The Causal Link between HPV and Cervical Cancer and Its Implications for Prevention of Cervical Cancer

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This article reviews epidemiologic evidence linking human papillomavirus (HPV) to cervical cancer. The authors conclude that over 90% of all cervical cancers can be attributed to certain HPV types—HPV 16 accounting for the largest proportion (roughly 50%) followed by HPV 18 (12%), HPV 45 (8%), and HPV 31 (5%). Recognition of this circumstance has far-reaching implications for primary and secondary prevention of this malignancy. At present, prophylactic and therapeutic HPV vaccines are under development, and HPV typing is being integrated into pilot study screening programs in a few developed countries. In developing countries, well conducted conventional screening programs remain the best approach for the control of cervical cancer until a safe and efficient HPV vaccine can be developed for use by the general population.

Certain types of human papillomavirus (HPV) are currently accepted as constituting the central etiologic factor of invasive cervical cancer and its precursor lesions. Consequently, vaccines directed at preventing HPV infection or inducing regression of HPV-associated neoplastic lesions are currently under development (*1*).

Because cervical cancer is the most common cancer of women worldwide after breast cancer (*2*), this recognition that certain HPVs are its chief etiologic agents implies identification of the most important carcinogen among women to date, since the main cause of breast cancer remains elusive.

In 1988 and 1991 we evaluated the epidemiologic evidence relating HPV with cervical cancer and concluded that although highly suggestive, the hypothesis of causality remained to be proved. Issues relating to study design and HPV detection methods were the major limitations of the then-available reports (*3–4*). In contrast, today the epidemiologic evidence for the association includes an impressive and largely consistent set of case-series and case-control studies together with some cohort studies.

We will restrict this review to studies fulfilling the following criteria:

(a) The study was restricted to invasive cervical cancer, carcinoma *in situ* (CIS), or moderate or severe grades (II or III) of cervical intraepithelial neoplasia (CIN). We felt this restriction important because the cytologic/histologic signs of HPV infection are considered by most pathologists to be indistinguishable from low-grade CIN (CIN I) lesions. Thus, CIN I lesions could be regarded as the morphologic manifestation of HPV-productive infection or a marker of exposure to HPV, rather than as a disease outcome.

(b) The study used accurate hybridization methods of HPV DNA detection—i.e.,

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methods based on Southern blot or polymerase chain reaction (PCR) techniques—and unfixed cervical specimens.

(c) If the study used a case-control or cohort approach, it met basic design requirements directed at avoiding or reducing bias and confounding factors.

CASE-SERIES

CIN II–III Studies

The prevalence of HPV DNA found in CIN II–III lesions varied from 39% to 97% in studies using the Southern blot (*5–11*) and from 75% to 100% in those using PCR-based methods (*12–14*). In all series, HPV 16 and 18 were the most commonly detected virus types, but a few lesions were observed to contain HPV types 6 or 11.

Invasive Squamous Cervical Cancer

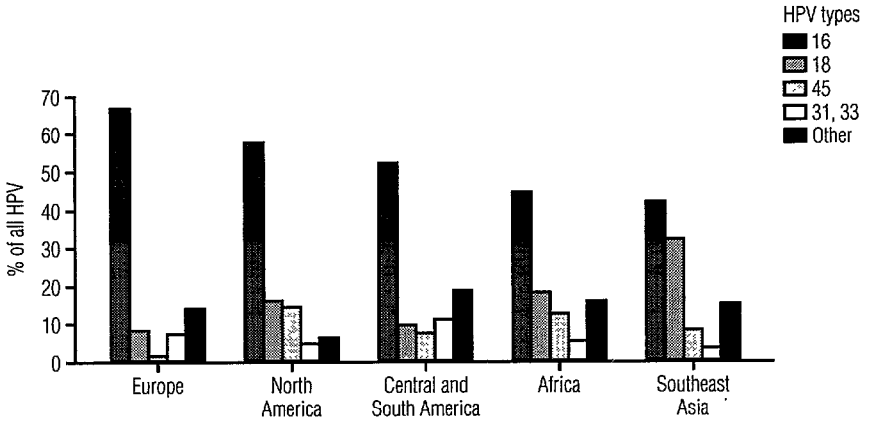
The largest study, the International Biological Study on Cervical Cancer (IBSCC), included over 1 000 biopsy specimens from 22 countries and used a PCR-based assay capable of detecting more than 25 HPV types. HPV DNA was detected in 92.7% of the tumors, the most frequently detected HPV types being HPV 16 (in 49.2% of the tumors), HPV 18 (11.7%), HPV 45 (8%), and HPV 31 (5%). HPV 16 was the predominant type in all countries except Indonesia, where HPV 18 was more common. A clustering of HPV 45 was apparent in western Africa, while HPVs 39 and 59 were detected almost exclusively in Latin America (*15*). Figure 1 shows the distribution of major HPV types by geographic region.

In reports preceding that of the IBSCC, HPV DNA had been detected in anywhere from 68% to 86% of the cases tested by Southern blot (*5–6, 10, 16*) and from 67% to 100% of those investigated with PCR-based methods (*17–26*).

Adenocarcinoma

In the IBSCC study, HPV DNA was detected in 95% of 43 adenocarcinomas and adenosquamous tumors; HPV 18 and HPV 16 accounted, respectively, for 51% and 24% of the HPV types detected (*15*). In previously reported smaller series, HPV DNA had been detected in 52% to 64% of the cases when the Southern blot method was used (*16, 27*) and in 88% when a PCR-based

Figure 1. HPV type-specific distributions among HPV positive individuals in different geographic regions. The distribution of each type or group of types is shown as a percentage of the total HPVs identified in each region.



method was employed (*17*). As in the IBSCC study, the most frequently detected HPV type was 18.

CASE-CONTROL STUDIES

CIN II–III Lesions

Table 1 summarizes the results of seven case-control studies meeting the inclusion criteria. Six of them used PCR-based methods and one used the Southern blot.

In Spain, Bosch *et al.* (*28*) compared 157 cases of histologically confirmed severe dysplasia (CIN III) or carcinoma *in situ* (CIS) with 193 controls matched by age, date of cytology, and recruitment center. The controls had cytologic diagnoses of nonspecific inflammatory changes or Pap grades I–II. The cytologic and histologic diagnoses of cases and controls were confirmed by a panel of cytopathologists. The mean age of both the cases and the controls was 36 years. The method used to detect HPV was PCR—based on consensus primers of the L1 region with probes for HPV types 6, 11, 16, 18, 31, 33, and 35. The HPV DNA prevalence found among the cases was 70.7%, as compared to 4.7% among the controls. Risk estimates were adjusted for age, geographic area, number of sexual partners, husband's number of sexual partners, age at first sexual intercourse, and presence of *Chlamydia trachomatis*. The adjusted odds ratio (ORa) and 95% confidence interval (CI) for the presence of HPV DNA was ORa = 56.9, CI = 24.8–130.6, while the attributable fraction (AF) was 72.4%. The ORa and 95% CI for specific HPV types were HPV 16: ORa = 295.5, CI = 44.8–1 946.6; HPV 31, 33, or 35: ORa = 28.9, CI = 5.5–152.8; HPV unknown: ORa = 18.7, CI = 6.6–54.8.

As part of the same project, a second study was conducted in the city of Cali, Colombia, where 125 histologically confirmed CIN III cases were compared with 181 controls using the same epidemiologic design and laboratory methods (*28*). The mean age of both the cases and the controls was 39 years. The HPV DNA prevalence found among the cases was 63.2%, as compared to 10.5% among the controls. Risk estimates were adjusted for age, geographic area, number of sexual partners, age at first sexual intercourse, presence of *C. trachomatis*, and smoking. The ORa and 95% CI for the presence of HPV DNA was ORa = 15.5, CI = 8.2–29.4, while the AF was 60.3%. The ORa and 95% CI for specific HPV types were HPV 16: ORa = 27.1, CI = 10.6–69.5; HPV 31, 33, or 35: ORa = 23.4, CI = 2.8–190.6; HPV unknown: ORa = 12, CI = 5.1–28.6. In both studies the ORas for CIN III increased with rising estimates of the amount of HPV DNA present.

The PCR assay used in these two studies employed the early version of L1 consensus primers and of the generic probe—which detected a narrower spectrum of HPV types. Therefore, some HPV types may have escaped detection. In addition, higher prevalences of HPV were observed when biopsy specimens were used instead of scrapes. Thus, it is likely that the adjusted odds ratios and attributable fractions shown in Table 1 are underestimates of the true ORas and AFs.

To try to identify cofactors that might determine progression from HPV DNA positivity to CIN III lesions, a case-control analysis of these data was restricted to cases and controls positive for HPV DNA. This analysis suggested that early age at first intercourse and high parity increased the risk of progression to CIN III (*29*).

Also, after taking into account the strong effect of HPV, seropositivity to *C. trachomatis* was found to be moderately associated with CIN III. The effect was consistent in both countries; in Spain the ORa was 2.3 (95% CI = 1.1–4.5), while in Colombia the ORa was 1.7 (95% CI = 1.1–2.7) (*29*).

In the United States (Portland, Oregon) Schiffman *et al.* (*30*) compared 50 cases of

Table 1. Data from seven case-control studies of CIN II–III lesions.

Author, country	Cases (No.)	Controls (No.)	Type of HPV test	Observed HPV prevalence (%)			Adjusted odds ratio (95% confidence interval)	HPV attributable fraction (%)
				HPV type	Cases	Controls		
Bosch *et al.*, 1993 (*28*), Spain	157 (CIN III, CIS)	193	PCR L1 consensus for HPV types 6, 11, 16, 18, 31, 33, & 35	All tested	70.7	4.7	56.9 (24.8–130.6)	72.4
				HPV 16	49.0	0.5	295.5 (44.8–1 946.6)	59.6
Bosch *et al.*, 1993 (*28*), Colombia	125 (CIN III, CIS)	181	PCR L1 consensus for HPV types 6, 11, 16, 18, 31, 33, & 35	All tested	63.2	10.5	15.5 (8.2–29.4)	60.3
				HPV 16	32.8	3.3	27.1 (10.6–69.5)	46.3
Schiffman *et al.*, 1993 (*30*), United States	50 (CIN II–III)	433	PCR L1 consensus for HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 57, 59, PAP 88, PAP 155, PAP 238A, PAP 251, PAP 291, & W13B	All tested	90	17.7	42 (15.3–124.3)	87.9
				HPV 16/18	62	2.9	180 (49–630)	83.8
Becker *et al.*, 1994 (*14*), United States	176 (CIN II–III)	311	PCR L1 consensus for HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 54, 56, 58, 59, PAP 88, PAP 238, & W13B	All tested	93.8	42.1	20.8 (10.8–40.2)	89.0
				HPV 16	52.4	8.6	9.9 (5.4–18.3)	44.0
Olsen *et al.*, 1995 (*31*), Norway	98 (CIN II–III)	221	PCR nested general primers for HPV types 6, 11, 16, 18, 31, 33, and others (unknown)	All tested	90.8	15.4	72.8 (27.6–191.9)	92.0
				HPV 16	65.3	6.3	182.4 (54.0–616.1)	92.0
Liaw *et al.*, 1995 (*32*), Taiwan	48 (CIN II–III or invasive cancer)	261	PCR L1 consensus for HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 68, PAP 88, PAP 155, PAP 238A, PAP 291, & W13B	All tested	91.7	9.2	122.3 (38.5–388.9)	91.8
				High-risk types	58.3	0.8	1 279.9 (185.5–8 829.8)	91.1
				Med.-risk types	31.3	4.2	98.8 (23.8–410.5)	80.4
				Low-risk types	2.1	4.2	4.8 (0.5–47.8)	13.8
Brisson *et al.*, 1994 (*33*), Canada	456 (CIN II–III)	408	Southern blot for HPV type 16	HPV 16	42.5	6.0	8.7 (5.1–15.0)	32.0

CIN II–III with over 400 controls selected randomly from among 17 654 women with normal cytology and no known history of CIN. Cervico-vaginal lavage was used to collect cytologic specimens, and the method used to detect HPV DNA was the L1 consensus primer PCR with 25 HPV type-specific probes (an improved version of the PCR assay used in the studies in Spain and Colombia). Risk estimates were adjusted for age and lifetime numbers of sexual partners. The ORa and 95% CI for specific HPV types were HPV 16 or 18: ORa = 180, CI = 49–630; HPV 6, 11, 42, or unknown: ORa = 10, CI = 3–36; HPV 31, 33, 35, 39, 45, 51, or 52: ORa = 22, CI = 4.8–97. A case-control analysis restricted to the HPV-positive women was reported for all grades of CIN but not for CIN II–III.

Also in the United States (New Mexico), Becker *et al.* (*14*) compared 176 women with a histologic diagnosis of high-grade dysplasia or CIN II–III with 311 women who had been referred to the same clinics for colposcopy, but who had a normal cytology or cervical biopsy. The age range of both groups was 18–40 years. HPV DNA was detected by dot blot and by PCR (the same method used in the Portland study) with 19 HPV type-specific probes. The ORa for HPV DNA after adjusting for age, age at first intercourse, lifetime number of partners, and ethnicity was 20.8 (95% CI = 10.8–40.2); for HPV 16 the ORa was 9.9 (95% CI = 5.4–18.3). Increased risk (but with a lower CI, less than 1.0) was observed for HPV types 18, 31, 33, 56, or 58. After adjusting for the presence of HPV and other variables showing significant associations, three factors (low educational level, smoking, and a history of sexually transmitted disease) remained significantly associated with the risk of CIN II–III. No analysis restricted to the HPV-positive women (to assess the role of cofactors) was reported.

A recent study conducted in Norway (*31*) included 98 cases with histologically confirmed CIN II–III and 221 age-matched and randomly selected controls from the general population. The mean age of the subjects with cases was 31.8 years, while that of the control subjects was 32.7 years. HPV DNA in cervical cells was detected with PCR using general nested primer pairs of the L1 region. These primers detect HPV types 6, 11, 16, 18, 31, and 33 as well as other unknown types. The ORa for the association between HPV and CIN II–III was 72.8 (95% CI = 27.6–191.9). The ORa for HPV 16, the most common type, was 182.4 (54.0–616.1). No analysis restricted to HPV-positive women (to assess the role of cofactors) was performed.

A small case-control study was conducted in rural Taiwan within the framework of a cervical cancer screening project (*32*). The study included 45 cases of histologically confirmed CIN II–III, 3 cases of invasive cervical cancer, and 261 cytologically normal controls. The mean age of the subjects with cases was 47 years, while that of the control subjects was 43 years. HPV DNA was detected with the same PCR-based assay used in the study by Schiffman *et al.* (*30*) with a few differences in the probes used. (Besides the probes used in the Schiffman study, the Taiwan study employed probes for HPV types 56 and 68.) The ORa for all HPV types was 122.3 (95% CI = 38.5–388.9). The ORa for HPV types classified as high-risk was higher (ORa = 1 280) than the one for medium-risk types (ORa = 99) or low-risk types (ORa = 5). In this study high-risk types were those most frequently found in cervical cancer (HPV types 16, 18, 31, and 45); medium-risk types included those less frequently found in cervical cancer (HPV types 26, 33, 35, 39, 51, 52, 55, 56, 58, 59, and 62); and low-risk types included those not generally found in cervical cancer (HPV types 6, 11, 40, 42, 53, 54, and 57). No analysis restricted to HPV-positive women was reported.

In a case-control study from Canada (Quebec), 456 cases with histologic diagno-

sis of high-grade CIN (II–III) were compared with 408 hospital controls who had no cytologic or histologic diagnosis of HPV, CIN, or cancer, but who had been referred to a colposcopy clinic. The mean age of the subjects with cases was 28.2 years and that of the control subjects was 31.2 years. HPV DNA detection was restricted to HPV 16 using the Southern blot. The ORa found for CIN II–III associated with HPV 16 (after adjusting for age, number of sexual partners, age at first intercourse, smoking, and oral contraceptive use) was 8.7 (95% CI = 5.1–15.0) (*33*). The ORas of CIN II–III increased with the amount of HPV 16 DNA detected. The role of cofactors among HPV-positive women was not assessed in this study.

Overall, the results of these seven studies (see Table 1) show that recent studies using highly sensitive PCR-based assays (*14, 30–32*) detected higher prevalences (90–94%) of HPV DNA among subjects with moderate to severe CIN cases than did studies using earlier versions of these assays (*28*), which detected HPV DNA in 63–71% of the cases.

In comparing the prevalence of HPV DNA among controls, one should consider age structure and the source of control patients in addition to the accuracy of the hybridization techniques. Thus, the higher HPV DNA prevalences among controls in the United States and Norway, compared to controls in Spain and Colombia, are probably explained by the younger ages of the study populations and the more sensitive PCR assays; while the higher prevalences of HPV 16 among controls in the Canada and New Mexico studies relative to those in the Portland study probably arose from the nature of the Canadian and New Mexico control groups (women referred to a colposcopy clinic rather than women with normal cytology and no history of CIN). In other words, selection bias cannot be totally excluded in the case of the Canada and New Mexico studies.

Invasive Cervical Cancer

Four case-control studies fulfilling the inclusion criteria, all of them using PCR-based assays, are described below and in Table 2.

In Spain and Colombia, Muñoz *et al.* (*34*) conducted two population-based case-control studies including 436 incident cases with a histologic diagnosis of invasive squamous cell cervical cancer and 387 controls randomly selected from the populations under study. HPV detection was done using PCR methods based on the L1 region consensus primers. Hybridization was performed sequentially with probes for HPV types 6, 11, 16, 18, 31, 33, and 35 under high stringency conditions. Subsequently, the filters were screened with a generic probe containing a mixture of amplimers (primers for amplification) of HPV 16 and 18 (*35*). The respective ORas for any HPV DNA and for DNA from HPV type 16 were 46.2 and 45.8 in Spain and 15.6 and 17.3 in Colombia. Over 65% of the cases in this study could be attributed to HPV. ORas for other types of HPV were as follows: for HPV 31, 33, or 35, ORa = 21.3 (95% CI = 6.1–75.6); and for HPV type unknown, ORa = 79.6 (CI = 11.1–572.4).

The PCR assay used in these studies was the same employed in the CIN III case-control studies in Spain and Colombia cited above (*28*). Thus, the ORs and AFs reported here are probably underestimates of the true ORs and AFs.

An additional analysis stratified by HPV status showed that among HPV-negative cases the risk factors identified were still related to sexual behavior. However, when HPV-positive cases were compared to HPV-positives and controls, the only significant differences were the use and the duration of use of oral contraceptives (*36*). This result is consistent with the PCR-based studies on CIN III in Spain and Colombia that reported some independent effect of parity, suggesting that hormonal circum-

Table 2. Data from four case-control studies of invasive cervical cancer.

Author, country	Cases (No. and type)	Controls (No. and type)	Type of HPV test	Observed HPV prevalence (%)			Adjusted odds ratio (95% confidence interval)	HPV attributable fraction (%)
				HPV type	Cases	Controls		
Muñoz *et al.*, 1992 (*34*), Spain	250 population-based	238 population-based	PCR L1 consensus for HPV types 6, 11, 16, 18, 31, 33, & 35	All tested	69	4.6	46.2 (18.5–115.1)	67.5
				HPV 16	45.8	3.1	45.8 (15.8–132.9)	58.1
Muñoz *et al.*, 1992 (*34*), Colombia	186 population-based	149 population-based	PCR L1 consensus for HPV types 6, 11, 16, 18, 31, 33, & 35	All tested	72.4	13.3	15.6 (6.9–34.7)	66.0
				HPV 16	50.6	9.2	17.3 (7.4–40.4)	60.0
Eluf-Neto *et al.*, 1994 (*39*), Brazil	199 hospital-based	255 hospital-based	PCR general and type-specific primers for HPV types 6, 11, 16, 18, 31, & 33	All tested	84.0	17.0	37.1 (19.6–70.4)	86.0
				HPV 16	53.8	5.3	74.9 (32.5–173)	79.7
Peng *et al.*, 1991 (*41*), China	101 hospital-based	146 clinic-based	PCR type-specific primers for HPV types 16 & 33	HPV 16 & 33	34.7	1.4	32.9 (7.7–141.1)	31.0

stances may play a role as a cofactor in development of the HPV chronic carrier state and/or in the progression from HPV to HPV-related neoplasia.

On the other hand, cigarette smoking and other sexually transmitted agents (herpes simplex type 2, cytomegalovirus, gonococcus, syphilis) were not associated with cervical cancer risk once the strong effect of HPV was taken into account (*37–38*).

A hospital-based case-control study including 199 histologically confirmed incident cases of invasive cervical cancer and 225 age-matched controls was conducted in Brazil (*39*). A PCR-based assay was used that employed a general primer and type-specific primers for HPV types 6, 11, 16, 18, 31, and 33. The OR and AF shown in Table 2 indicate that these more sensitive PCR methods (as suggested by the higher fraction of HPV-positive cases) yielded a higher AF estimate (86%) than did the Spain and Colombia studies (66–68%). An analysis restricted to the HPV-positive cases and controls identified high parity and long-term use of oral contraceptives as cofactors (*40*).

This Brazil study is part of an IARC coordinated multi-center case-control study also being conducted in Mali, Morocco, Thailand, and the Philippines. The main strength of this project is that the same protocol and questionnaire are being used at all the study sites and that the HPV DNA detection is being performed at a central laboratory using a PCR-based method. It is expected that this undertaking will provide valuable information about the risks associated with the various HPV types and cofactors.

Another hospital-based study conducted in China included 101 cases and 146 controls. HPV DNA detection, using a PCR-based method, was restricted to HPV types 16 and 33. The prevalences of these two HPV types were found to be relatively low in both cases (34.7%) and controls (1.4%), and the corresponding ORa (adjusted for age, income, residence, age at first marriage, and smoking) was 32.9 (*41*). The small sample size and low observed prevalences of HPV precluded an analysis for cofactors among HPV-positive women.

These four case-control studies give consistent results. As already noted, the higher HPV DNA prevalence found in Brazil as compared to Spain and Colombia was probably test-related. Among controls, higher HPV prevalences were observed among residents of countries at relatively high risk for cervical cancer (Brazil and Colombia) than among those of low-risk countries (Spain and China). No formal case-control studies on cervical adenocarcinoma have been reported.

Case-control studies suffer from inherent temporal ambiguity concerning exposure and disease outcome. Thus, the higher prevalence of HPV DNA among cases than among controls could be interpreted in two ways:

(a) If we assume that a single measurement of HPV DNA is a good marker of chronic persistent infection with HPVs, HPV DNA detected at case and control recruitment could be taken to indicate an HPV infection that preceded cancer development.

(b) HPV DNA could be more readily detected in tumor cells than in normal cells or could indicate opportunistic infection with HPV.

Direct evidence in support of the first possibility can only be derived from long-term follow-up studies. (A few such studies will be reviewed below.) However, indirect evidence with respect to possible opportunistic infection may be obtained by observing HPV DNA prevalence trends relative to the time since last sexual intercourse, because sexual intercourse is the major route of HPV transmission. Data from our studies in Spain and Colombia show a stable high rate of HPV DNA positivity not only among women with cervi-

cal cancer who reported being sexually active at the time of the interview, but also among those who had their last sexual intercourse many years before entry into the study (*28, 34*).

The possibility that HPV DNA is more readily detected in tumor cells is unlikely because the HPV DNA prevalence in precursor lesions (CIN II–III) is as high as it is in invasive cervical cancer lesions. Against the argument of HPV being an opportunistic infection, there is a great deal of laboratory data indicating that HPV DNA and transcripts of specific HPV types are usually detected in tissue specimens from cervical cancer and its precursor lesions; that high-risk HPV types are able to immortalize human cells; and that their oncoproteins interfere with the functions of negative cellular regulators.

COHORT STUDIES

Although several cohort or follow-up studies have been reported, only those having CIN II–III as an end-point, using Southern blot or PCR-based hybridization assays for HPV DNA detection, and fulfilling the aforementioned basic design criteria are considered here.

Three such studies have been reported from the United States. In the first, a cohort of 241 cytologically normal women was recruited from a sexually transmitted disease clinic and tested for HPV types 6, 11, 16, 18, 31, 33, and 35 using dot blot and Southern blot methods. They were followed up every 4 months for an average of 25 months. HPV DNA positivity was associated with an adjusted relative risk (RR) for CIN II–III of 11.0 (95% CI = 3.7–31.0). The risk was highest among those positive for HPV types 16 and 18 and for those who were HPV DNA positive in repeated tests. The cumulative incidence of CIN II–III among the HPV DNA positive women was 28% at two years, as compared to 3% among the HPV DNA negative women. Most of the incident CIN II–III cases occurred within the first two years of follow-up (*42*).

In a second study, 206 women—173 with low-grade squamous intraepithelial lesions (SIL) and 33 with high-grade SIL—who participated in an intervention trial were followed up every two months for six months. DNA from HPV type 16 was detected at study entry and at each follow-up examination by Southern blot. By multivariate modelling and adjustment for age, race, smoking, oral contraceptive use, and plasma levels of micronutrients, HPV 16 was found related to progression to high-grade SIL with a relative risk of 1.19 (95% CI = 1.03–1.38) (*43*).

In the third study, 70 women with a histologic diagnosis of dysplasia were followed at three-month intervals for 15 months. These women were enrolled in a double-blind randomized trial to assess the efficacy of β-carotene for the treatment of CIN. HPV DNA was detected by both Southern blot and a PCR-based assay. Persistent SIL (the continual occurrence of SIL on two consecutive visits) was associated with persistent HPV infection, especially with a persistently high viral load detected by Southern blot (ORa = 4.1, 95% CI = 1.4–12.3).[3] The ORs were adjusted for randomized grouping (*44*), an adjustment made because the women, participating in a randomized trial, were randomized to β-carotene or a placebo.

In the Netherlands, a cohort of 342 women with abnormal cytology (Pap class IIIb or lower, i.e. CIN III or lower) were followed up every 3–4 months for an average of 16 months (*45*). During the follow-up visits the following examinations were performed: cytology, colposcopy without biopsy, and HPV DNA testing for 27 HPV types using an accurate PCR technique.

[3] The ORa 4.1 refers to a persistently high viral load defined as HPV DNA detectable by both Southern blot and PCR on two consecutive visits.

Nine (3.0%) of the 298 women with an original cytologic diagnosis of Pap IIIa (CIN I–II) progressed to CIN III (diagnosed by colposcopy and histology); all nine were positive for DNA of high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, or 58) at enrollment and during the follow-up. The authors reported that the progression rate was higher among women positive for high-risk HPV types than among women positive for low-risk HPV types or negative for HPV.

Three retrospective cohort studies based on archival cytologic or histologic slides have been reported.

In the first, carried out in Amsterdam, 18 women with cervical cancer whose 27 previous smears were diagnosed as cytologically negative were tested for HPV DNA using a PCR-based assay. HPV DNA was detected in the smears of 16 of the 18; the smears of the remaining 2 subjects were not suitable for PCR assay. No control group was included in the study (*46*).

In the United Kingdom, a cohort of 93 untreated women with cervical abnormalities was identified from a randomized control trial undertaken some years ago. The patients were followed every four months by colposcopic and cytologic examinations for a median period of 26 months. HPV 16 and 18 were detected in the baseline biopsy sections by a PCR-based assay. HPV (16 or 18) was detected in 47 (51%) of the women, and its presence was associated with an increased risk of progression (OR = 2.3, 95% CI = 1.2–4.3) (*47*).

In Sweden, smears from 30 women with invasive cervical cancer (18 squamous cell carcinomas and 12 adenocarcinomas) and from 58 with *in situ* carcinomas positive for HPV DNA were compared with smears from a control group. For the subjects with cancer cases, the smears were taken 1.5 to 7 years prior to the diagnosis of cancer. HPV DNA, detected with a nested PCR-based assay, was found in 67% of the smears preceding the cancer from women with cases and in 11% of the smears from control women (OR = 16, 95% CI = 6.8–38.0) (*48*).

The above results suggest that persistent infection with high-risk HPV types precedes the development of CIN II–III and predicts a high risk of developing it. The main limitation of this study design is that in most settings follow-up is interrupted at stages CIN II–III for treatment of these lesions, and therefore the role of HPV in the progression to invasive cancer cannot be investigated. In addition, it is known that a certain proportion of CIN II–III lesions regress spontaneously.

Various other cohort studies are in progress in Colombia, Costa Rica, India, the United States, and the United Kingdom; but their results have not yet been reported.

CONCLUSIONS

The epidemiologic data reviewed above indicate that the association between certain HPV types and cervical cancer fulfills the accepted criteria of causality proposed by Sir Bradford Hill:

(a) The association is very strong, with ORs over 15 in all methodologically sound case-control studies using reliable means of HPV DNA detection. The strength of the association rules out the possibility that it can be explained by chance, bias, or confounding factors.

(b) It is consistent, because equally strong associations have been found in countries where the risks of contracting cervical cancer are high and in others where they are low.

(c) Results from several cohort studies indicate that infection with certain HPV types precedes the development of CIN II–III lesions.

(d) The association is specific for certain HPV types considered high-risk varieties. Out of the 30 HPV types that infect the uterine cervix, HPV 16 is associated with the highest proportion of cervical cancers, fol-

lowed by HPV 18.

(e) The epidemiologic evidence is supported by a great number of laboratory investigations indicating that the HPV types implicated in cervical neoplasia have a carcinogenic potential.

These conclusions have been endorsed by an international multi-disciplinary group that met recently in Lyon to evaluate the carcinogenicity of HPV (*49*).

Results from the reviewed case-control studies and our international prevalence survey of HPV DNA in invasive cervical cancer indicate that over 90% of these tumors can be attributed to certain HPV types. The fact that only a small minority of the persistent HPV infections progress eventually to cancer indicates that there should be other factors or cofactors that promote the progression to malignancy. Indeed, if we consider the small fraction of cervical cancers in which HPV DNA has not been detected as truly HPV-negative cases, we should conclude that HPV is neither a necessary nor a sufficient cause of cervical cancer.

Two types of cofactors may be of importance:

(a) Host factors that could modulate the effect of HPV—such as genetic factors (HLA or MHC haplotypes), genetic or induced immunosuppression, or endogenous hormonal factors reflected in the associations with high parity detected in our studies—as well as early age at first sexual intercourse, which could be regarded as a surrogate for early age at first HPV infection.

(b) Exogenous factors. In our studies in Spain, Colombia, and Brazil, only long-term use of oral contraceptives emerged as such a cofactor among HPV-positive women. However, our observations need to be confirmed in other populations and in larger studies. Our ongoing multicenter study in which a larger number of women with HPV-positive invasive cervical cancer will be compared with HPV-positive control women is expected to produce valuable information on the role of cofactors.

Our studies also suggest that the above cofactors probably influence the progression from persistent HPV infection to CIN III more than the progression from CIN III to invasive cervical cancer. In fact, a comparison of the risk factors identified for CIN III and invasive cancer in Spain and Colombia did not reveal any risk factor that was consistently different enough between CIN III and invasive cancer to suggest a role in the progression of CIN III to invasive cancer (*50*).

Finally, the role of etiologic factors independent of HPV has not been considered, as it is still uncertain whether the small proportion of cervical cancers apparently negative for HPV DNA are truly negative or are false negatives that might turn out to be HPV-positive when more sensitive methods of HPV DNA detection are available. In any case, if a subgroup of HPV-negative cervical cancers is finally identified, it would probably account for less than 5% of all cervical cancers.

IMPLICATIONS

The knowledge that certain types of HPV account for over 90% of all cervical cancers has far-reaching implications for the primary and secondary prevention of this malignancy. Prophylactic, therapeutic, and prophylactic-therapeutic HPV vaccines are now under development, and a few phase I trials with therapeutic ones are under way (*1*).

Vaccines

Prophylactic Vaccines

These vaccines, directed at preventing HPV infection, should be based on structural proteins or late antigens called L1 or L2.

Various vaccines against animal papillomavirus have been developed, and they have been shown effective in preventing papillomavirus infection and tumors associated with these viruses in cattle, rabbits, and beagle dogs. The synthetic production of virus-like particles (VLPs) of papillomavirus (PV) has led to major advances in the development of such vaccines. VLPs of PV are produced through overexpression of the major capsid protein (L1) in various expression vectors. VLPs have the same surface topography as infectious viral particles and present the conformational epitopes required for generating high titers of neutralizing antibodies; but they do not contain the potentially oncogenic viral DNA. Vaccination experiments have shown VLPs in rabbits and dogs to have protective efficacy against challenge with cottontail rabbit PV (*51*) and canine oral PV (*52*), respectively. Thus, the VLPs are currently the immunogen of choice in seeking an HPV vaccine to prevent genital HPV infection, and various VLP vaccines are now under development. The efficacy and safety of these vaccines should be assessed in phase I, II, and III trials before their use is permitted in the general population.

Therapeutic Vaccines

HPV's E6 and E7 oncoproteins are the natural targets for an immune attack. Various recombinant HPV vaccines are being developed on this principle, and some are already being tested in small-scale phase I trials (*1*).

In the United Kingdom, a vaccine has been based on a recombinant vaccinia vector expressing mutated E6 and E7 from both HPV 16 and HPV 18. The mutated oncoproteins have lost their oncogenic potential while retaining their immunogenic properties. The vaccine has been used in treating seven patients with invasive cervical cancer who are also receiving chemotherapy and radiotherapy. No side-effects of vaccination have been observed after nine months (*53*).

In Australia, a vaccine based on bacterial fusion proteins for HPV 16 E7 with algammulin adjuvant has been administered to five patients with stage 2b, 3, or 4 cervical cancer for which no potentially curative therapy is available. Three of the five patients made antibodies to E7; no adverse effects of vaccination have been observed (*54*).

A similar trial has started in the Netherlands using peptides related to HPV 16 E7 that bind to MHC class I molecules for use in HLA–A0201 cervical cancer patients (*55*). Although previous vaccination experiments have shown this peptide to protect mice against challenge with a syngeneic HPV 16-carrying tumor cell line, it remains to be seen whether this approach is successful in humans. Such skepticism is justified by evidence indicating that most cervical cancers have defects in MHC class I expression or processing.

Combined Prophylactic and Therapeutic Vaccines

Full-length HPV 16 L2–E7 chimeric proteins that are incorporated into L1 VLPs have been generated (*56*). Women with either normal or abnormal cytology who are positive for high-risk HPVs could constitute the target population for this vaccine.

HPV Testing in Screening Programs

In secondary prevention, HPV testing can be used as a primary screening tool or to support cytologic examination.

Use in Screening

HPV testing fulfills some but not all of the requirements for screening:

(a) HPV infection precedes morphologic lesions for a prolonged period of time (most

probably for years), during which the viral presence is detectable.

(b) The test to detect HPV is highly reliable. New technology being developed should soon standardize PCR-based methods, including methods for making quantitative estimates of the viral load. The task of HPV detection, which is expected to yield low false negative rates, may be transferred to clinical laboratories.

(c) Specimens for HPV DNA detection are obtained through noninvasive procedures that should be as acceptable to the population at large as cytology-based screening programs. Furthermore, the logistics required for such screening can be superimposed over the arrangements already in place.

(d) The current cost of HPV-based screening programs should be reduced through standardization and commercialization of the assays.

Two arguments can be made against the use of HPV tests in screening programs. One, perhaps the strongest, is that there is no effective treatment once infection is detected. Increased cytologic surveillance of the carriers of high-risk HPV types seems the only option to date. Use of condoms has not been shown to prevent HPV transmission, and recommending reduction in a person's number of sexual partners does not seem a feasible option.

The second argument is that, while cervical cancer is almost inevitably preceded by HPV infection, the natural history of the infection is not well understood. Namely:

(a) It is established that most HPV infections will resolve spontaneously with time, but it is unclear which factors intervene in establishing a chronic carrier state.

(b) The potential for progression of an LgSIL lesion (perhaps the early visible manifestation of the HPV infection) is highly variable, and morphologically it is impossible to predict progression.

(c) The paradigm of cervical cancer progression from low-grade lesions to invasive cancer through discrete intermediate stages (CIN I-II-III-CX) has been challenged. New interpretations have been proposed suggesting that "de novo" high-grade lesions can appear within short time intervals, as a consequence of unknown circumstances of the HPV infection (HPV type, viral load, immune status, etc.).

Use in Supporting Cytology

A few studies have explored the value of HPV testing as an adjuvant to cytologic examination. The results have been encouraging, suggesting that HPV testing may in fact be of great use in predicting high-grade lesions when cytologic examination fails.

Cuzick *et al.* (*57*) investigated a group of women referred to colposcopy clinics because of mild to moderate cytologic abnormality. The final diagnosis was established by colposcopy-directed biopsy; HPV 16 testing was done using a semi-quantitative PCR system. Of 55 women with mild to moderate cytology, 27 were normal on the biopsy; of these, 26 yielded a negative or low-intensity HPV 16 hybridization signal. In contrast, 18 of the women were found on biopsy to have proven CIN III; and of these, 11 yielded high levels of HPV 16 DNA. The suggestion is that HPV testing could prove useful in supporting cytologic triage of low-level lesions.

The same group (*58*) used HPV testing plus conventional cytology to assess the detection rate of high-grade CIN lesions in 2 009 women having routine screening. They concluded that HPV testing could usefully augment but not replace conventional cytology.

Ho *et al.* (*44*), on the basis of an intensive follow-up study including frequent retesting, suggested that the factors associated with neoplastic progression were HPV type, viral load, and persistence of HPV infection (as determined by repeated positivity).

The Free University in Amsterdam has pioneered a large population-based screening scheme in which the risk of progression to cervical cancer is predicted by either an abnormal cytology (Pap IV/V) or by detection of high-risk HPV types by a highly sensitive PCR system. If the investigators' hypothesis is confirmed, women at low risk (95% of the screening population) could lengthen the intervals between screenings considerably, reducing the costs of unnecessary testing and concentrating diagnostic resources on the high-risk population (*59*). This important study should soon clarify several issues concerning the value of HPV-based screening programs, the hybridization test to be used, the most adequate screening intervals, and the cost-effectiveness of the new strategy.

Conclusions about Screening

Overall, in developed countries it appears that integration of HPV testing into screening programs may improve the efficacy of such programs. On the other hand, in developing countries well-conducted conventional screening programs appear to remain the best approach for control of cervical cancer until standardized and inexpensive methods for HPV typing are available.

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