NO EXCESS RISK OF CERVICAL CARCINOMA AMONG WOMEN SEROPOSITIVE FOR BOTH HPV16 AND HPV6/11

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Human papillomavirus (HPV) types 16 and 18 are the major risk factors for cervical carcinoma, whereas HPV types 6 and 11 cause benign genital lesions. We wanted to study the joint effect of simultaneous infections with the oncogenic and non-oncogenic HPV types on risk of subsequent development of cervical carcinoma. A cohort of 530,000 women who had donated blood samples to Nordic serum banks between 1973 and 1994 was followed up by linkage to national cancer registries. We identified 182 prospective cases with invasive cervical carcinoma and selected 538 matched controls at random. HPV 6, 11, 16, 18 and 33 seropositivity was used as a marker for the different HPV infections, and seropositivity for Chlamydia trachomatis and cotinine were used as markers for risk-taking sexual behavior and smoking respectively. The adjusted odds ratio (OR) of cervical squamous-cell carcinoma (SCC) was 2.2 for HPV6/11 among HPV16 seronegatives and 5.5 for HPV16 among HPV6/11 seronegatives. Assuming multiplicative joint effect, the expected OR for seropositivity to both HPV6/11 and HPV16 would have been 12, but the observed OR was 1.0. The antagonistic interaction was statistically significant (p = 0.001) and present also under deterministic considerations of possible misclassification bias. Antagonistic interactions were also detected for combinations of HPV16 and HPV18 and of HPV16 and HPV33. The results are in line with the concept that HPV-specific immunity protects against SCC and supports primary prevention of SCC by vaccination against the HPVs. Int. J. Cancer 80:818–822, 1999.

MATERIAL AND METHODS

The study cohort consists of about 530,000 women, who donated blood samples to 3 population-based serum banks in Finland, Norway and Sweden. The women eligible for the study were free of cancer at the time of serum sampling.

The Finnish Maternity Cohort has collected blood samples during early pregnancy (first trimester), in order to screen for congenital infections in Finland, since 1983 (Bardy et al., 1993). The blood samples are drawn at the maternity clinics, and almost all (over 98%) the pregnant women have donated blood samples to the bank. At the end of 1993, the bank contained 710,000 blood samples taken from 390,000 women and stored at −25°C. The donors of 15,000 samples could not be identified because of incorrect or missing personal identification number.

The JANUS project was initiated in Norway in 1973 to search in the pre-noburity sera for changes indicative of chronic disease development at early stages (Jellum et al., 1995). By the end of 1991, 424,000 serum samples had been collected from 293,000 donors (males and females) to the JANUS serum bank and stored at −25°C. The 28,000 voluntary Red Cross blood donors with 77,000 blood samples were excluded. All the women included in the present study (about 124,000, with 174,000 samples) were recruited during routine health examinations, particularly in connection with evaluation of risk factors for chronic diseases. During

Different human papillomavirus (HPV) types are phylogenetically very old, and co-evolution with the human species has established an equilibrium between the different types causing genital infections in stable populations (Shah and Howley, 1996). Longitudinal studies have shown that infection with HPV types 16 and 18 increases the risk for subsequent development of cervical intraepithelial neoplasia (CIN) (IARC, 1995) and cervical carcinoma (Lehtinen et al., 1996; Dillner et al., 1997). On the contrary, benign genital lesions caused by HPV types 6 and 11 do not predispose to cervical carcinoma (IARC, 1995). At the individual level, concomitant or subsequent genital infections with non-oncogenic and oncogenic HPV types are common (Shah and Howley, 1996; IARC, 1995), but little is known about the joint effects of the different HPV types on the risk of cervical carcinoma. To evaluate this in depth we applied HPV serology, which provides type-restricted measures of past exposure to HPV types 6, 11, 16, 18 and 33 (Dillner et al., 1996), to a nested case-control study on risk factors for invasive cervical carcinoma (ICC) with a maximum follow-up of 16 years (Dillner et al., 1997).

Abbreviations: HPV, human papillomavirus; SCC, squamous-cell carcinoma; ICC, invasive cervical carcinoma; CIN, cervical intraepithelial neoplasia; CMI, cell-mediated immunity; OR, odds ratio; CI, confidence interval; |, on condition that.

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phase I (mainly years 1974–1978), the participation rate was 85% in the study area, comprising one county each from western, mid- and northern Norway. During phase II (late 1980s until 1991), the study area included various counties, and the participation rate was 75%.

The Västerbotten project was initiated in a northern Swedish county in 1986 (Dillner et al., 1994). Each year, all residents aged 30, 40, 50, 60 or 69 years are invited to participate in a health-promoting project, including the donation of biological samples for future medical research. The participation rate is about 65%. In 1993 the bank contained 15,000 blood samples taken from 15,000 women and stored at −80°C; 297 women could not be fully followed up, due to death (n = 127) or migration (n = 170).

The Finnish Cancer Registry and the Cancer Registry of Norway are nationwide, and the regional cancer registry at the Oncological Centre in Umeå covers the 4 northern-most counties in Sweden (Engeland et al., 1993). All the registries are population-based. They receive notifications from hospitals, pathology and haematology laboratories and physicians, and achieve close to 100% coverage in reporting.

Cases with cervical carcinoma were identified by linking the data files of the serum banks and the cancer registries using personal identification numbers. By late 1994, a total of 208 women diagnosed with invasive cervical carcinoma at least half a month after the enrollment had been reported to the cancer registries; 26 cases had not enough sera, or could not be located. If multiple serum samples were available, the earliest pre-diagnostic sample was chosen. For each case, 3 controls were matched for gender, age at serum sampling (±2 years), storage time of the serum sample (±2 months), and country. In Norway, the samples were matched also for county because of different sampling fractions by county. All controls were cancer-free at the time of diagnosis of the case. If 3 controls per case could not be found, the matching criteria on age and storage time were widened until the controls were found. The age at serum sampling of 6 controls differed more than 4 years from that of the case, and difference in the storage time was more than 6 months for one control. The serum samples of 8 controls could not be located. Thus, the final numbers of cases and controls were 182 and 538 respectively.

IgG antibodies to oncogenic (16, 18 and 33) and non-oncogenic (6 and 11) HPV types were determined by the standard ELISA method using HPV capsids, monoclonal anti-human IgG and horseradish-peroxidase-labelled goat anti-mouse IgG as conjugate (Dillner et al., 1996, 1997). The cutoff level of 0.100 absorbance units was used as an indication of exposure to HPV types 6, 18 and 33, and, correspondingly, cutoff levels of 0.158 and 0.200 absorbance units were used for HPV types 16 and 11. IgG antibodies specific to Chlamydia trachomatis were determined by the micro-immunofluorescence method using the following Chlamydia trachomatis serovars: B-group (B-E-D), C-group (C-H-I-J) and an intermediate group (G-F-K) (Wang and Grayston, 1970). Those seropositive for Chlamydia trachomatis were defined as having titres of at least one group ≥ 16. Serum cotinine was measured by radioimmunoassay using a hapten-specific monoclonal antibody (Parish et al., 1995). Serum cotinine levels ≥ 20 ng/ml were considered to indicate smoking. All laboratory analyses were performed at different times on different, masked serum aliquots.

Odds ratios (OR) and 95% confidence intervals (CI) were estimated by conditional logistic regression with EGRET software (Statistics and Epidemiology Research Corporation, Seattle, WA). A model with HPV16 and a model with different parameters for the effect of HPV16 in the absence and the presence of a possible effect modifier, HPV6/11, were used to test for effect modification. No independent effect on risk was assumed for the effect modifier in the model. The 2 hierarchical models used to test for multiplicative vs. non-multiplicative interaction of 2 factors were one with the main effects only and another with main effects and a joint effect. Possible confounders, other HPV types, Chlamydia trachomatis and cotinine, were included in these models. Difference between the likelihood ratio statistics related to the hierarchical models with one degree of freedom served as test statistic for tests for effect modification and non-multiplicative interaction.

Misclassification bias in the OR for the joint effect of HPV16 and HPV6/11 was considered using frequencies of misclassification-corrected joint distributions of seropositivity for HPV16 and HPV6/11 or HPV6 or 11 (HPV6/11). Misclassification-corrected joint distributions were calculated using the observed joint distribution of SCCs (Table I), and assuming different levels of sensitivity (S16 and S6/11), specificity (W16 and W6/11) and cross-reactivity (P16/6/11 and P6/11/6) for the HPV16 and HPV6/11 ELISA methods (T16 and T6/11) respectively. The cross-reactivity, P6/11/16, is the probability that someone is classified as HPV16-seropositive by the T16 method because of antibodies induced by HPV6/11 infection. For the calculations, the following definitions and assumptions were made. T16 identifies true HPV16-seropositives with probability, S16 · T16 misclassifies true HPV16-seropositives with probability, 1-W16 T16 classifies HPV6/11 seropositivity of HPV6/11 antibodies with cross-reactivity, P6/11/16, equally likely in the presence or the absence of HPV16 antibodies. The probabilities of correct and misclassified diagnoses of HPV16 seropositivity are:

\[
\text{P(observe HPV16{16} & HPV6/11{11})} = S_{16} + (1 - S_{16})P_{16/6/11},
\]

\[
\text{P(observe HPV16{16} & HPV6/11{11})} = S_{16},
\]

\[
\text{P(observe HPV16{16} & HPV6/11{11})} = 1 - W_{16}(1 - P_{16/6/11}),
\]

\[
\text{P(observe HPV16{16} & HPV6/11{11})} = 1 - W_{16}.
\]

The probabilities for observing HPV16 seronegativity given the true event are complement probabilities of those given above. In the probability formulae for observing HPV6/11 seropositivity, 16 and 6/11 are interchanged in the subscripts. It was further assumed about the tests that T16 succeeds or fails in identification of the HPV16 antibodies whether or not T6/11 succeeds or fails to identify HPV6/11 antibodies and vice versa (see Appendix).

RESULTS

Before diagnosis the cases were followed up on average 5 years. Of the cases, 149 (82%) were cervical squamous-cell carcinomas (SCCs), 32 were cervical adenocarcinomas and one was unspecified carcinoma. OR for seropositivity for HPV6/11 and/or one of the oncogenic HPV types 16, 18 and 33 were calculated for all invasive cervical carcinomas, and for SCC (Table I). The OR of ICC for HPV16 seropositivity in the absence of HPV6/11 antibodies, adjusting for antibodies to HPV18 and HPV33, was 3.8 (95% CI 1.9–7.6). The OR of ICC was 6.3 (95% CI 2.9–14). Conversely, the OR of ICC and SCC for HPV6/11 seropositivity in the absence of HPV16 antibodies, adjusting for antibodies to HPV18 and HPV33, were 1.7 (95% CI 1.0–2.9) and 2.3 (95% CI 1.3–4.1). The OR among those seropositive for both HPV16 and HPV6/11 did not show increased risk of ICC or SCC as compared with those seronegative for both HPV16 and HPV6/11 (OR = 0.8, 95% CI 0.2–2.8, OR = 1.0, 95% CI 0.3–4.0 respectively). Further adjustment for cotinine and for cotinine and antibodies to Chlamydia trachomatis had only a marginal effect on these estimates (Table I). No combinations of HPV18 or HPV33 and HPV6/11 yielded point estimates significantly different from unit risk (Table I).

The interaction between seropositivity to HPV16 and HPV6/11 was antagonistic for SCC (OR = 1.0, expected multiplicative OR = 12, p for interaction = 0.001) (Table II). Similar significant shifts from marginal expected risk to observed unit risk were not found for comparable combinations of HPV18 and HPV6/11 or HPV33 and HPV6/11 seroposities and seronegatives (Tables I, II). The interaction between seropositivity to HPV16 and HPV18 or to...
reported in Table II. Furthermore, at no combinations of sensitivity between HPV16 and HPV6/11 prove less antagonistic than that indicated in Table III, at no combinations did the interaction specificity (0.96 to 0.99) and cross-reactivity (0.01 to 0.04). As considered by assuming different levels of sensitivity (0.5 to 0.8), to a lesser extent (Table II).

Finally, the impact of non-differential misclassification was considered by assuming different levels of sensitivity (0.5 to 0.8), specificity (0.96 to 0.99) and cross-reactivity (0.01 to 0.04). As indicated in Table III, at no combinations did the interaction between HPV16 and HPV6/11 prove less antagonistic than that reported in Table II. Furthermore, at no combinations of sensitivity and specificity did cross-reactivity have a material effect on the point estimates.

We found an antagonistic interaction between HPV16 and HPV6/11 in cervical carcinogenesis. Because most of the invasive cervical carcinomas were squamous-cell carcinomas, the results for ICC and SCC are practically the same. We also found that HPV6/11 seropositivity was associated with increased risk of SCC among HPV16-seronegatives. The smaller effect of HPV6/11 on the risk for SCC (OR = 2.2) may have been masked in earlier studies by HPV16, due to cross-sectional design prone to differential misclassification bias (IARC, 1995; Franco, 1991), smaller amount of material (Shah et al., 1997) and/or less satisfactory analysis. On the other hand, the fact that HPV6/11 DNA is not found in cervical cancer tissue (IARC, 1995) makes it unlikely that the association is a simple cause-effect relationship. When the data were modelled assuming that there actually was no solitary effect of HPV6/11 on SCC (i.e., setting the OR to 1), the OR for HPV16 became 3.1. In the no-solitary-effect-assumption model, the OR for HPV16 became 4.8 among HPV6/11-seronegatives, but 0.8 among HPV6/11-seropositives (p for effect modification = 0.015). Thus, both the null hypotheses of no multiplicative interaction and no effect modification by HPV6/11 were rejected, and an antagonistic interaction between HPV16 and HPV6/11 in cervical carcinogenesis was disclosed.

One third of the Finnish and less than one fourth of the Norwegian donors had serial samples. In this study, only one sample was analyzed per subject. To maximize the follow-up time between serum sampling and diagnosis of the cancer, the first pre-diagnostic serum sample was analyzed. A study with repeated

TABLE I – ODDS RATIOS (OR) WITH 95% CONFIDENCE INTERVALS (CI) FOR ANY INVASIVE CERVICAL CARCINOMA AND CERVICAL SQUAMOUS-CELL CARCINOMA IN RELATION TO SERO-POSITIVITY FOR HUMAN PAPILLOMA VIRUS (HPV) TYPE 6 OR 11 AND/or HPV TYPES 16, 18 OR 33 IN A COHORT OF 530,000 NORDIC WOMEN

| Seropositivity | Number of | OR\(^1\) | 95% CI | Number of | OR\(^1\) | 95% CI |
|---------------|-----------|---------|--------|-----------|---------|--------|
| HPV6/11 HPV16 | Cases      |        |        | Controls  |         |        |
| No            | 428       | 1.2     |        | 356       | 1.2     |        |
| Yes           | 120       | 1.0     | 0.97   | 24        | 1.2     | 0.87   |
| HPV16         | No        | 54      | 1.6    | 54        | 1.2     | 0.8    |
| Yes           | 2          | 0.5     | 0.2    | 2         | 0.8     | 0.4    |
| HPV6/11 HPV18 | No        | 13      | 0.7    | 1.0       | 0.2     | 0.4    |
| Yes           | 6         | 1.4     | 0.5    | 4         | 0.8     | 0.4    |
| HPV18         | No        | 6        | 1.7    | 0.9      | 0.3    | 0.1    |
| Yes           | 19        | 1.7     | 0.9    | 15        | 0.7    | 0.4    |
| HPV6/11 HPV33 | No        | 407     | 1.2    | 1.0      | 0.3    | 0.1    |
| Yes           | 123       | 3.2     | 1.4    | 2.2       | 1.0    | 0.5    |
| HPV33         | No        | 56      | 1.2    | 0.7      | 0.3    | 0.1    |
| Yes           | 21        | 1.3     | 0.7    | 1.7       | 0.8    | 0.4    |

1Adjusted for seropositivity to other HPV types (HPV18/33, HPV16/33, or HPV16/18), cotinine and Chlamydia trachomatis. – Reference group, seronegative for the index HPV types.

TABLE II – HUMAN PAPILLOMA VIRUS (HPV)-, COTININE- AND CHLAMYDIA TRACHOMATIS-ADJUSTED JOINT EFFECTS OF SEROPOSITIVITY FOR HPV TYPE 6 OR 11 AND HPV TYPES 16, 18 AND 33 FOR ANY INVASIVE CERVICAL CARCINOMA AND CERVICAL SQUAMOUS-CELL CARCINOMA IN A COHORT OF 530,000 NORDIC WOMEN

| Joint effect of | All carcinomas | Squamous-cell carcinoma |
|-----------------|----------------|-------------------------|
|                 | OR\(^2\) 95% CI | OR\(^2\) 95% CI         |
| HPV16 and HPV6/11 | 0.7 0.2–2.5 | 5.4 0.003     | 1.0 0.3–4.0   |
| HPV16 and HPV18  | 1.2 0.3–4.0 | 6.8 0.016     | 1.3 0.4–4.8   |
| HPV16 and HPV33  | 1.3 0.4–4.4 | 4.5 0.077     | 1.6 0.5–5.3   |
| HPV18 and HPV6/11| 1.4 0.5–4.6 | 2.3 0.517     | 1.2 0.3–4.4   |
| HPV33 and HPV6/11| 1.6 0.6–4.3 | 1.7 0.916     | 2.2 0.7–6.4   |

1Expected multiplicative odds ratio. –2p value of likelihood ratio test statistic for multiplicative vs. non-multiplicative interaction.

TABLE III – MISCLASSIFICATION-CORRECTED ODDS RATIOS (OR) AND EXPECTED MULTIPlicative Odds Ratios (OR\(^e\)) FOR THE Joint Effect OF SEROPOSITIVITY TO HUMAN PAPILLOMA VIRUS (HPV) TYPE 6 OR 11, AND HPV TYPE 16 FOR CERVICAL SQUAMOUS-CELL CARCINOMA, BY DIFFERENT LEVELS OF SENSITIVITY, SENSITIVITY AND CROSS-REACTIVITY

| Sensitivity | Specificity | Cross-reactivity | OR | OR\(^e\) |
|-------------|-------------|------------------|----|---------|
| 0.5         | 0.98        | 0.01             | 1.2| 820     |
| 0.5         | 0.98        | 0.02             | 0.9| 670     |
| 0.5         | 0.99        | 0.01             | 1.4| 160     |
| 0.65        | 0.98        | 0.01             | 1  | 0.9     |
| 0.65        | 0.98        | 0.02             | 1  | 0.9     |
| 0.65        | 0.99        | 0.01             | 1  | 0.9     |
| 0.8         | 0.96        | 0.01             | 1  | 0.9     |
| 0.8         | 0.96        | 0.02             | 1  | 0.9     |
| 0.8         | 0.96        | 0.04             | 1  | 0.9     |
| 0.8         | 0.98        | 0.01             | 0.7| 31      |
| 0.8         | 0.98        | 0.02             | 0.7| 31      |
| 0.8         | 0.99        | 0.01             | 0.9| 21      |

Specificity and sensitivity of HPV6/11 and HPV16 antibody assays, and cross-reactivity of HPV6/11 to HPV16 and of HPV16 to HPV6/11 have been set equal. – Misclassification-corrected frequency less than zero; 0.8 specificity, 0.027 cross-reactivity yield an OR of 0 and an expected OR of 290.

HPV16 and HPV33 on cancer risk was also antagonistic, although to a lesser extent (Table II).

DISCUSSION

We found an antagonistic interaction between HPV16 and HPV6/11 in cervical carcinogenesis. Because most of the invasive cervical carcinomas were squamous-cell carcinomas, the results for ICC and SCC are practically the same. We also found that HPV6/11 seropositivity was associated with increased risk of SCC among HPV16-seronegatives. The smaller effect of HPV6/11 on the risk for SCC (OR = 2.2) may have been masked in earlier studies by HPV16, due to cross-sectional design prone to differential misclassification bias (IARC, 1995; Franco, 1991), smaller amount of material (Shah et al., 1997) and/or less satisfactory analysis. On the other hand, the fact that HPV6/11 DNA is not found in cervical cancer tissue (IARC, 1995) makes it unlikely that the association is a simple cause-effect relationship. When the data were modelled assuming that there actually was no solitary effect of HPV6/11 on SCC (i.e., setting the OR to 1), the OR for HPV16 became 3.1. In the no-solitary-effect-assumption model, the OR for HPV16 became 4.8 among HPV6/11-seronegatives, but 0.8 among HPV6/11-seropositives (p for effect modification = 0.015). Thus, both the null hypotheses of no multiplicative interaction and no effect modification by HPV6/11 were rejected, and an antagonistic interaction between HPV16 and HPV6/11 in cervical carcinogenesis was disclosed.

One third of the Finnish and less than one fourth of the Norwegian donors had serial samples. In this study, only one sample was analyzed per subject. To maximize the follow-up time between serum sampling and diagnosis of the cancer, the first
samples is better, without a doubt, but would in our case have resulted in a smaller sample size, with insufficient power for interaction analyses.

Non-differential misclassification of HPV status may underestimate relative risks for cervical carcinoma, especially when assessing the magnitude of risk associated with the major risk factors HPV16 or HPV18 (Franco, 1991). Assumptions of the diagnostic test performance were based on careful test validation (Dillner et al., 1996, 1997) and the literature (de Gruijl et al., 1997; Carter et al., 1996; Wideroff et al., 1996). The fact that the antagonism was more striking under the different alternatives of sensitivity, specificity and cross-reactivity investigated than without correcting for misclassification suggests that the antagonism cannot be totally explained by non-differential misclassification, as long as the deterministic assumptions made are in line with the literature.

Differential misclassification bias may result if controls have more frequent transient HPV infections than the cases. Determination of cumulative HPV exposure by capsid antibodies helps to avoid this bias, since it is a marker of (non transient) persistent HPV infection (de Gruijl et al., 1997). While HPV16 is more likely to persist than HPV6/11, antibody responses to both are likely to reflect a non-transient infection. Furthermore, the longitudinal study design and sampling as close to the start of the persistent exposure as possible decrease this bias.

Seroprevalence of Chlamydia trachomatis increases with the number of life-time sexual partners (Dillner et al., 1996) and can therefore be considered to reflect risk-taking sexual behaviour. The inclusion of the other types of HPV was of further assistance in this issue. Tobacco exposure is measured more accurately by cotinine analyses than by self-reporting (Parish et al., 1995). However, cotinine does not necessarily reflect smoking history, and variations in the age of starting smoking and in the daily dose may cause further uncontrolled variations in the estimation. Nevertheless, lack of complete control of these confounders is not likely to account for the observed antagonism between the HPV types 16 and 6/11.

Matching for storage time was necessary for vitamin analyses considered in other papers, to control for season of serum sampling and degradation with storage time. The consequence of matching both for age at serum sampling and for storage time was that the controls donated serum samples at the same time as their case. Storage temperature of the serum samples was controlled by Weiss (1994), and personal identification numbers for linkage between the registries and the serum banks (Pukkala, 1992). Moreover, there was a valid and concomitant assessment of type-specific exposure to past HPV infections (Carter et al., 1996; Dillner et al., 1996; Wideroff et al., 1996). These conditions allowed by far the largest follow-up study with invasive cervical carcinoma as an end point. The result, i.e., less than expected risk of SCC among women seropositive to both HPV16 and HPV6/11, is in line with current theoretical considerations on competition of different (sero)types of a pathogen (Lipsitch, 1997) and interference between different pathogens in the carcinogenic process (Donato et al., 1998). It suggests an explanation as to why a history of genital warts, the major causes of which are HPV6 and HPV11, is not usually associated with increased risk for cervical carcinoma (IARC, 1995). It also suggests that cross-protective immunity between different genital HPV types may protect against cervical carcinoma, irrespective of whether the benign HPV types are effect modifiers or causes, and supports the concept that HPV vaccination may prevent cervical cancer.

Why then were women with antibodies to both HPV6/11 and HPV16 less likely to develop cervical carcinoma than women with antibodies to only one of these types? The interactions of HPV6 and HPV11, and HPV16 oncoproteins E6 and E7 with the tumour-suppressor gene p53 and Rb products are qualitatively different (Shah and Howley, 1996). However, there is no evidence that during concomitant infections multiple HPV types can infect the same cell, and a molecular antagonistic mechanism at the cellular level is therefore unlikely.

The lack of antagonistic interaction between HPV6/11 and HPV18 may give a clue. HPV 16 can exist both in epithelial and in integrated form, while HPV18 escapes immunological surveillance by integration into the host chromosomes (Shah and Howley, 1996; IARC, 1995). Capsid antibodies to HPV6/11 and 16 do not cross-neutralize in vitro (Roden et al., 1996) and it is therefore unlikely that they induce cross-protection from infection. It is also unlikely that virion antibodies play any role in clearing established viral lesions, since the virion proteins are not expressed on the cell surface (de Gruijl et al., 1997). HPV capsid antibodies are primarily a marker of persistent infection that, among HPV16-DNA-positive HPV16-seropositive women, is associated with greater risk of CIN than among HPV16-DNA-positive HPV16-seronegative women (de Gruijl et al., 1997; Carter et al., 1996; Wideroff et al., 1996), and indicate increased risk for subsequent development of cervical carcinoma up to 20 years before cancer diagnosis (Lehtinen et al., 1996; Dillner et al., 1997; Shah et al., 1997).

We suggest that HPV6/11 and HPV16 capsid antibodies are also a marker of a cross-protective cervical mediated immune (CMI) response to HPV infections. HPV type specificity of the CMI response is not well known, but some of the early viral proteins (E1 and E2), required for episcopal maintenance of the virus and expressed throughout the infected lesion, are highly conserved among HPV types (Shah and Howley, 1996). CMI is generally considered important in clearing the established viral lesions, and existence of a cellular immunity cross-reactive between HPV types would appear a plausible explanation for the observed antagonism.

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**APPENDIX**

An example of how the misclassification-corrected joint distributions of seropositivity for HPV16 and HPV6/11 were calculated. Let us solve the misclassification-corrected joint distribution of seropositivity for HPV16 and HPV6/11 under assumptions of 0.65 sensitivity and 0.98 specificity of HPV16 and HPV6/11 antibody assays, and 0.01 cross-reactivity of HPV6/11 to HPV16 and HPV16 to HPV6/11. The observed frequencies are sums over the products of misclassification-corrected frequencies and conditional probabilities given in Appendix Table I. Let us denote the misclassification-corrected frequencies by $m$, seronegativity by $1$, sensitivity for HPV16 and HPV6/11 antibody assays, and $0.01$ cross-reactivity of HPV6/11 to HPV16.

To calculate the misclassification-corrected numbers of controls (336.1, 55.31, 67.07, $m_{10}$) and cases (14.05 and 23.58, in same order as the cases), the observed numbers of cases to the right-hand side of the equations:

$$0.9801m_{00} + 0.34304m_{10} + 0.34304m_{01} + 0.12006m_{11} = 90$$

$$0.0099m_{00} + 0.63706m_{10} + 0.006965m_{01} + 0.22644m_{11} = 24$$

$$0.0099m_{00} + 0.006965m_{10} + 0.63706m_{01} + 0.22644m_{11} = 24$$

$$0.0001m_{00} + 0.012935m_{10} + 0.012935m_{01} + 0.42706m_{11} = 3$$

There is only one solution to the set of equations above: $m_{00} = 67.07$, $m_{01} = 34.5$, and $m_{11} = 4.92$. To calculate the misclassification-corrected numbers of controls ($336.1$, $55.31$, $14.05$ and $23.58$, in same order as the cases), the observed numbers of cases are replaced by the observed numbers of controls.

| APPENDIX TABLE I | PROBABILITIES OF CORRECT AND MISCLASSIFIED DIAGNOSIS OF $x$ AND $y$ |
|------------------|---------------------------------------------------------------|
| **Observed** $(x, y)$ | **True** $(x, y)$ | **$(+, +)$** |
| $(-, -)$ | $W_x W_y$ | $(1 - S_x)A_y$ | $A_y (1 - S_y)$ | $(1 - B_y)(1 - B_x)$ |
| $(-, +)$ | $(1 - W_x)W_y$ | $S_x A_y$ | $(1 - A_y)(1 - S_y)$ | $B_y (1 - B_x)$ |
| $(+, -)$ | $W_x (1 - W_y)$ | $(1 - S_x)(1 - A_y)$ | $A_x S_y$ | $(1 - B_x)B_y$ |
| $(+, +)$ | $(1 - W_x)(1 - W_y)$ | $S_x (1 - A_y)$ | $(1 - A_y)S_y$ | $B_x B_y$ |

$S_x$ and $S_y$ are sensitivities, $W_x$ and $W_y$ specificities, and $P_{1|x}$ and $P_{1|y}$ cross-reactivities of $x$ to $y$ and of $y$ to $x$ correspondingly. $A_x$ and $A_y$ are abbreviations of $W_y(1 - P_{1|x})$ and $W_x(1 - P_{1|y})$ correspondingly. $B_x$ and $B_y$ are abbreviations of $S_x + (1 - S_x)P_{1|x}$ and $S_y + (1 - S_y)P_{1|y}$ correspondingly.