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The IARC Commitment to Cancer Prevention: The Example of Papillomavirus and Cervical Cancer

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Abstract Every year approximately half a million women worldwide develop cervical cancer (CC) of whom 80% live in poor countries where population-based screening programmes are virtually non-existent. The role of sexually transmitted agents in the aetiology of cervical cancer has been suspected for more than a century, but knowledge in this field has rapidly expanded only in the last 20 years, after major improvements were made in detection methods for human papillomavirus (HPV). A dozen types of HPV have been identified in 99% of biopsy specimens from CC worldwide and the relative risk estimates for HPV in case-control studies of CC are in the 50 to 100 range. A meta-analysis done at the International Agency for Research on Cancer (IARC) included a total of 10,058 CC cases from 85 published studies. The most common HPV types identified in CC were, in order of decreasing prevalence, HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70. Over two-thirds of CC cases were associated with an infection of either HPV 16 (51.0%) or HPV 18 (16.2%). Despite the overwhelming importance of HPV, other factors contribute to the rare occurrence of CC after HPV infection. Nine case-control studies from the IARC have confirmed the adverse effect of long-term use of oral contraceptives, high parity, smoking and sexually transmitted infections (i.e. *Chlamydia trachomatis* and herpes simplex virus-2) after adjustment for, or stratification by, HPV infection. Ten surveys of HPV infection in population-based samples of approximately

15,000 women in four continents have shown that: (1) the prevalence of HPV infection varies greatly (between 2% and nearly 30%); and (2) the age distribution also varies widely, pointing to cohort effects. There is no effective medical treatment for HPV, but a prophylactic vaccine, based on late (L) 1 HPV 16 proteins, has been shown to be safe, highly immunogenic and efficacious in preventing persistent HPV infections. A multivalent vaccine against the most common oncogenic HPV types may thus ultimately represent the most effective way to prevent CC worldwide either alone, or in combination with screening. It may, however, take several years before this approach becomes a reality. Thus, early detection of CC precursor lesions by screening, and their treatment, will remain the most important measures for the control of CC for the foreseeable future.

1 Introduction

Every year approximately half a million women worldwide develop cervical cancer (CC) of whom 80% live in poor countries where population-based screening programmes are virtually non-existent. Screening with cervical cytology has greatly helped to reduce the incidence of, and death from, CC in developed countries through the detection and treatment of cervical precancerous lesions (many years before CC occurs), so that they do not progress to invasive cancer, and possibly death (Cuzick et al. 2000). However, the risk of CC remains high in many developing countries, mostly due to the lack or inadequacy of existing prevention programmes.

The role of sexually transmitted agents in the aetiology of CC has been suspected for more than a century, but knowledge in this field has rapidly expanded only in the last 20 years, after major improvements were made in detection methods for human papillomavirus (HPV) (Cuzick et al. 2000). A dozen types of HPV have been identified in 99% of biopsy specimens from CC worldwide (Walboomers et al. 1999) and the relative risk estimates for HPV in case-control studies of CC are generally greater than 100 (International Agency for Research on Cancer 1995).

There is no effective medical treatment for HPV, but a prophylactic vaccine, based on late (L) 1 HPV 16 proteins, has been shown to be safe and highly immunogenic (with anti-HPV IgG titres many times higher than those that follow natural infection, Villa et al. 2002). It has also proved to be efficacious in preventing persistent HPV infections in a trial of 1,523 HPV 16-naive young women in the United States (Koutsky et al. 2002). A multivalent vaccine against the most common oncogenic HPV types may thus ultimately represent the most effective way to prevent CC worldwide, either alone or in combination with screening.

It may, however, take several years before this approach becomes a reality. Thus, early detection of CC precursor lesions by screening and their treatment will remain the most important measures for the control of CC for the foreseeable future. However, cytology-based screening is cost intensive, and the

organisation of pap smear-based screening programmes in many high-risk developing countries is a major challenge in the face of limited health care resources and other competing health priorities. Thus, simple, effective, low-cost and low-technology alternatives to cervical cytology for CC prevention are urgently needed for high-risk countries. Visual inspection of the cervix uteri with acetic acid and with Lugol's iodine, which are based on the ability of the trained health personnel to detect acetowhite areas or yellow non-iodine intake areas in the cervical transformation zone, are currently being evaluated in experimental settings by the International Agency for Research on Cancer (IARC) as alternatives to cervical cytology (Sankaranarayanan et al. 2003; Sankaranarayanan and Wesley 2003).

The IARC has contributed substantially to progress in the HPV field through international collaborative studies, especially:

- Case-series investigations where the range of HPV types in cancer specimens can be identified
- Case-control studies, where relative risk for HPV and other risk factors can be computed
- Population-based surveys where the prevalence of, and risk factors for, HPV in women with different cytological findings can be studied
- Design of possible trials of new vaccines against HPV in order to accelerate the introduction of such vaccines in developing countries

Major recent achievements of IARC studies and plans for future studies will be reviewed, with a special focus on those which are essential to translate our knowledge on HPV into successful vaccination programmes.

2
Methods and Results

2.1
Distribution of Different HPV Types in Invasive and Pre-invasive CC Carcinomas

Prophylactic vaccines against particular HPV types hold great promise for reducing the global burden of CC. However, some 15 oncogenic HPV types have been suggested to be associated with CC, and the relative prevalence of these types may vary by region (Muñoz et al. 2003). We wanted, therefore, to identify worldwide and regional priorities for HPV types to be included in potential vaccines. Given that the final outcome in vaccine efficacy trials will be the prevention of pre-cancerous lesions (Plummer and Franceschi 2002), we have also tried to determine if the distribution of HPV types in high-grade squamous intra-epithelial lesions (HSIL) is representative of those that

go on to cause cancer, or if certain types are more likely to progress to malignancy.

All published studies presenting type-specific HPV prevalence data on CC and/or HSIL were identified and classified by geographical region (Clifford et al. 2003a). Worldwide and regional prevalence was estimated for each HPV type by performing a meta-analysis of all studies presenting data on each particular type. HPV type-specific prevalence was estimated independently for squamous cell (SCC), and adeno- and adenosquamous carcinoma (ADC). The relative risk for individual HPV types to progress from HSIL to malignancy was investigated by comparing HPV type distribution in HSIL and SCC.

The meta-analysis included a total of 10,058 CC, and 4,151 histologically verified HSIL cases drawn from 85 and 52 published studies, respectively. The most common HPV types identified in CC were, in order of decreasing prevalence, HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70 (Fig. 1). Over two-thirds of CC cases were associated with an infection of either HPV 16 (51.0%) or HPV 18 (16.2%). The next most prevalent types were HPV 45 (2%–8%), HPV 31 (2%–7%) and HPV 33 (3%–5%) in all regions except Asia where HPV types 58 (6%) and 52 (4%) were more prevalent than elsewhere. The HPV 16 family of viruses was more commonly found in SCC, whereas the HPV 18 family was more likely to be found in ADC. This study reinforces the view that HPV 16 and HPV 18 are the most important HPV types for vaccination in all regions. However, the relative priorities for these types vary somewhat by region.

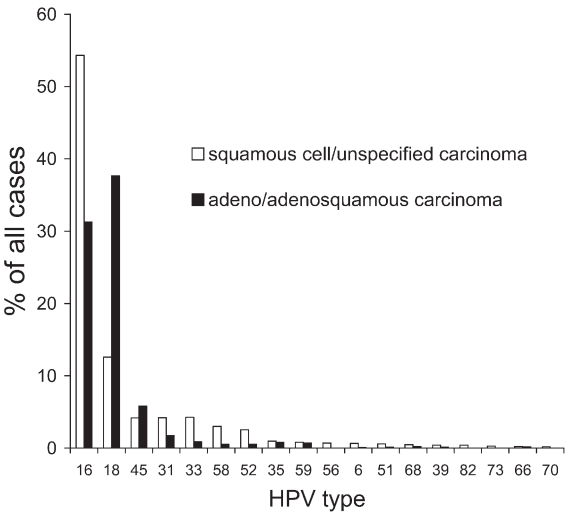

Fig. 1. Type-specific prevalence of human papillomavirus (*HPV*) in 10,058 worldwide cases of invasive cervical cancer by histological type. (Clifford et al. 2003a)

Table 1. Comparison of overall and type-specific HPV prevalence between SCC and HSIL cases (Clifford et al. 2003b)

HPV type	SCC		HSIL		SCC:HSIL prevalence ratio[a]	
	n	HPV %	*n*	HPV %		
All	8,550	87.6	4,191	84.0	1.04	(1.03,1.06)
16	8,594	54.3	4,191	45.6	1.19	(1.15,1.24)
18	8,502	12.6	4,191	7.2	1.74	(1.52,2.04)
33	8,449	4.3	4,155	7.3	0.59	(0.52,0.67)
45	5,174	4.2	1,835	2.5	1.70	(1.25,2.68)
31	7,204	4.2	3,889	9.1	0.46	(0.42,0.52)
58	5,646	3.0	2,084	6.6	0.45	(0.39,0.55)
52	5,304	2.5	2,062	4.8	0.53	(0.44,0.68)
35	6,223	1.0	2,704	4.4	0.22	(0.18,0.27)
59	4,488	0.8	1,489	1.5	0.54	(0.37,0.99)
56	4,493	0.7	1,872	3.2	0.22	(0.17,0.30)
51	4,580	0.6	1,981	3.2	0.19	(0.15,0.25)
68	4,148	0.5	1,437	1.1	0.43	(0.28,0.91)
39	3,899	0.4	1,841	1.0	0.39	(0.26,0.76)
66	4,799	0.2	1,670	2.2	0.10	(0.08,0.15)

HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell/unspecified carcinoma of the cervix.
[a] With 95% confidence intervals.

HPV type-specific prevalence data was summarised in a similar manner for HSIL, and the distribution of HPV types compared across SCC and HSIL (Clifford et al. 2003b). HPV 16 was the most common type in both SCC (54.3%) and HSIL (45.6%), but was more prevalent in SCC (SCC:HSIL ratio=1.19). HPV 18 and HPV 45 were also more prevalent in SCC than in HSIL, whereas the opposite was true for all other high-risk types (Table 1). Thus, HSILs infected with HPV 16, 18 or 45 appear to have greater potential for progression and any beneficial effect identified by randomised trials from the proportion of HSIL preventable by HPV 16 or HPV 16/18 vaccines may be an under-estimate of the beneficial effect of the vaccine on the prevention of invasive cervical cancer (ICC).

3 Major Risk Factors Other Than HPV

The role of cofactors that may influence the rare progression from HPV infection to CC retains a great importance. Such cofactors may be in the environment, the host or the virus itself. The identification of cofactors for HPV not only improves our understanding of the aetiology of CC, but may also be useful from a prevention standpoint.

Between 1985 and 1997, twelve case-control studies of CC were conducted by the IARC in 10 countries: Brazil (Eluf-Neto et al. 1994), Colombia and Spain (Muñoz et al. 1992; Bosch et al. 1993), Paraguay (Rolón et al. 2000), Peru (Santos et al. 2001), Mali (Bayo et al. 2002), Morocco (Chaouki et al. 1998), the Philippines (Ngelangel et al. 1998), Thailand (Chichareon et al. 1998) and India (Franceschi et al. 2003a; Rajkumar et al. 2003). These studies, which were published separately, have now been pooled in order to investigate the role of cofactors. Pooling of the data was facilitated by the common protocol used in all studies, which included a personal interview, collection of a blood sample and cervical scrapes for the identification of HPV DNA.

The following cofactors were investigated: oral contraceptive (OC) use, parity, smoking, HPV type, and the sexually transmitted infections herpes simplex virus-2 (HSV-2) and *Chlamydia trachomatis*. Many of these cofactors have been studied previously, and in some cases have been suspected for decades to be associated with CC risk. However, previous studies have not controlled for the strong confounding effect of HPV infection. The primary advantage of the pooled case-control study over previous studies is the use of accurate polymerase chain reaction (PCR)-based assays for the detection of HPV DNA from a wide range of types. The second advantage of the study is its size. In total, 2,506 women with CC and 2,491 control women were interviewed. Of these, 1,739 cases and 259 controls were HPV DNA positive. The large study size allows rarer cofactors to be studied that could not be adequately addressed in a single study. Finally, exposure to HSV-2 and *C. trachomatis* was assessed using gold standard assays.

Our current understanding of HPV as a necessary cause of CC implies that any cofactor must act in one of two ways: either by increasing the risk of acquiring HPV infection (and it would therefore be found to be associated with HPV infection among control women), or by increasing the risk of progression from infection to cancer. Risk factors for acquisition of HPV are discussed in Sect. 4. Risk factors for progression, which are reported here, were mainly evaluated by restricting the analysis to HPV-positive cases and HPV-positive controls.

Results are reported separately for each cofactor, but the analyses followed a common pattern. All analyses were adjusted for age and centre, which were frequency-matching variables, number of sexual partners, age at first intercourse and pap smear history, which are potential confounding factors. Confounding by the other cofactors listed here was also investigated.

3.1
Oral Contraceptive Use

In the multi-centric study, data were combined from 10 of the 11 studies to investigate the role of OCs. Among HPV-positive women, use of OCs was associated with an odds ratio (OR) of 1.42 [95% confidence interval (CI): 0.99–2.04] (Moreno et al. 2002). For duration of use, no increased risk was observed for users of less than 5 years compared with never-users (OR=0.73; 95% CI: 0.48–1.12) but a significantly increased risk was observed for use of 5 years or longer (OR=3.42; 95% CI: 2.00–5.84).

The role of OCs as a cofactor for CC was further investigated in a systematic review (meta-analysis) of published data, carried out in collaboration with the Cancer UK Epidemiology Unit, Oxford (Smith et al. 2003). The review included 28 studies and 12,531 women with ICC or in situ carcinomas of the cervix.

The excess risk increase for OC use of less than 5 years, 5–9 years, and 10 years or more were 10%, 60% and 120%, respectively. The results were broadly similar in developed and developing countries, for ICC and in situ CC, for SCC and ADC. In addition, they did not differ depending upon whether findings had been adjusted for HPV status, number of sexual partners, cervical screening, smoking and use of barrier contraceptives. The association with OC use was, however, consistently stronger in cohort than case-control studies. The limited available evidence (Fig. 2) suggests that the relative risk of CC may decrease after cessation of OC use (Smith et al. 2003).

Questions concerning the persistence of any effect of OC is critical when considering the absolute risk of CC among past users, hence the public

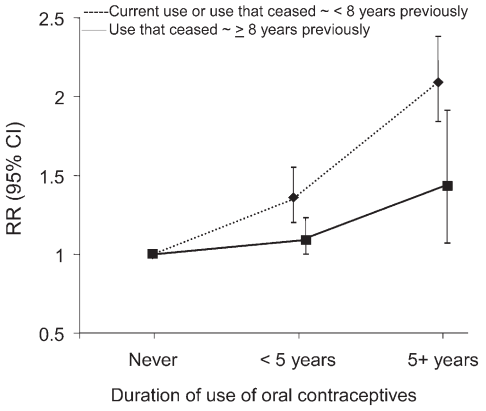

Fig. 2. Summary results on the relative risk (*RR*) with 95% confidence intervals (*CI*) of cervical cancer, according to time since last use and duration of use of oral contraceptives. (Smith et al. 2003)

health implications of our findings. Systematic reviews like ours are severely hampered by the lack of published data cross-classifying women by duration of use and time since last use. Henceforth, a decision has been made to promote a collaborative re-analysis of individual data from all relevant studies on CC, with the support of the WHO Human Reproduction Unit. All contributors have been contacted and asked to provide original data. Over 15,000 CC cases and 30,000 healthy women will be analysed.

3.2 Parity

Data from ten of the IARC case-control studies were used to examine the effect of parity (Muñoz et al. 2002). Among HPV-positive women, a direct association was found between the number of full-term pregnancies and risk of SCC. The OR for seven or more full-term pregnancies was 3.82 (95% CI: 1.90–7.67) compared with nulliparous women, and the trend was highly significant ($p<0.001$). No significant association was found between parity and ADC. Number of abortions and age at menarche and menopause were unrelated to the risk of CC of any histological type. It was concluded that high parity increases the risk of SCC among HPV-positive women and the decline in parity seen in most countries might partly explain the reduction in CC.

3.3 Smoking

Data from ten studies were combined to examine the effect of smoking (Plummer et al. 2003). Any degree of smoking was associated with an increased risk of SCC (OR=2.08; 95% CI: 1.33–3.27) compared with never smoking among HPV-positive women. There was no difference in risk between current and ex-smokers. The prevalence of smoking among women in the populations studied was low, and this precluded an investigation of dose-response effects by number of cigarettes per day or duration of smoking. We concluded that smoking increases the risk of SCC. No clear conclusions could be drawn for ADC due to small numbers. An important public health implication of this finding is that the widespread increase in smoking rates among young women may have an impact on CC incidence.

3.4 Herpes Simplex Virus-2

Data from seven studies were combined to examine the effect of infection with HSV-2 (Smith et al. 2002). In five studies, serum antibodies against herpes simplex virus-1 (HSV-1) and HSV-2 were tested by Western blot, which is considered the reference gold standard. In the other two studies, HSV-2 IgG antibodies were tested using a commercial kit (Gull/Pre-Meridian

HSV-2 ELISA). Among HPV-positive women, HSV-2 positivity was associated with increased risk of SCC (OR=2.19; 95% CI: 1.41–3.40) and ADC (OR=3.37; 95% CI: 1.47–7.74). Further adjustment for number of sexual partners, age at first sexual intercourse, infection with *C. trachomatis*, and use of OCs did not substantially reduce the OR for HSV-2. The principal advantage of this study is that the HSV assays used are type-specific, and so can distinguish between HSV-2 infections (which are almost exclusively genital) and HSV-1 infections (which are primarily non-genital).

3.5
Chlamydia trachomatis

Serum antibodies to *C. trachomatis* were tested in seven studies by a microimmunofluorescence assay, which is considered the gold standard measurement (Smith et al. 2004). Since antibodies against *C. trachomatis* are persistent, this assay measures cumulative exposure to past infections rather than current infection. The OR for the presence of *C. trachomatis* antibodies was 1.7 (95% CI: 1.1–2.5) in HPV-positive women. Additionally, a significant trend in risk ($p<0.001$) was observed with increasing *C. trachomatis* antibody titre. As with HSV-2, further adjustment for sexual variables did not eliminate the association with *C. trachomatis*.

3.6
The Male Role

Seven IARC case-control studies on CC also allowed the evaluation of HPV penile infection in the husbands of 445 women with ICC, 165 women with in situ carcinoma and 717 control women. The strongest variation in penile HPV infection was by country, with percentages among the husbands of control women ranging between 3% in Spain to 39% in Brazil. Having over 50 lifetime sexual partners (compared to only one) was associated with an OR of 2.3 (Franceschi et al. 2002). Male circumcision was associated with a reduced risk of penile HPV infection (OR=0.4) and of CC in monogamous women (OR=0.7) (Castellsagué et al. 2002).

Table 2 shows the comparison of the associations between cervical carcinoma and OC use, parity (among parous women only), smoking, HSV-2 and *C. trachomatis* according to three different models. Adjustment for HPV or restriction to HPV-positive women did not change most of the ORs in Table 2, with the possible exception of those for OC use. This highlights the difficulty of taking the strong effect of HPV infection on CC risk into account. The lack of substantial impact of adjustment for HPV or restriction to HPV-positive cases and controls suggest that either: (1) the currently available marker of HPV status (i.e. the presence of HPV DNA in cervical cells) is inadequate (e.g. because it has a different meaning in cases with CC and

Table 2. ORs[a] and 95% CIs[b] for squamous cell carcinoma of the cervix according to OC use, parity, smoking, HSV-2 and *Chlamydia trachomatis* serology, and by different models in the pooled analysis of IARC case-control studies

	All women	All women HPV-adjusted	HPV-positive women
	OR (95% CI)	OR (95% CI)	OR (95% CI)
OC use (years)			
Never	1.0 (0.9–1.1)	1.0 (0.8–1.2)	1.0 (0.8–1.3)
1–5	1.0 (0.9–1.2)	0.8 (0.6–1.1)	0.8 (0.6–1.0)
5–9	1.3 (1.1–1.6)	1.4 (0.9–2.0)	2.4 (1.3–4.5)
≥10	1.6 (1.3–1.9)	1.6 (1.1–2.3)	2.6 (1.4–4.6)
No. of full-term pregnancies			
1–2	1.0 (0.8–1.2)	1.0 (0.8–1.3)	1.0 (0.7–1.4)
3–4	1.5 (1.3–1.7)	1.5 (1.2–1.8)	1.4 (1.1–1.9)
5–6	1.8 (1.6–2.2)	1.8 (1.4–2.4)	1.6 (1.2–2.2)
≥7	2.1 (1.8–2.5)	1.7 (1.3–2.3)	2.2 (1.6–3.2)
Smoking			
Never	1	1	1
Ever	1.6 (1.3–1.9)	2.4 (1.8–3.2)	2.1 (1.3–3.3)
HSV-2			
Negative	1	1	1
Positive	1.8 (1.5–2.3)	1.5 (1.1–2.2)	1.9 (1.2–2.8)
C. trachomatis			
Negative	1	1	1
Positive	2.1 (1.7–2.6)	1.8 (1.3–2.5)	1.9 (1.3–2.8)

CI, confidence interval; HPV, human papillomavirus; HSV, herpes simplex virus; OC oral contraceptive; OR, odds ratio.
[a] Adjusted for age, centre, education, parity, age at first intercourse, sexual partners, pap smears, smoking, and OC use.
[b] Confidence intervals for OC use and number of full-term pregnancies are floating confidence intervals.

control women); or (2) HPV infection does not confound or modify the associations observed between CC and OC use, number of full-term pregnancies and smoking.

4 HPV Infection in Healthy Women

The incidence rates of CC vary more than ten-fold worldwide. Even after excluding countries where screening programmes have contributed to lowering rates, CC incidence ranges between less than 10/100,000 women in some parts of China, North Vietnam and Kuwait to more than 35/100,000 in Sub-Saharan Africa and some areas in India and Latin-America (Parkin et al. 2002). It is unclear to what extent such variation is attributable to differ-

ences in HPV prevalence at a population level. IARC has, therefore, promoted a series of population-based surveys of the prevalence of HPV DNA and serum IgG against HPV virus-like particles (anti-VLPs) in different parts of Latin America, Asia and Africa and in Spain and Italy. Type-specific HPV prevalence data have become even more important in the light of recent developments in prophylactic vaccines against specific types of HPV.

Similar prevalence surveys among random samples of women drawn from the general population have been completed and reported in South Korea (Busan, Shin et al. 2003), Thailand (Songkla in the South and Lampang in the North, Sukvirach et al. 2003), Vietnam (Hanoi and Ho Chi Minh City, Anh et al. 2003), Argentina (Cordoba, Matos et al. 2003), Colombia (Bogota, Molano et al. 2002a; Molano et al. 2002b; Molano et al. 2003a; Molano et al. 2003b), Mexico (Morellos State, Lazcano-Ponce et al. 2001), Nigeria (Thomas et al. 2004) and Spain (de Sanjosé et al. 2003). Additional surveys have been started in Turin, Italy; Santiago, Chile; Ambillikai, India; and Kampala, Uganda. Questionnaire information, and samples of exfoliated cervical cells and blood were collected. Type-specific prevalence of HPV DNA from cervical cells was analysed using GP5+/6+ primers, whereas anti-VLPs for HPV 16, 18, 31, 33 and 58 and HSV-2 were assessed using enzyme-linked immunosorbent assay. It is important to bear in mind that it turned out to be very difficult, notably in Asia, to perform pelvic examinations on unmarried/virgin women. The following findings are, therefore, truly representative of HPV DNA prevalence among married/sexually active women in the 15- to 65-year age range.

Korea. Overall HPV prevalence among 863 sexually active women was 10.4% for HPV DNA and 19.8% for anti-VLPs. The HPV types found most frequently were HPV 70, 16 and 33. The concordance between HPV DNA and anti-VLPs at an individual level was modest, but risk factors for the two HPV markers were similar. Risk factors for detection of HPV DNA or anti-VLPs were: number of lifetime sexual partners (OR for $\geq$4 vs 1=3.5 and 5.4 respectively), seropositivity for HSV-2 antibodies (OR=2.6 and 2.5, respectively), and being single or divorced. HPV DNA (but not anti-VLPs) was elevated among women whose husbands were thought by their wives to have extra-marital sexual relationships and those who had undergone a vasectomy.

Thailand. 1,035 women from Lampang, in the North, and 706 from Songkla, in the South were studied. HPV DNA and anti-VLPs were more common in Lampang (8.0% and 29.2%, respectively) than in Songkla (3.8% and 10.9%, respectively), in agreement with a North-South gradient in CC incidence in Thailand. The most common HPV types were HPV 16, 52, and 70. Risk factors for HPV infection were young age (<25 years, OR=2.5), HSV-2 seropositivity (OR=2.1), and a husband's extra-marital sexual relationships

(OR=2.1). Risk factors did not differ between high- and low-risk types and women below and above 45 years of age.
Vietnam. 922 women from Ho Chi Minh City and 994 from Hanoi were studied. HPV DNA prevalence was 10.9% and 2.0%, respectively. The most common types were HPV 16, 58 and 18. The major risk factors for HPV DNA detection were indicators of sexual habits, most notably the presence of HSV-2 antibodies (OR=2.4), nulliparity (OR=3.0) and current use of OCs (OR=3.2). Women in Hanoi showed the lowest HPV prevalence found so far in HPV surveys. In contrast to other populations, no HPV peak was detected in young women.

Argentina. In all, 987 women were studied. The prevalence of HPV DNA among sexually active women was 17.7%. The most common types were HPV 16, 35 and 18. Among women below age 45 the main risk factors for HPV detection were increasing lifetime number of sexual partners (OR=3.0; 95% CI: 1.9–4.8 for $\geq$3 vs 1), and severe vaginal discharge. OC use was associated with a significant reduction in HPV detection. None of these risk factors were associated with infections in women above age 45.

Colombia. In Bogota, 1,859 cytologically normal women were studied. The overall prevalence of HPV DNA was 14.8% and the commonest types were HPV 16, 58 and 56. There was a positive association between HPV detection and age less than 20 years (OR vs 35–44 years=9.6), three or more sexual partners (OR=2.1) and OC use (OR=1.4). In women below age 25, high education and intercourse with casual partners were associated with infection risk.

A subset of 227 women in Bogota with normal cytology, but positive for HPV DNA at study enrolment and at least one follow-up visit was studied (Molano et al. 2003a). The aim of the analysis was to search for determinants of HPV infection clearance. Results indicated that infections with HPV 16 (hazard ratio=0.6; 95% CI: 0.4–0.8), but not with high-risk HPV types other than HPV 16, had a significantly lower clearance rate than infections with low-risk types. Infections with a single type and multiple infections had similar clearance rates. There was an indication that parous women cleared HPV infections less efficiently than nulliparous women, but OC users may have less persistence of infection.

Nigeria. We interviewed and obtained a sample of cervical cells from 932 sexually active women aged 15 years or older from Idikan, an inner-city area of Ibadan, Nigeria. Thirty-one different HPV types were identified for an HPV prevalence of 26.3% overall. High-risk HPV types predominated, most notably HPV 16, 31, 35 and 58. One-third of infections involved more than one HPV type. Contrary to most populations studied so far, HPV prevalence was high not only among young women, but also in middle-aged and old

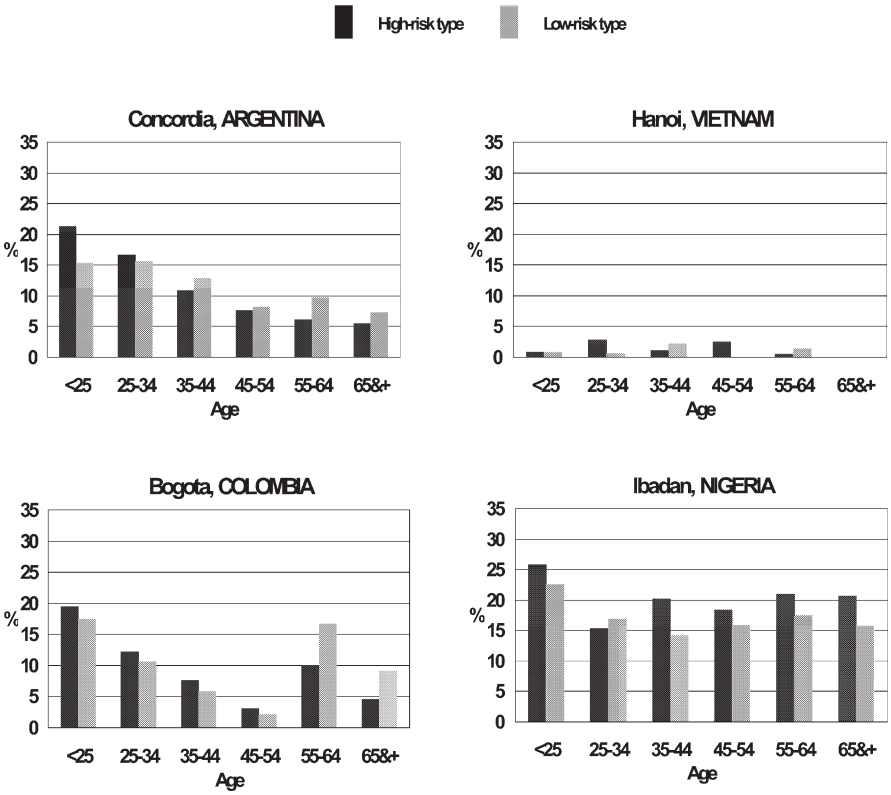

Fig. 3. Prevalence of cervical human papillomavirus HPV DNA in healthy women by age and HPV types: IARC, 1995–2002

women. Illiterate women (OR=1.7; 95% CI: 1.1–2.5) also showed increased HPV-positivity. Associations were found also with HSV-2 antibodies (OR=1.6; 95% CI: 1.1–2.1) and a husband's extra-marital relationships (OR=1.6; 95% CI: 1.0–2.6). High prevalence of HPV in all age groups may be a distinctive feature of populations where HPV transmission continues into middle age and CC incidence is very high.

Figure 3 shows the distribution of HPV infection (all types) in four locations of IARC surveys. They represent good examples of the four main age patterns that we and other investigators (Herrero et al. 2000) have identified worldwide:

1. The most common (e.g. in Argentina, Matos et al. 2003) includes a peak of HPV prevalence below age 25, when women start being exposed to HPV infection through first sexual intercourse.
2. In some countries, notably in Latin America, (e.g. in Colombia), a second increase in HPV prevalence is sometimes found among middle-aged women.

3. Steady low prevalence can be found in all age groups in some countries (e.g. North Vietnam) where HPV infection is rare.
4. Steady high prevalence can be detected in all age groups in the countries at highest risk for CC (e.g. Nigeria).

The extent to which differences in sexual habits, ability to clear HPV infection, and cohort effects account for this variation in the age curve of HPV prevalence in different world populations is not yet well understood.

University Students in Korea. In order to introduce a prophylactic vaccine against HPV, it is essential to understand the age at which women and men first acquire the infection, but very little is known about the prevalence of HPV infection among young adults in Asia. Therefore, we invited 900 female and 600 male students in Busan, South Korea to participate in a survey that included self-collection of vaginal cells or physician-performed collection of genital exfoliated cells in males (Shin et al. 2004). The prevalence of 25 different HPV types was evaluated, using a PCR-based detection and genotyping assay, among 672 female (median age=19) and 381 male students (median age=22).

HPV DNA was detected more frequently among female (15.2%) than among male (8.7%) students. High-risk types predominated in both genders. HPV prevalence was 38.8% among sexually active female students and 10.6% among sexually active male students. Being a current smoker (OR=3.8; 95% CI: 1.7–8.3) and reporting multiple sexual partners (OR for $\geq$4 vs 1 partner=6.9; 95% CI: 2.8–16.8) were the strongest risk factors for HPV detection in females. Among males, associations with sexual habits were in the same direction as in females, but they never attained statistical significance. Circumcision was frequently reported by males but did not seem to protect against HPV infection.

Young women in South Korea start sexual intercourse relatively late (median age=18) but HPV prevalence quickly rises to levels comparable to those found in college students in the United States (Winer et al. 2003) and Northern Europe (Woodman et al. 2001). The high participation of our study suggests that trials on new vaccines against HPV may be feasible among university students in South Korea (Shin et al. 2004).

5
HPV Vaccines in the Prevention of CC

Prophylactic vaccines to prevent HPV infection and therapeutic vaccines targeted at the HPV tumour antigens are in clinical trials (Galloway 2003). Early findings from a trial from Merck (Koutsky et al. 2002) have shown that prophylactic vaccines against HPV have a very high efficacy, at least in the

short term. It has provided added urgency to the evaluation and deployment of suitable HPV vaccines in areas of the world where CC is most common and particularly where screening programmes will be very difficult to setup and maintain (Franceschi et al. 2003b; Plummer and Franceschi 2002).

In principle, after HPV vaccines have become available for large-scale use, their effectiveness as a strategy for CC control can be measured either by monitoring secular trends in CC incidence or by conducting randomised trials. The former approach has greatly contributed to establishing the efficacy of the vaccine against hepatitis B virus (HBV) in the prevention of primary liver cancer (Huang and Lin 2000), but it is unlikely to provide convincing evidence of effectiveness. In fact, CC rates are subject to strong secular trends that are independent of intervention measures. A few phase III trials of HPV prophylactic vaccines are now being started by different pharmaceutical industries, but they are very expensive studies involving frequent and complicated investigations. It would be important, however, to start simpler trials designed to demonstrate the effectiveness of an HPV vaccine in field conditions, i.e. in developing or intermediate countries which suffer the major burden of mortality from CC, as soon as possible. Such trials may capture a difference in the most severe, and rarest, pre-invasive cervical lesions (i.e. the real target of any HPV vaccine) over a prolonged follow-up (20 years at least).

Relevant trials could be conducted in any country, but in order to accelerate the adoption of HPV vaccination in the populations that need it most, priority should be given to developing countries, most notably Asia, where 50% of worldwide CC cases occur. These trials should be large and of long duration (20 years at least) in order to capture a difference in the most severe pre-invasive cervical lesions, which take many years to develop. Consequently, the design must be simple and cost-effective. The trial design may be summarized as follows:

- Vaccination of young women before they become exposed to HPV (i.e. in conservative societies, before marriage).
- Long-term "opportunistic" monitoring of serious side effects (e.g. through monitoring of hospital admissions).
- No measurement of cervical outcomes until the subjects are of sufficient age to benefit from screening.

A fundamental difference between these trials and the phase III trials currently being conducted is that there are no plans for early gynaecological examination for the purposes of the trial only. The lack of early examination is beneficial to the participants since women under the age of 30 have a very low risk of CC and cervical intraepithelial neoplasia (CIN) III, but may undergo over-treatment of transient HPV infections which may manifest themselves clinically as CIN I. A corollary of the lack of early gynaecological ex-

aminations is that a population-based screening programme must be in place for the study participants in due time (e.g. when they reach the age of 30–35). This will ensure two things: first, that the control group receives an adequate standard of care, and second, that an outcome measurement, at the age when CIN III peaks, is taken for as many subjects as possible. A second requirement for this study is the ability to follow-up subjects over a long period, and in particular to accurately identify the treatment group decades after randomisation.

Different locations for such trials are conceivable, but it would be desirable that they include (and provide some information about) very different populations. We have considered the possibility of implementing this design in two areas: a rural area in Southern India and an urban one in South Korea, which are presented here in respect to their different strengths and weaknesses (Plummer and Franceschi 2002; Franceschi et al. 2003b).

Some areas of Southern India have a very high risk for CC. The age-standardised rate for Chennai (Madras) was 30.1 per 100,000 in the late 1990s (Parkin et al. 2002). This makes it an attractive location for CC prevention trials. Long-term follow-up of subjects is probably not feasible in an urban setting due to the very marked population movement in developing countries. In a rural setting, the most appropriate study design is a community intervention study with randomisation by village. This provides a simple mechanism for identifying the treatment group of a subject many years after randomisation, since it suffices to know a subject's place of birth. A cluster randomised trial also presents the only feasible opportunity to randomise males and thus to evaluate the usefulness of vaccinating both sexes. Men very rarely develop severe HPV-related diseases (e.g. cancer of the penis and the anus). They may therefore not respond to individual randomisation, but may agree to do so in the context of a community intervention. The efficacy of male vaccination will have to be evaluated in terms of its contribution to the decrease of pre-cancerous lesions in women.

To this extent, "discordant" couples (i.e. couples where only the husband or wife is vaccinated) will be most informative. The target population for a trial in southern India is unmarried women, i.e. women below age 19, as very early marriage is still common in rural India. The sample size of this trial should include approximately 80,000 women. These calculations are based on an assumption of vaccination at age 15 with a 10-year interval between vaccination and the first screening examination, with CIN III as an endpoint. The incidence rates for CIN are imputed from the incidence rates for ICC by assuming that CIN III occurs 5 years earlier and at a rate three times higher (hence 2/3 of CIN III will regress without progression to cancer). Loss to follow-up has not been factored into these calculations, since a realistic assessment of the rate of loss depends on the specific design of the study. However, in order to take into account loss to follow-up, a possible decline in CC incidence and the overwhelming difficulty of replicating com-

munity-based intervention trials, the target power of the study needs to be very high. These calculations use a target power of 99%, under the assumption that the chance to be able to replicate such huge trials is minimal.

South Korea is no longer considered a developing country but, on account of the recency of the economic and medical development, is still an intermediate-risk country for CC. The age-standardised rate for Busan county was 21.1 per 100,000 in the late 1990s, which is two- to four-fold higher than in most Western countries (Parkin et al. 2002). However, a few characteristics of the population and the health system in South Korea may be greatly beneficial to the implementation of a clinical trial. Recent IARC HPV surveys (Shin et al. 2003, 2004) have shown that infection with HPV occurs later in Busan than in Western countries, and it may thus be possible to offer HPV vaccines to women in the 18- to 22-year age range. A majority of young women in this age range in South Korea attend higher education and may thus be readily contacted, individually randomised, and offered the vaccine in university health facilities that have shown themselves to be open to collaboration in a study by IARC (Shin et al. 2004).

Most importantly, each person in South Korea has a unique national identity number, which will greatly facilitate long-term follow-up. Finally, the South Korean government has a strong commitment to implementing population-based screening programmes in the near future, including cytological screening for the prevention of CC in women aged 30 or older. Thus, the follow-up process in such a large trial of a prophylactic vaccine against HPV may benefit from the present development of national screening programmes.

6 Discussion

Many challenges remain in respect to the efficacy and efficiency of prophylactic vaccines against HPV (Galloway 2003).

Despite successful results in animal models and humans, it is not clear which elements of the human immune system are important in preventing or resolving HPV infections. High levels of circulating neutralizing antibodies induced by VLP vaccines have been shown to provide a high degree of protection against incident and persistent infection, but the duration of the protection is unknown. Ways to enhance mucosal immunity and cell-mediated immunity are being evaluated (e.g. intra-nasal or oral immunization, Galloway 2003).

Obviously, so-called chimaeric vaccines (i.e. vaccines able to prevent HPV infection and induce clearance of the infection at an early stage) would be a more preferable solution. They would substantially anticipate the benefits of vaccinations that, in the case of prophylactic vaccines, would take three or

four decades to become apparent. In fact, therapeutic vaccines may benefit not only sexually inexperienced women who have not yet been infected by HPV, but also older women who may be already harbouring HPV-related cervical lesions.

Furthermore, while safety and efficacy are essential for a vaccine, ways to reduce costs and increase vaccine coverage must also be considered. They will include formulating an oral vaccine, creating a stable vaccine that does not require an expensive cold-chain and/or one that can be produced in developing countries. Finally, it is worth bearing in mind that the sexually transmitted nature of HPV infection will probably enter into the public debate, as will the gender issue (i.e. the current restriction of current HPV vaccine to women as a target population). While the efficacy and opportunity of vaccinating boys as well as girls will have to be evaluated, ways to tackle an open discussion on HPV infection will have to be found in developing as well as developed countries.

The challenges above notwithstanding, HPV vaccine development holds great promise for reducing the mortality and morbidity of cervical neoplasia in the world's women.

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