1)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Reactive	Normal	Missing		Normal (Normal)	Positive	18;51		
1.9	Colpo								Normal	
13.8	Screen	Reactive	Normal	Reactive		Normal (Metaplasia)		18		
26.5	Screen	Reactive		Reactive		Normal (Metaplasia)		18;55		
38.5	Screen	Reactive		Normal		Normal (Normal)&		18		
50.5	Screen	Inadequate		Inadequate		Normal (Normal)		18		
60.7	Screen	Reactive		Normal		Normal (LSIL)		18;56		
86.6	Screen	Reactive		Normal	Reactive	Normal (HSIL)		18;73		
96.5	Colpo$^{\Delta}$								Unsat	

Patient L (Age = 28; AFS = 14; #Partners = 1)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
98.1	Post-colpo	CIN1							Normal	
104.3	Post-colpo	CIN3							Normal	
105.9	Post-colpo	Reactive							Normal	LEEP = Cancer

Patient M (Age = 63; AFS = 18;#Partners = 1)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	ASCUS	Reactive		Normal	Positive	16		
1.8	Colpo							16	Normal	
13.3	Screen	Normal	ASCUS	CIN2		Normal		16;45		
16.5	Colpo								Normal	
26.2	Post-colpo								Normal	
106.0	Screen			HSIL (CIN3)	HSIL (CIN2)	Normal		16		
106.2	Colpo								Normal	
107.1	Colpo									Hyst = Cancer

Patient N (Age = 39; AFS = 15; #Partners = 9)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	Normal	CIN1		Normal (Metaplasia)‡	Negative	16; 58		
26.6	Screen	CIN1	CIN3	Cancer		Normal (Normal)‡		16		
29.2	Colpo								HSIL	Biopsy = CIN3
34.1	Post-colpo								HSIL	LEEP = CIN2
43.1	Post-colpo								Normal	
55.1	Post-colpo	Reactive							Normal	
112.2	Screen			HSIL (CIN3)	HSIL (CIN2)	Normal		16		
115.8	Colpo								LSIL	LEEP = Cancer

Patient O (Age = 38; AFS = 15; #Partners = 5)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	CIN2	Missing	CIN3		Normal (Cancer)	Negative	16;71		
1.8	Colpo							16	LSIL	Biopsy = CIN3
5.1	Post-colpo									LEEP = CIN3
76.0	Colpo$^{\Omega}$				CIN3				Cancer	Biopsy = Cancer

Patient P (Age = 46; AFS = 16; #Partners = 4)

Time (Months)	Visit Type	Pap (CR)	Pap (JHU)	LBC (US)	LBC (CR)	Cervigram (Review)	HCT	HPV (PCR)	Colpo	Histology
0.0	Screen	Normal	ASCUS	Normal		Normal[&]	Negative	52		
3.5	Colpo							52	Normal	
13.2	Screen	CIN3	CIN2	CIN3		Normal (Normal)[‡]		52		
15.2	Colpo								Nom	
16.4	Post-colpo								HSIL	LEEP = CIN3
81.0	Colpo				Cancer					
82.4	Colpo				Cancer				Cancer	Biopsy = Cancer