Research Article

Cancer Epidemiology, Biomarkers & Prevention

# Seroprevalence of Antibodies against Human Papillomavirus (HPV) Types 16 and 18 in Four Continents: the International Agency for Research on Cancer HPV Prevalence Surveys

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## Abstract

**Background:** Few human papillomavirus (HPV) seroprevalence studies have been carried out in women from low-resource countries.

**Methods:** Seroprevalence of antibodies against HPV16 and HPV18 was assessed in 7,074 women ≥15 years of age (median 44 years) from eight world areas. Serum antibodies against HPV16 and HPV18 were tested for using enzyme-linked immunosorbent assay. HPV DNA was assessed using a general primer GP5 +/6+–mediated PCR.

**Results:** HPV16 and HPV18 seroprevalence both ranged from <1% (Hanoi, Vietnam) to ≥25% (Nigeria). Of women who were HPV16 or HPV18 DNA-positive, seropositivity for the same type was 39.8% and 23.2%, respectively. Seropositivity for either type was directly associated with markers of sexual behavior. HPV16 and/or 18 (HPV16/18)–seropositive women had an increased risk of having cytologic abnormalities only if they were also HPV DNA-positive. A high international correlation was found between HPV16/18 seroprevalence and overall HPV DNA prevalence ($r$ = 0.81; $P$ = 0.022). However, HPV16/18 seroprevalence was substantially higher than the corresponding DNA prevalence in all study areas (although to different extents) and, contrary to DNA, tended to increase from young to middle age, and then decline or remain fairly constant. In all study areas, the vast majority of the information on the burden of exposure to HPV16/18 derived from serology.

**Conclusions:** The correlation between HPV DNA and HPV serology was not very good at an individual woman level, but high at a population level.

**Impact:** HPV serology is a poor marker of current infection or related lesions, but it can contribute, together with DNA, in evaluating the variations in the burden of HPV infection worldwide. *Cancer Epidemiol Biomarkers Prev; 19(9); 2379–88. ©2010 AACR.*

## Introduction

Human papillomavirus (HPV) infection is considered a necessary cause of cervical cancer (1), with types 16 and 18 accounting together for at least 70% of cervical cancer in all world regions (2). The gold standard for diagnosing cervical HPV infection is the detection of HPV DNA in exfoliated cervical epithelial cells. As most HPV infections are transient and clear within 2 years (3), HPV DNA testing mainly reflects current HPV infection status. Therefore, HPV DNA is not a good measure of cumulative lifetime exposure to the virus.

The serum antibody response to HPV virus-like particles (VLP), on the other hand, remains relatively stable

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over time, even after clearance of an HPV infection (4). It is therefore a candidate marker for cumulative HPV exposure, thus complementing HPV DNA detection. Cervical HPV infection, however, does not always lead to the development of detectable antibodies against HPV (HPV antibodies). Among HPV DNA-positive women, no more than half are also HPV-seropositive (5-8).

The vast majority of HPV seroprevalence studies have been carried out in women from high-resource countries (3, 6, 8-14). Few studies have focused on low-resource areas, such as Central and South America (7, 15-17), Asia (5, 18) or Africa (19), where the highest HPV DNA prevalence in the world can be found, notably among middle-aged women (20).

In the present article, we report on the seroprevalence and risk factors for HPV16 and HPV18 seropositivity in eight, predominantly low-resource areas included in the International Agency for Research on Cancer (IARC) HPV Prevalence Surveys.

## Materials and Methods

### Contributing studies and data collection

Study protocols and questionnaires were developed by the IARC, and field work was carried out between 1997 and 2000 in eight areas in four continents. Population sampling methods of participating women have been previously described for the individual study areas: Barcelona, Spain (21); Concordia, Argentina (22); Busan, Korea (23); Lampang and Songkla, Thailand (24); Hanoi and Ho Chi Minh City, Vietnam (25); and Ibadan, Nigeria (26). In summary, in each study area an attempt was made to obtain a random age-stratified sample of the population that included at least 100 women in each 5-year age group, from 15 to 19 up to 65 years and older. Participation ranged from 48% in Songkla, Thailand (where most nonparticipants were not found at the address given by the population list), to 94% in Hanoi, Vietnam.

Exclusion criteria were pregnancy at time of recruitment, previous hysterectomy, and physical or mental incompetence. The number of women per study area in this analysis sometimes differs from that in the original reports due to different selection criteria (i.e., information on both HPV DNA and HPV serology had to be available for inclusion in the present study). We further restricted our present analyses to women who reported to have had sexual intercourse.

Trained interviewers administered a face-to-face questionnaire that included information on sociodemographic characteristics, history of Papanicolaou (Pap) smear, reproductive and contraceptive history, menstrual factors, and smoking habits. Information on sexual behavior was collected and included lifetime number of sexual partners, age at first sexual intercourse, and husband's extramarital sexual relationships with other women or with female sex workers.

All participants signed informed consent forms according to the recommendations of the IARC and the local ethical review committees, which approved the study.

### Gynecologic examination, specimen collection, and cytology

Study participants underwent a pelvic examination performed by gynecologists or midwives. Samples of exfoliated cells from the ectocervix were collected with one or two wooden Ayre spatulas, and from the endocervix with a cytobrush (Cervibrush, CellPath). After the preparation of a Pap smear, the remaining exfoliated cervical cells were placed in tubes with PBS and stored on ice. Cells were centrifuged at 3,000 × *g*, and the resulting pellets were resuspended in PBS and frozen between −20°C and −80°C until they were shipped to the IARC, Lyon, France, for storage. Pap smears were stained and read locally and classified according to the Bethesda or equivalent system. A 10 mL sample of blood was also collected from each consenting participant. Blood samples were centrifuged at 1,500 × *g*, aliquoted into plasma, buffy coats, and red blood cells, and stored at −20°C. All samples were sent on dry ice to the IARC for storage. Women with abnormal cytologic findings, that is, with atypical squamous cells of undetermined significance or worse, were referred for further diagnostic procedures and treatment according to standard local protocols.

### Serologic testing for antibodies against HPV VLPs

The semiquantitative measurement of serum IgG antibodies against HPV16 and HPV18 VLPs was assessed at the Institut National de la Santé et de la Recherche Médicale U618 Laboratory, Faculty of Pharmacy, Tours, France, using enzyme-linked immunosorbent assay (ELISA) as previously described (7). Briefly, HPV16 and HPV18 VLPs were produced in Sf21 insect cells using recombinant baculoviruses encoding the L1 gene of HPV16 and HPV18 and purified by isopyknic ultracentrifugation (7, 27, 28). Flat-bottomed wells of microtiter plates were coated overnight at 4°C with 200 to 800 ng of VLP preparation or 200 ng of bovine serum albumin (BSA) in PBS (pH 7.4). Each plasma sample was tested at a dilution of 1:10 against both the VLP types and BSA at the same time on the same plate, and the optical density was read. Background reactivity found in the BSA-coated wells was subtracted from the optical density found in each of the VLP-coated wells. Negative values were adjusted to zero. The cutoff level for HPV16 and HPV18 antibodies was based on the mean plus 3 SDs of the optical density values observed in sera from 20 Colombian infants (age range 1–2 years). These values were 0.325 for HPV16 and 0.370 for HPV18, and it was therefore decided to set the cutoff at 0.4 for both HPV16 and HPV18 antibodies, as in previous studies (23, 24, 29). An alternative analysis was also done using a different cutoff based on an iterative approach as described by Clifford et al. (5). The cutoffs produced by the iterative approach were 0.50 for HPV16 and 0.53 for HPV18. The results of this

alternative classification of seropositivity yielded substantially similar results (data not shown).

### HPV DNA testing methods

HPV DNA testing was done in the Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands, as described in individual study publications. The overall presence of HPV DNA was determined by performing a general primer GP5+/6+–mediated PCR (30). HPV positivity was assessed by hybridization of PCR products in an enzyme immunoassay using two HPV oligoprobe cocktails that, together, detect the following HPV types: HPV6, 11, 16, 18, 26, 31, 33–35, 39, 40, 42–45, 51, 52–59, 61, 66, 68, 70, 71 (equivalent to CP8061), 72, 73 (equivalent to MM9), 81 (equivalent to CP8304), 82 (IS39 and MM4 subtypes), 83 (equivalent to MM7), and 84 (equivalent to MM8) and CP6108. Subsequent HPV typing was done by enzyme immunoassay or reverse-line blot analysis of GP5+/6+ PCR products, as described previously (31, 32).

### Herpes simplex virus type-2 serum antibodies

Testing for the presence of type-specific IgG antibodies against herpes simplex virus type-2 (HSV-2) in plasma was conducted blindly in a central laboratory in Seattle, Washington, using a HSV-2 ELISA assay developed by Focus Technology (formerly MRL; ref. 33). All HSV-2–positive sera were retested to confirm results.

### Statistical analyses

The lifetime number of sexual partners was calculated by combining the information on regular and occasional partners. Current smokers were defined as women who had smoked at least one cigarette per day for a continuous period of 1 year or more. α-Species 9 includes HPV types 16, 31, 33, 35, 52, 58, and 67. α-Species 7 includes HPV types 18, 39, 45, 59, 68, 70, and 85 (1).

Unconditional logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) of HPV seropositivity according to several characteristics, including known risk factors for cervical cancer, and positivity for HPV16 and HPV18 DNA or for either of the two types (HPV16/18). ORs were adjusted for age (<25, 25–34, 35–44, 45–54, ≥55 years), study area, and lifetime number of sexual partners (1, 2, ≥3) as indicated. Tests for linear trend of ORs were done, giving an increasing score for each level of the categorized variable and fitting them into the model as continuous variables.

To analyze seropositivity by HPV DNA positivity, women were classified according to the presence of HPV16 and HPV18 DNA, or that of other high- or low-risk types of the same respective α-species (α-species 9 for HPV16 and α-species 7 for HPV18). Women with multiple infections were classified hierarchically as follows: (*a*) positive for HPV16 or HPV18, if one of these types was present; (*b*) positive for other types belonging to α-species 9 or 7; or (*c*) positive for types not belonging to α-species 9 or 7.

To assess the international correlation between HPV seroprevalence and DNA prevalence, linear regression models were fitted to the grouped data from each of the eight study areas. The strength of the ecological correlation between HPV16 or HPV18 seroprevalence and DNA prevalence of any HPV type was assessed using the Spearman rank correlation coefficient.

## Results

A total of 7,074 sexually active women with valid serology and HPV DNA results were included in this study. Table 1 shows HPV16 and HPV18 seropositivity according to HPV DNA status. HPV16 seropositivity was 9.7% in HPV DNA-negative women, 18.4% in women HPV

**Table 1.** ORs and corresponding 95% CIs of HPV16 or HPV18 seropositivity, by HPV DNA status (IARC HPV Prevalence Surveys)

| HPV DNA status | Total *n* | HPV seropositivity | |
|---|---|---|---|
| | | *n* (%) | OR (95% CI) |
| | | HPV16 | |
| Negative | 6,359 | 614 (9.7%) | 1 |
| Positive: types not belonging to α-species 9 | 386 | 71 (18.4%) | 1.3 (1.0–1.7) |
| Positive: types—other than 16—belonging to α-species 9 | 206 | 63 (30.6%) | 2.3 (1.7–3.2) |
| Positive: HPV16 | 123 | 49 (39.8%) | 4.1 (2.8–6.1) |
| | | HPV18 | |
| Negative | 6,359 | 514 (8.1%) | 1 |
| Positive: types not belonging to α-species 7 | 523 | 100 (19.1%) | 1.6 (1.2–2.0) |
| Positive: types—other than 18—belonging to α-species 7 | 136 | 16 (11.8%) | 0.9 (0.5–1.6) |
| Positive: HPV18 | 56 | 13 (23.2%) | 2.2 (1.1–4.2) |

NOTE: ORs were adjusted for age and study area.

**Table 2.** ORs and 95% CIs of HPV16 or HPV18 seropositivity by selected socioeconomic and behavioral characteristics (IARC HPV Prevalence Surveys)

| Characteristics | n* | HPV16-seropositive | | HPV18-seropositive | |
|---|---|---|---|---|---|
| | | (%) | OR (95% CI) | % | OR (95% CI) |
| Overall | 7,074 | 11.3 | | 9.1 | |
| Study area | | | | | |
| Spain | 908 | 0.8 | | 3.1 | |
| Argentina | 902 | 15.7 | | 7.9 | |
| Lampang, Thailand | 1,018 | 15.1 | | 12.2 | |
| Songkla, Thailand | 704 | 2.7 | | 2.7 | |
| Korea | 860 | 5.9 | | 9.0 | |
| Hanoi, Vietnam | 957 | 0.6 | | 0.2 | |
| Ho Chi Minh City, Vietnam | 803 | 20.9 | | 11.6 | |
| Nigeria | 922 | 27.1 | | 24.8 | |
| Age (y) | | | | | |
| <25 | 881 | 9.7 | 1 | 8.1 | 1 |
| 25–34 | 1,314 | 11.9 | 1.3 (0.9–1.7) | 9.4 | 1.1 (0.8–1.5) |
| 35–44 | 1,419 | 12.3 | 1.5 (1.2–2.1) | 8.9 | 1.2 (0.8–1.6) |
| 45–54 | 1,370 | 12.4 | 1.4 (1.1–1.9) | 10.0 | 1.2 (0.9–1.6) |
| ≥55 | 2,090 | 10.3 | 1.2 (0.9–1.6) | 8.9 | 1.1 (0.8–1.5) |
| Test for trend | | | 0.3; $P = 0.56$ | | 0.3; $P = 0.61$ |
| Education level | | | | | |
| None | 1,335 | 14.3 | 1 | 12.1 | 1 |
| Primary | 3,028 | 10.1 | 0.8 (0.7–1.1) | 9.0 | 1.1 (0.8–1.4) |
| Secondary | 2,709 | 11.0 | 0.8 (0.6–1.1) | 7.8 | 0.9 (0.6–1.2) |
| Test for trend | | | 4.37; $P = 0.04$ | | 1.73; $P = 0.19$ |
| Smoking status | | | | | |
| Never | 5,921 | 11.2 | 1 | 9.1 | 1 |
| Ever | 1,150 | 11.9 | 1.2 (1.0–1.6) | 9.0 | 1.3 (1.0–1.6) |
| Marital status | | | | | |
| Married | 5,526 | 10.9 | 1 | 8.9 | 1 |
| Widowed | 811 | 11.0 | 0.9 (0.7–1.2) | 9.9 | 1.0 (0.7–1.3) |
| Separated/divorced | 326 | 17.8 | 1.5 (1.1–2.0) | 12.3 | 1.3 (0.9–1.9) |
| Single | 409 | 11.5 | 1.6 (1.1–2.4) | 7.8 | 1.0 (0.7–1.6) |
| Lifetime number of sexual partners | | | | | |
| 1 | 5,588 | 9.6 | 1 | 7.9 | 1 |
| 2 | 976 | 15.1 | 1.2 (1.0–1.5) | 13.0 | 1.2 (0.9–1.5) |
| ≥3 | 492 | 22.8 | 1.9 (1.5–2.4) | 15.0 | 1.4 (1.0–1.8) |
| Test for trend | | | 22.0; $P < 0.001$ | | 5.45; $P = 0.02$ |
| Husband's extramarital sexual relationships | | | | | |
| No | 2,749 | 8.6 | 1 | 6.8 | 1 |
| Yes/unknown/uncertain | 4,298 | 13.1 | 1.1 (0.9–1.3) | 10.6 | 1.1 (0.9–1.3) |
| Age at first sexual intercourse (y) | | | | | |
| ≥22 | 2,365 | 10.6 | 1 | 7.9 | 1 |
| 19–21 | 2,280 | 10.7 | 0.9 (0.8–1.1) | 9.5 | 1.2 (1.0–1.6) |
| <19 | 2,427 | 12.4 | 1.0 (0.8–1.2) | 9.8 | 1.2 (1.0–1.6) |
| Test for trend | | | 0.0; $P = 0.99$ | | 3.32; $P = 0.07$ |
| Years since first sexual intercourse | | | | | |
| <15 | 2,320 | 11.4 | 1 | 8.8 | 1 |
| 15–30 | 2,344 | 11.8 | 0.9 (0.6–1.2) | 8.7 | 1.2 (0.8–1.7) |
| >30 | 2,408 | 10.6 | 0.8 (0.5–1.3) | 9.8 | 2.0 (1.2–3.2) |
| Test for trend† | | | 0.56; $P = 0.46$ | | 7.21; $P = 0.007$ |

(*Continued on the following page*)

**Table 2.** ORs and 95% CIs of HPV16 or HPV18 seropositivity by selected socioeconomic and behavioral characteristics (IARC HPV Prevalence Surveys) (Cont'd)

| Characteristics | n* | HPV16-seropositive | | HPV18-seropositive | |
|---|---|---|---|---|---|
| | | (%) | OR (95% CI) | % | OR (95% CI) |
| HPV18 seropositivity | | | | | |
| Negative | 6,431 | 9.0 | 1 | — | — |
| Positive | 643 | 34.1 | 3.1 (2.6–3.8) | — | — |

NOTE: ORs were adjusted for age, study area and lifetime number of sexual partners.
*Some figures do not add up to the total due to missing data.
†The result of the heterogeneity test, based on a direct comparison between HPV16- versus HPV18-seropositive women, excluding women positive for both seromarkers, was $\chi^2_1 = 4.46$ ($P = 0.03$).

DNA-positive for types not belonging to α-species 9, 30.6% in women HPV DNA-positive for α-species 9 types other than HPV16, and 39.8% for women HPV16 DNA-positive (OR for this category versus HPV DNA-negative women, 4.1; 95% CI, 2.8–6.1). Compared with HPV DNA-negative women (8.1%), HPV18 seropositivity was significantly higher in HPV18 DNA-positive women (23.2%; OR, 2.2; 95% CI, 1.1–4.2) and in women who were HPV DNA-positive for types not belonging to α-species 7 (19.1%; OR, 1.6; 95% CI, 1.2–2.0), but not in women who were HPV DNA-positive for α-species 7 types other than HPV18 (11.8%; OR, 0.9; 95% CI, 0.5–1.6; Table 1). Similar results were found when women with multiple HPV infections were excluded from the analyses (data not shown).

Table 2 shows, separately, the distribution and association of HPV16 and HPV18 seropositivity with socioeconomic and behavioral characteristics after adjustment for age group, study area, and lifetime number of sexual partners. HPV16 seroprevalence ranged from 0.6% in Hanoi, Vietnam, to 27.1% in Nigeria. Likewise, the lowest HPV18 seroprevalence was found in Hanoi, Vietnam (0.2%), and the highest in Nigeria (24.8%). In Argentina and Ho Chi Minh, Vietnam, HPV16 seroprevalence was significantly greater than HPV18 seroprevalence, whereas the opposite was true in Spain and Korea ($P < 0.05$). In all other study areas, seroprevalence for the two HPV types was similar.

Single women (OR, 1.6; 95% CI, 1.1–2.4), as well as those who were separated or divorced (OR, 1.5; 95% CI, 1.1–2.0), were more often HPV16-seropositive, but not HPV18-seropositive, than married women. A significant increase in HPV16 and HPV18 seropositivity was found with increasing lifetime number of sexual partners ($P$ trend <0.001 for HPV16 and 0.02 for HPV18). The ORs for three or more compared with one lifetime sexual partner were 1.9 (95% CI, 1.5–2.4) for HPV16 and 1.4 (95% CI, 1.0–1.8) for HPV18 seropositivity, respectively. HPV18 but not HPV16 seropositivity increased significantly with years since first sexual intercourse (HPV18: $P$ trend, 0.007; ORs for >30 compared with <15 years since first sexual intercourse, 2.0; 95% CI, 1.2–3.2; $\chi^2_1$ for heterogeneity, 4.46; $P = 0.03$). The results were substantially similar when years since first sexual intercourse was analyzed in four categories; that is, <5, 5–14, 15–30, and >30 years.

Smoking showed an association of borderline statistical significance with HPV16 seropositivity (OR for ever versus never smoking, 1.2; 95% CI, 1.0–1.6) and HPV18 seropositivity (OR for ever versus never smoking, 1.3; 95% CI, 1.0–1.6). HPV16 and HPV18 seropositivity were significantly associated (OR, 3.1; 95% CI, 2.6–3.8) with each other (Table 2). Oral contraceptives and condom use were not found to be associated with either HPV16 or HPV18 seropositivity (data not shown).

The association of HSV-2 seropositivity and abnormal cytologic results with different combinations of HPV DNA (any type) and antibodies against HPV16/18 is shown in Table 3. The risk of HSV-2 seropositivity was highest in women positive for both HPV DNA and antibodies (OR, 2.9; 95% CI, 2.2–3.8), followed by those positive for DNA alone (OR, 2.2; 95% CI, 1.8–2.7) or antibodies alone (OR, 1.7; 95% CI, 1.4–2.0), compared with women negative for both markers. Compared with women negative for both HPV antibodies and DNA, HPV16/18–seropositive women had an increased risk of having abnormal cytologic results only if they were also HPV DNA-positive (OR, 6.9; 95% CI, 4.7–10.0). The risk of having cervical abnormalities for women who were HPV DNA positive only was 5.8 (95% CI, 4.3–7.9; Table 3). Analyses in Table 3 were repeated in strata of younger and older women, producing similar results (data not shown).

Figure 1 shows the international correlation between HPV16/18 seroprevalence and overall HPV DNA prevalence among women of any age. A high positive correlation was found ($r = 0.81$, $P = 0.022$). Similar results were observed when HPV16/18 seroprevalence was correlated with HPV16/18 DNA prevalence (data not shown). However, in some countries HPV16/18 seroprevalence was lower (Argentina) or higher (Lampang, Thailand, and Ho Chi Minh, Vietnam) than in other study areas with comparable HPV DNA prevalence (Fig. 1). The

**Table 3.** ORs and 95% CIs of association of HPV DNA, any type, and/or antibodies against HPV16 and/or HPV18 with HSV-2 seropositivity and cytologic results (IARC HPV Prevalence Surveys)

| | HSV-2-seropositive vs-seronegative | | | Cytology abnormal vs normal | | |
|---|---|---|---|---|---|---|
| **HPV** | ***n*** | **HSV-2 pos** | **OR (95% CI)** | ***n*** | **Abn** | **OR (95% CI)** |
| DNA–/antibody– | 5,140 | 1,311 | 1 | 5,387 | 171 | 1 |
| DNA–/antibody+ | 944 | 456 | 1.7 (1.4–2.0) | 972 | 38 | 1.1 (0.8–1.6) |
| DNA+/antibody– | 449 | 232 | 2.2 (1.8–2.7) | 466 | 79 | 5.8 (4.3–7.9) |
| DNA+/antibody+ | 246 | 157 | 2.9 (2.2–3.8) | 249 | 52 | 6.9 (4.7–10.0) |

NOTE: ORs were adjusted for age, study area, and lifetime number of sexual partners. DNA positivity refers to any type of HPV; antibody positivity refers to HPV16 and/or HPV18.

correlation coefficients between seroprevalence and HPV DNA prevalence were similar when women below the age 35 and 35 years or older were evaluated separately (data not shown).

Figure 2 shows age-specific seroprevalence and DNA prevalence for HPV16/18 by study area. Seroprevalence in all areas was more elevated than DNA prevalence although to a different extent in different countries. Contrary to DNA prevalence, HPV16/18 seroprevalence tended to increase, in most study areas, from young to middle age, and then decline or remain fairly constant.

Positivity for HPV16/18 DNA only, HPV16/18 DNA and serology, and serology only were shown cumulatively in the same histogram (Fig. 3). Among women positive for at least one of the two markers of HPV infection, 87% were positive for antibodies against HPV16/18 only.

## Discussion

In the present pooled analysis of the IARC HPV Prevalence Surveys, we found that HPV16 and HPV18 seroprevalence varied greatly across study areas, from less than 1% each in Hanoi, Vietnam, to 25% or more in Nigeria. These differences are consistent with the broad range of HPV16 and HPV18 seroprevalence previously observed by other authors (5, 14, 17, 34) and by us with respect to HPV DNA prevalence in the same studies (20).

At the individual woman level, our findings showed that women who were HPV16 or HPV18 DNA-positive were substantially more likely to be seropositive for antibodies against the same type, compared with HPV16 or HPV18 DNA-negative women. Among HPV16 or HPV18 DNA-positive women, however, a substantial proportion were seronegative for the same type (i.e., 60% for HPV16 and 77% for HPV18). The only moderate agreement between HPV antibodies and DNA that we found in our study women has also been reported by others (5, 17). The excess of seropositivity detected in women who were DNA-positive for a type other than HPV16 or HPV18 compared with women DNA-negative for any HPV type might reflect the fact that HPV antibodies

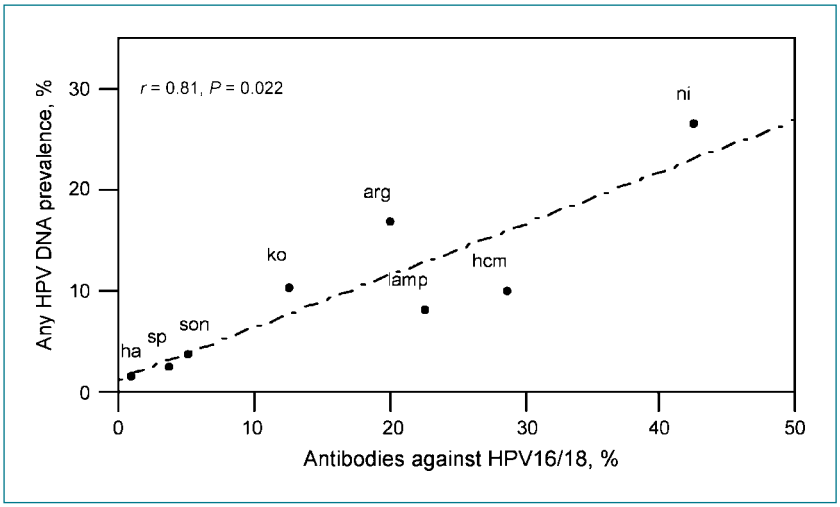


**Figure 1.** International correlation between prevalence of antibodies against HPV16/18 and overall HPV DNA prevalence. IARC HPV Prevalence Surveys. arg, Argentina; ha, Hanoi, Vietnam; hcm, Ho Chi Minh, Vietnam; ko, Korea; lamp, Lampang, Thailand; ni, Nigeria; son, Songkla, Thailand; sp, Spain.

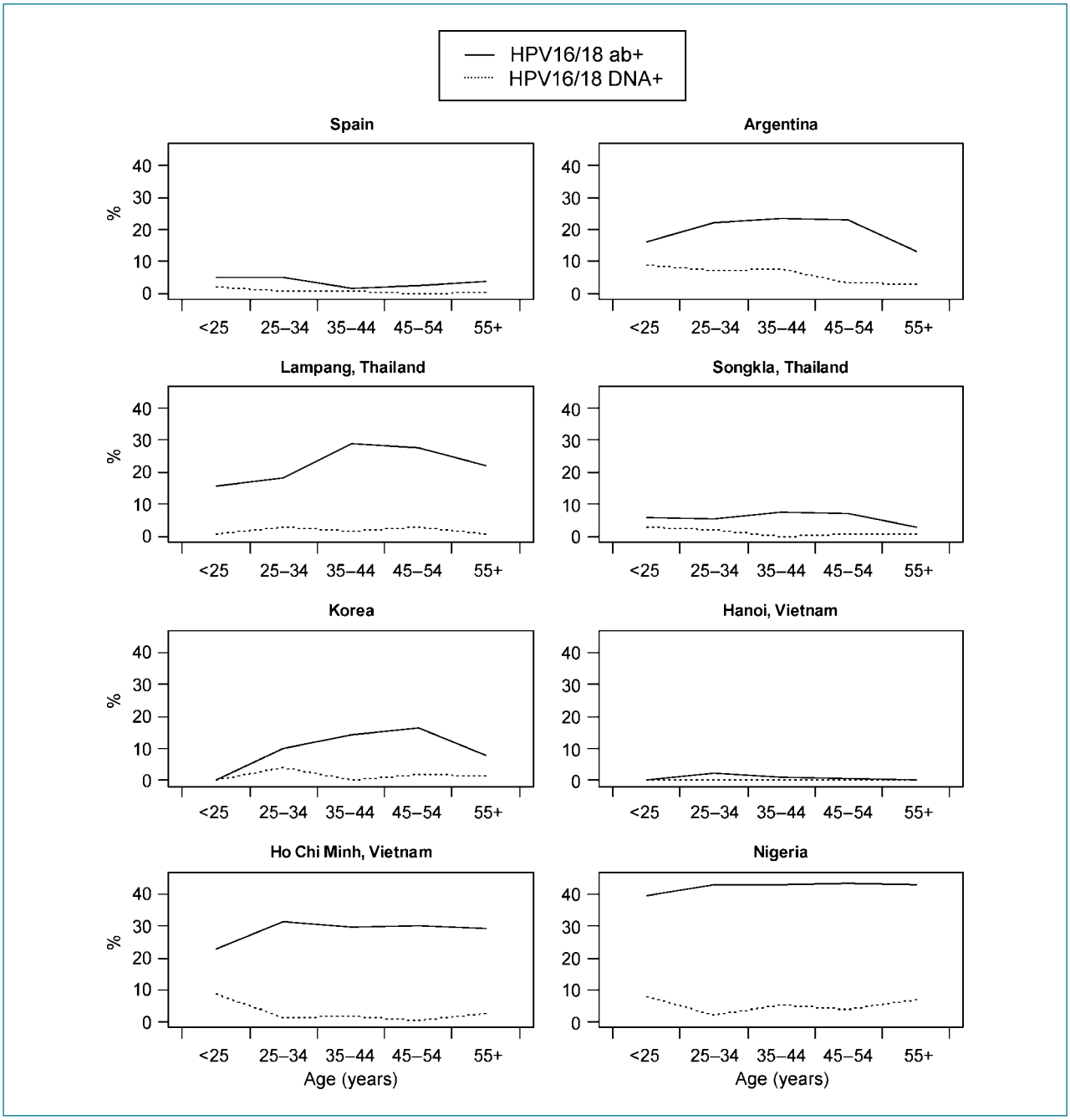


**Figure 2.** Age-specific prevalence of HPV16/18 DNA and antibodies against HPV16/18 by study area. IARC HPV Prevalence Surveys. ab, antibodies.

are a proxy of high exposure to HPV infection in general. Alternatively, it might suggest a certain degree of cross-reactivity between serotypes, notably in α-species 9. Finally, a high proportion of HPV16- or HPV18-seropositive women were DNA-negative for the same type (93.9 and 98.0, respectively), as could be expected given the transient nature of HPV DNA positivity.

Previous analyses of the same study populations revealed a much higher prevalence of HPV16 DNA compared with HPV18 DNA in each study area (21-26). Conversely, our present findings showed that HPV16 seroprevalence was, in most areas, similar to or lower than HPV18 seroprevalence. Explanations for this discrepancy include the possibility that HPV18-infected women seroconvert more often or remain seropositive longer than HPV16-infected women.

HPV seropositivity was previously found to be linked with several characteristics, mainly sexual activity

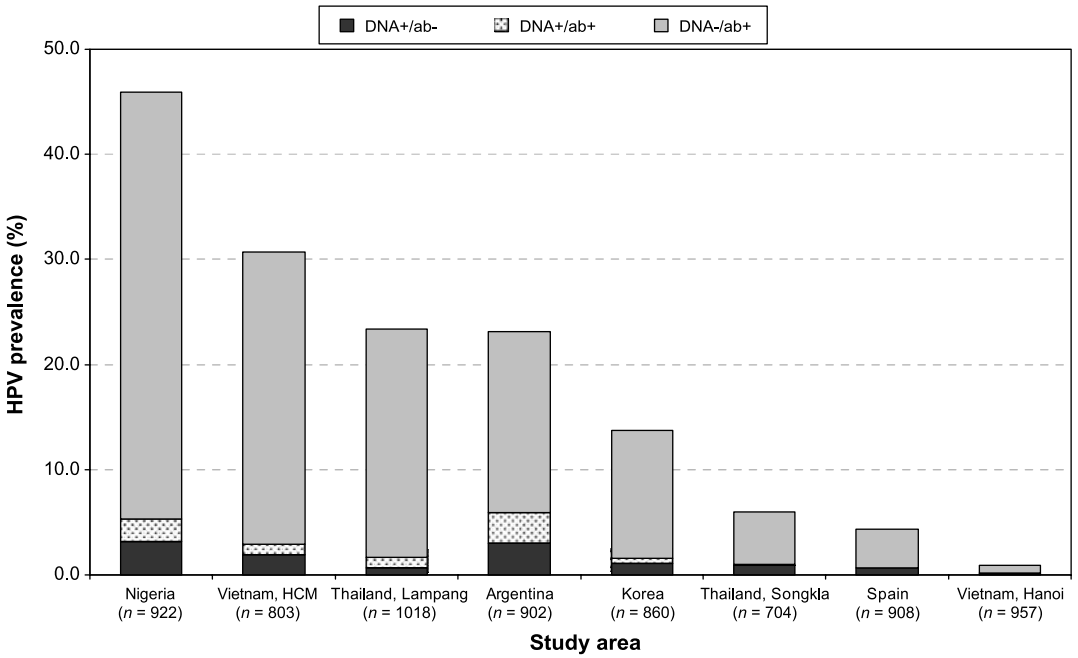


**Figure 3.** Prevalence of different combinations of HPV16/18 DNA and antibodies against HPV16/18 by study area. IARC HPV Prevalence Surveys. ab, antibodies; HCM, Ho Chi Minh.

indicators such as lifetime number of sexual partners, HSV-2 seropositivity, and marital status, as well as to oral contraceptive use and smoking (5, 8, 14, 17, 23, 35-40). In the present study, a clear dose-response relationship was observed for both HPV16 and HPV18 seropositivity with lifetime number of sexual partners, the most important risk factor for cervical HPV DNA prevalence in the same populations (41). Smoking, and being single, separated, or divorced increased the risk of HPV16 seropositivity even after adjustment for age, study area, and lifetime number of sexual partners. Smoking was previously found to be associated with HPV DNA prevalence in the same study populations (42). Also consistent with findings for HPV DNA positivity (41), husband's extramarital sexual relationships and early age at first sexual intercourse were not associated significantly with HPV16 or HPV18 seropositivity. HPV18, but not HPV16 seropositivity, increased significantly with years since first sexual intercourse.

Some authors reported a moderate association of HPV seropositivity with the presence of cytologic cervical abnormalities (43) and cervical cancerous or precancerous lesions (35, 44-47). Our analyses of different combinations of HPV16/18 seropositivity and overall HPV DNA positivity clearly showed that seropositive women were at a significantly increased risk of having cervical cytologic abnormalities only if they were also HPV DNA-positive. In addition, given HPV DNA status, HPV16/18 seropositivity did not predict the risk of having cytologic abnormalities. Conversely, the risk of HSV-2 seropositivity, a useful marker of sexual behavior, increased from women with neither to women with one or both markers of HPV.

Age-specific HPV16/18 seroprevalence showed a tendency to increase slightly up to middle age and then to stabilize or decrease, in agreement with previous HPV serology findings (17). In contrast with seroprevalence, HPV DNA prevalence was, in most study areas, highest at younger ages, and declined thereafter (20). The discrepancy between the increase in HPV seroprevalence and the decrease in DNA prevalence at younger ages observed in most study areas could be explained by a need for repeated exposure or persistent infection to induce a detectable antibody response (17). A decline in HPV seroprevalence at older ages in some study areas could represent waning of seropositivity after HPV exposure ceases (17).

The reason for the discrepancy between seroprevalence and DNA prevalence observed in some study areas is unclear. The Guanacaste cohort study showed that serologic status was a dynamic result of seroconversion and clearance (48). Only 55% of 1,216 women HPV16-seropositive at enrollment remained positive at 5 to 7 years of follow-up, while 6% seroconverted.

A correlation analysis showed, however, that HPV16/18 seroprevalence was fairly consistent with the prevalence of overall HPV DNA or HPV16/18 DNA across study areas. The magnitude of the correlation coefficients was similar in younger and older women.

Limitations of the present study are common to all HPV serologic studies (49) and include the lack of agreement on the level of antibodies that is necessary to indicate seroconversion. HPV-negative reference sera are difficult to find, and the levels of HPV antibodies are low and continuously distributed (5). Therefore, the choice of the cutoffs for seropositivity is always relatively arbitrary. For the sake of consistency, we used type-specific cutoffs that were based on the distribution of the optical density values in Colombian children; the same cutoffs have been used previously to assess seropositivity in different studies (23, 24, 29), some of which are included in this pooled analyses (23, 24). The choice of higher or lower cutoffs would have decreased or increased HPV seropositivity. The use of an alternative cutoff based on an iterative approach (5) led to similar findings with respect to the association of seropositivity with women's characteristics and the presence of HPV DNA.

A strength of our study was the use of the same protocols and methods for the evaluation of HPV DNA and serology in all study areas. This allowed the comparison of HPV seroprevalence across a wide range of populations and the assessment of the correlation between the prevalence of HPV DNA and antibodies. However, on account of the different characteristics of the serologic assays, a direct comparison of results with those from studies that used different serologic assays was not possible. For instance, the ELISA assay we used cannot distinguish between neutralizing and nonneutralizing antibodies.

In summary, the use of standardized methods for HPV DNA and serology testing showed that, although the correlation between the presence of HPV DNA and antibodies against HPV16/18 was not very good at an individual woman level, it was high at a population level. HPV serology is a poor marker of current infection or related cytologic lesions, but it can contribute, together with DNA, in evaluating the distribution of HPV infection worldwide.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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