2. Studies of Cancer in Humans

2.1 Methodological concerns

(*a*) *Choice of disease end-point*

To obtain epidemiological evidence of the risk for cervical cancer due to a specific type of human papillomavirus (HPV), the choice of disease end-point must be appropriate. The risk for invasive cancer is examined optimally by a case–control design or among historical cohorts in which archived specimens are tested.

Prospective studies that follow women forward in time must ethically rely on surrogate end-points, the choice of which is critical. For studies of HPV infection, invasive cancer and grade 3 cervical intraepithelial neoplasia (CIN3; which subsumes diagnoses of severe dysplasia and carcinoma *in situ*) are considered to be the primary disease end-points. The inclusion of CIN3 as a surrogate for invasive cancer permits prospective studies that would otherwise be unethical, because it is a condition that often requires medical treatment, whith thus interrupts the natural history of the disease. CIN3 is the immediate precursor of invasive cervical cancer, and the two diseases share a similar

cross-sectional virological and epidemiological profile (except for an earlier average age at diagnosis of CIN3) and demonstrate good histopathological reproducibility (Shah *et al.*, 1980; Walker *et al.*, 1983; Muñoz *et al.*, 1992, 1993). Therefore, CIN3 is a practical surrogate end-point for cervical cancer, although a proportion of cases of CIN3 regress rather than invade. However, in cohort studies, new diagnoses of small CIN3 lesions may represent diseases that were missed at the time of enrolment when HPV was assayed. This may lead to misclassification bias and spurious risk estimates.

While the choice of CIN3 is imperfect, less severe and more common grades of neoplasia, particularly CIN1, are clearly unreliable and are too closely linked to newly acquired infections with a broad range of HPV types to serve as surrogate end-points of cervical cancer. CIN2 is probably the result of a mixture of newly acquired infections and incipient CIN3; it is often treated, but can represent 'over-called' low-grade lesions.

Virologically, the persistence of HPV for several years cannot be used as an accurate surrogate of type-specific carcinogenicity because persistence is a necessary but not sufficient characteristic of carcinogenicity (Ho *et al.*, 1995; Nobbenhuis *et al.*, 1999).

(*b*) *Impact of study design*

When an etiological fraction of cervical cancers that can be attributed to a specific HPV type is small, it is more difficult to conduct prospective studies because of limitations of statistical power. At present, type-specific prospective evidence of carcinogenicity is readily available for HPV 16 and, to a lesser extent, for HPV 18. Because of the latency between average age at first HPV infection (late teens to early twenties) and average age at diagnosis of CIN3 (approximately 25–30 years of age), large longitudinal cohorts are only now attaining sufficient follow-up time to permit a reasonable assessment of a few additional HPV types. To overcome this limitation, some of the longest-term studies published to date have been based on HPV typing of archived slides from screening programmes, using a nested case–control approach. However, techniques for assaying the full spectrum of HPV types in these old specimens have not been fully validated. Since each individual HPV type is relatively uncommon, most prospective studies have combined all putative carcinogenic HPV types to assess the possible clinical utility of a pooled-probe HPV test.

As a result, epidemiologists often rely on cross-sectional designs to estimate the risk for individual HPV types. Estimation of the absolute risk, incidence rate and even the lifetime cumulative incidence rate of cervical cancer among infected compared with uninfected women and among women infected with each type of HPV alone would be ideal. The risk associated with each HPV type could then be estimated with adjustment for potential confounding due to co-infection with other HPV types. However, the correct estimation of these risks would require lifetime longitudinal follow-up of huge cohorts of women, while cross-sectional designs that use prevalence risk estimates suffer from unavoidable limitations due to the lack of a reliable measurement of lifetime exposure to HPV infection.

Case–control designs typically rely on the assessment of HPV DNA at the time of diagnosis for cases and at a similar age for controls. Since persistence of HPV DNA is a hallmark of cervical cancer/CIN3, the vast majority of cases are found to be HPV DNA-positive. In contrast, the low prevalence in controls reflects both recently acquired infections and an unknown, small fraction of infections from previous years (most of which proved to be transient after 1–2 years).

(*c*) *Problem of multiple infections*

HPV 16 and HPV 18 were classified previously as Group 1 carcinogens (IARC, 1995). In an assessment of whether additional types are also carcinogenic, possible confounding must be taken into account because different HPV types are frequently co-transmitted sexually. Multiple infections (i.e. infections with more than one HPV type) have been found in more than 25% of infected women in many surveys, but available polymerase chain reaction (PCR) assays are less sensitive and reproducible for the detection of multiple-type rather than single-type infections. When considering the possible carcinogenicity of a specific HPV type, any association with cancer due to co-infection with HPV 16 or HPV 18 must be ruled out. Possible strategies to address this type of confounding include the exclusion of all HPV 16- or HPV18-infected individuals, stratification for HPV type or group and multivariate statistical modelling. However, these strategies are constrained by the need to investigate concurrently approximately 40 relatively rare anogenital types of HPV. Thus, even very large studies typically lack statistical power to evaluate all combinations adequately. This problem is most apparent when assessing the possible carcinogenicity of uncommon types that occur mainly in combination with other HPV types.

(*d*) *Choice of method for HPV testing*

DNA testing is the reference standard for the detection of current HPV infection. There is a wealth of evidence from case–control studies that putative carcinogenic types assessed as a pooled group are associated with an increase in the risk for invasive cancer and CIN3. The collective strength of this evidence led the Food and Drug Administration in the USA to license Hybrid Capture 2, which allows the detection of 13 high-risk and five low-risk HPV types, as an adjunctive screening method. Similarly, several studies have employed PCR-based methods that still pool putative carcinogenic types, which prevents individualized assessment of the carcinogenicity of specific types. Thus, the assessment of type-specific carcinogenicity relies exclusively on studies that employ PCR with probes for individual types. However, each PCR-based system has selective differential sensitivity for individual HPV types; this could affect the risk estimates because of differential misclassification of cases and controls. Infections in cases result in lesions that are the site of viral replication, and tend to have higher viral loads than infections that do not cause obvious lesions such as those that occur in controls. Thus, a PCR system with relatively low sensitivity for a given HPV type tends to detect infections of that type

differentially in cases compared with controls, and thus overestimates the odds ratio for that specific type.

Serological data have yielded useful information for the assessment of exposure to HPV. HPV serology based on virus-like particles (VLPs) is a relatively type-specific but insensitive measure of exposure. Therefore, seropositive women appear to have been truly exposed to HPV, although the anatomical site of infection cannot be ascertained. Extremely large archives of serum specimens have permitted nested case–control studies of cervical cancer and CIN3 with an exceptional statistical power that is currently lacking in studies of DNA. Serology is included here to define HPV-exposed study populations for the consideration of etiological co-factors such as tobacco smoking or *Chlamydia trachomatis*. Finally, serology is discussed with reference to sites other than the cervix for which valid comprehensive DNA sampling is problematic.

(*e*) *Heterogeneity of definitions of initial cytomorphology*

Cohort and case–control studies have previously emphasized the distinction among HPV-infected women between those with normal versus mildly abnormal cytology. However, different types of HPV infection cause overlapping and pleiomorphic cellular changes that are sometimes pathognomonic (e.g. koiloctytotic atypia) but are often equivocal or lacking. Moreover, the interpretations of mild and equivocal HPV-related cytology differ greatly between geographical regions and assessors. A normal Papanicolaou (Pap) test in one geographical region might be called equivocal or even a low-grade squamous intraepithelial lesion (LSIL) in another region (Scott *et al.*, 2002a). Thus, for epidemiological studies to assess HPV type-specific carcinogenicity, a necessary requirement is that the study subjects are tested for a specific type of HPV and that cases have a confirmed diagnosis of CIN3 or cancer. For type-specific analyses, it is not necessary to focus excessively on the subtler, variable issues of whether control or cohort subjects had completely normal cytology.

(*f*) *HPV types, cervical cancer and cancers at other sites*

Since the association between HPV and cervical cancer is well established, the sections on cervical cancer focus on evaluating specific HPV types. In these sections, a limited number of highly stringent HPV DNA detection techniques were considered to be adequate to provide evidence of an association. For cancers at sites other than the cervix, their relationship with HPV is not well established. There are fewer studies on the association between HPV and cancers at sites other than the cervix, and the number of cases reported is much smaller. To allow for a preliminary assessment of the association between HPV and these cancers, a wider variety of techniques and methods were considered to be acceptable for presentation in their respective sections.

2.2 Cancer of the cervix

2.2.1 *Historical perspective*

Early studies on HPV and cervical cancer reported largely on HPV 16 and 18, which were the first two cancer-associated types that were isolated and used to design the initial testing systems (IARC, 1995). Developments in the technology used in large epidemiological and clinical studies evolved in two directions. Clinically designed testing systems generated cocktails of HPV probes, with the understanding that the individual risk for any HPV type of the high-risk group was clinically equivalent. Typically, these studies reported on the presence or absence of HPV DNA of the high-risk or low-risk cocktails (Peyton *et al.*, 1998; Vernon *et al.*, 2000; Castle *et al.*, 2002b). Research-oriented testing systems developed type-specific procedures and these were used to refine the understanding of type-specific risk and to make advancements in studies of HPV transmission, in the definition of HPV persistence and in investigations of HPV DNA at other organ sites (Manos *et al.*, 1989; Jacobs *et al.*, 2000).

As described in Section 1, the ability to identify multiple types of HPV in one specimen and the presence of some cross-reactivity has introduced some additional variability in the interpretation of the available data. The literature that related HPV and HPV types to cervical cancer up to 1994–95 was reviewed previously (IARC, 1995). It was concluded that there was sufficient evidence for the carcinogenicity of HPV types 16 and 18 (Group 1), HPV types 31 and 33 were classified as probably carcinogenic to humans (Group 2A) and an undefined group of other HPVs were evaluated as possibly carcinogenic to humans (Group 2B). Some evidence was suggestive that the same association existed for other HPV types, although the number of studies was limited. The only correlation observed between HPV type and clinical outcome was an increased relative frequency of HPV 18 in cervical adenocarcinoma compared with the more common squamous-cell carcinoma.

The case–control study in Spain and Colombia coordinated by IARC was instrumental in showing highly significant and high odds ratios with three different HPV DNA testing methods, namely the southern blot hybridization procedure, which was considered as the standard at the time, the first version of a testing cocktail intended for clinical use (Virapap) and the initial PCR systems based on the MY09/11 primers (Bosch *et al.*, 1992; Muñoz *et al.*, 1992). HPV type-specific risk estimates were provided for HPV 16, 18, the combination of HPV 31, 33 and 35 and for unidentified HPV types (Bosch *et al.*, 1992; Muñoz *et al.*, 1992; IARC, 1995). The high prevalence of HPV DNA among cases triggered intense research into the viral status of the apparently HPV-negative cases. Stringent laboratory analyses of case series of cervical cancer led to the conclusion that HPV is a necessary cause of cervical cancer (IARC, 1995; Walboomers *et al.*, 1999). These prompted analyses restricted to HPV-positive women for the evaluation of other risk factors. The study also showed that the risk factor profiles of the apparently HPV-negative and HPV-positive cervical cancer cases were notably similar, that the risk profile was identical for the established pre-invasive and invasive conditions and that the results

were consistent in two countries with a contrasting incidence of cervical cancer (Bosch *et al.*, 1992; Muñoz *et al.*, 1993; Moreno *et al.*, 1995).

2.2.2 *Data on pooled HPV types*

(*a*) *Cross-sectional studies and studies with short-term follow-up*

(i) *Risk for ≥ CIN2/3 from primary screening data*

Table 22 presents data from primary cervical cancer screening studies on the association between HPV positivity and the risk for ≥ CIN2/3. In some studies, verification of high-grade disease by colposcopy and histology was restricted to women who had positive cytological or HPV test results. In particular, all women screened in three studies were verified independently of screen test results and were therefore theoretically free from verification bias (Belinson *et al.*, 2001b; Blumenthal *et al.*, 2001; Sankaranarayanan *et al.*, 2004b) whereas verification bias was at least partially corrected in the statistical analyses in two studies (Schneider *et al.*, 2000; Kulasingam *et al.*, 2002).

Assessment of outcome is potentially hampered by the misclassification that is inherent to the use of an imperfect diagnostic gold standard. Nevertheless, relative risks associated with HPV status can be computed with acceptable reliability as the ratio of the risk for ≥ CIN2/3 in HPV-infected versus non-HPV-infected subjects. This relative risk is equivalent to the ratio of positive predictive value over the complement of the negative predictive value (relative risk = positive predictive value/(1-negative predictive value)).

The relative risk for ≥ CIN2 that is associated with HPV positivity varied from 5.6 (Blumenthal *et al.*, 2001) to 256 (Cuzick *et al.*, 1999). In one study in which verification bias was not taken into account, the relative risk was infinite (Clavel *et al.*, 2001) since the Hybrid Capture 2 result predicted all detected cases of ≥ CIN2. The relative risk was higher when the outcome of ≥ CIN3 was considered, and varied from 27 (Kulasingam *et al.*, 2002) to 530 (Petry *et al.*, 2003).

(ii) *Triage of women with atypical squamous cells of undetermined significance (ASCUS) or LSIL*

Using a meta-analytical approach, Arbyn *et al.* (2002, 2004a,b, 2005) documented the diagnostic performance of two management options for women with an equivocal Pap smear to detect women who need follow-up — reflexive high-risk HPV DNA testing versus repeat cytology. Two different triage groups were considered: (*a*) women with equivocal Pap smears, reported as ASCUS or borderline; and (*b*) women with LSIL or mild dyskaryosis. In these meta-analyses, diagnostic accuracy for the outcome of histologically confirmed CIN2 or CIN3 or worse was the focus of interest. The selection of studies considered was restricted to those in which ≥ CIN3 was the reported outcome and sensitive HPV DNA detection systems were used such as the Hybrid Capture 2 and PCR tests. Relative risks were computed as for the primary screening data.

The inter-study variation of the relative risks and the pooled measures are displayed graphically by the forest plots in Figures 10 for ASCUS and 11 for LSIL and are further

Table 22. Association between ≥ CIN2/3 and HPV status in women participating in cervical cancer screening

Reference, study location	Method of detection	Outcome	No. of women	Test posivity rate	Proportion of ≥ CIN2/3		Relative risk[a]	Verification bias
					HPV+	HPV–		
Cuzick *et al.* (1999), United Kingdom	HC2[b]	≥ CIN2	1703	0.073	0.160	0.001	255.84	Not corrected
Schiffman *et al.* (2000), Costa Rica	HC2	≥ CIN2	8554	0.139	0.102	0.002	45.09	Not corrected
		≥ CIN3	8554	0.075	0.096	0.001	109.05	Not corrected
Schneider *et al.* (2000), Germany	HC2[b]	≥ CIN2	4761	0.104	0.204	0.003	68.90	Partially corrected
Wright *et al.* (2000), South Africa	HC2	≥ CIN2	1365	0.208	0.137	0.007	19.36	Not corrected
Belinson *et al.* (2001b), China	HC2	≥ CIN2	1997	0.228	0.180	0.002	73.35	None
Blumenthal *et al.* (2001), Zimbabwe	HC2	≥ CIN2	2073	0.429	0.189	0.034	5.59	None
Clavel *et al.* (2001), France	HC2	≥ CIN2	5671	0.134	0.094	0.000	939.20[c]	Not corrected
Kulasingam *et al.* (2002), USA	HC2	≥ CIN2	4075	0.219	0.096	0.015	6.28	Partially corrected
		≥ CIN3	4075	0.279	0.018	0.001	27.31	Partially corrected
Salmerón *et al.* (2003), Mexico	HC2	≥ CIN2	7732	0.094	0.129	0.001	130.86	Not corrected
		≥ CIN3	7732	0.079	0.120	0.001	215.76	Not corrected
Petry *et al.* (2003), Germany	HC2	≥ CIN3	7592	0.047	0.073	0.000	530.44	None corrected

Table 22 (contd)

Reference, study location	Method of detection	Outcome	No. of women	Test posivity rate	Proportion of ≥ CIN2/3		Relative risk[a]	Verification bias
					HPV+	HPV–		
Sankaranarayan *et al.* (2004b), India	HC2	≥ CIN2	18085	0.070	0.128	0.005	28.32	None
		≥ CIN3	18085	0.070	0.089	0.002	55.27	None

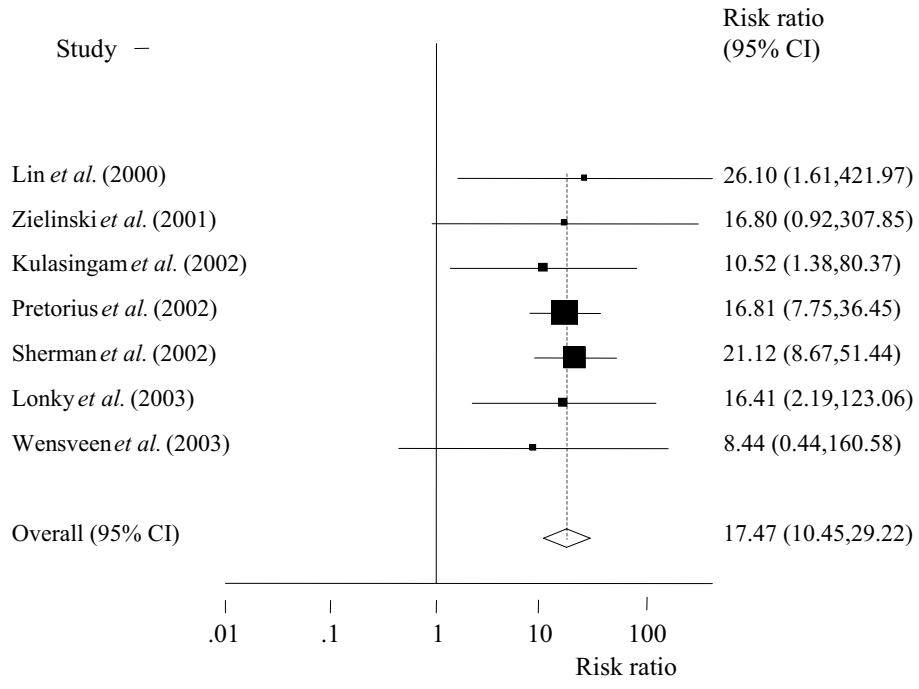
CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2: targets HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68; +, positive; –, negative

[a] Relative risks were calculated by the Working Group using data from Lörincz and Richart (2003), IARC (2005) and from the original publications or requested directly from the authors.

[b] PCR system that includes identification of 14 types (13 types as in HC2 + HPV 66)

[c] RR computed using Yates correction, by adding 0.5 to each cell of the 2 × 2 contingency table. This correction is required for studies where the risk for ≥ CIN2/3 in the HPV-negative group is zero. Risk for ≥ CIN2/3 if HPV positive corresponds with the positive predictive value of HPV testing for the presence of underlying ≥ CIN2/3. Risk for ≥ CIN2/3 if HPV negative corresponds with 1–negative predictive value.

Figure 10. Meta-analysis of the prediction of histologically confirmed ≥ CIN3 in women with an index Pap smear that showed ASCUS: relative risk for HPV-positive women versus HPV-negative women

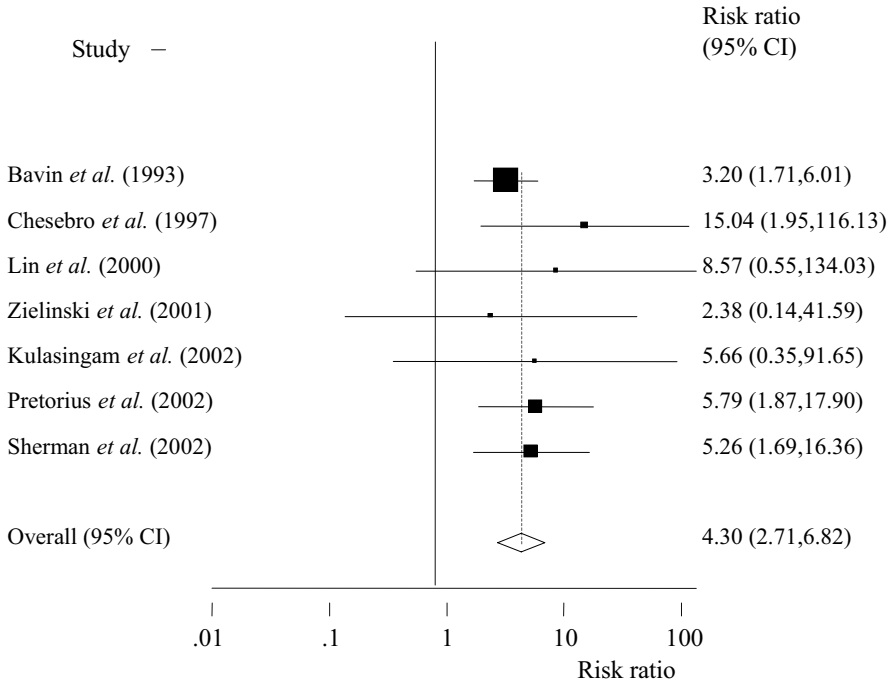


The meta-analysis is restricted to studies in which Hybrid Capture 2 or sensitive PCR was used to detect HPV DNA.

ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; Pap, Papanicolaou test

documented in Tables 23 and 24, respectively. Overall, HPV-positive women with ASCUS from seven pooled studies had a risk for ≥ CIN3 that was 17.47 times (95% confidence interval [CI], 10.45–29.22) higher than that of HPV-negative women (Figure 10). The relative risk was substantially lower in LSIL triage settings (4.30; 95% CI, 2.71–6.82) (Figure 11). Relative risks were pooled using random-effect meta-analytical models (Dersimonian & Laird, 1986; Sutton *et al.*, 2000). The risk for ≥ CIN3 in some studies was equal to zero in the HPV-negative group, which yielded a relative risk of infinity (∞). Such studies cannot be incorporated into a meta-analysis. For these studies, 0.5 was added to the nominator and 1 to the denominator in both the HPV-negative and HPV-positive groups. This correction yielded a considerable underestimate of the relative risk, especially in small studies. Nevertheless, the underestimation in the pooled relative risk was generally smaller than when studies with a relative risk of ∞ should have been discarded.

Figure 11. Meta-analysis of the prediction of histologically confirmed ≥ CIN3 in women with an index Pap smear showing LSIL; relative risk for HPV-positive women versus HPV-negative women



CIN, cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou test

Most triage studies were cross-sectional in design or involved a follow-up time that only lasted from the assessment of HPV status to the verification of outcome. The largest triage study (Castle *et al.*, 2005) included 5060 women who had ASCUS or LSIL. Oncogenic HPV-positive women who had ASCUS or LSIL had a 2-year absolute risk for CIN3 of approximately 15% or 17%, respectively. Women who had ASCUS or LSIL cytology who were HPV 16 DNA-positive at baseline had a 2-year cumulative absolute risk for ≥ CIN3 of 32.5% (95% CI, 28.4–36.8%) and 39.1% (95% CI, 33.8–44.7), respectively, thus the risk estimates seemed to be substantially lower than the cross-sectional relative risks. The difference in cross-sectional and longitudinal cumulative relative risks might indicate that cross-sectional studies overestimate a surplus of prevalent disease which ultimately regresses. This time-dependent effect was also observed in large cohort studies that focused on the natural history of HPV infection and precancerous cervical lesions (Liaw *et al.*, 1999; Kjaer *et al.*, 2002; Schlecht *et al.*, 2003c).

Increased risk for severe dysplasia was associated with a high risk for ≥ CIN2. The largest contribution to the relative risk derives from HPV 16 infection (Castle *et al.*, 2005)

Table 23. Triage of ASCUS: short-term outcome of ≥ CIN3 in high-risk HPV-positive versus HPV-negative women with ASCUS

Reference	Follow-up period	HPV test method	HPV types targeted	No. of women	Test posivity rate	Proportion of ≥ CIN3		Relative risk	Relative risk[a]
						HPV+	HPV–		
Lin, C.-T. *et al.* (2000)[b]	Cross-sectional	HC2	13 HR types (2)	74	0.527	0.359	0.000	∞	26.1
Zielinski *et al.* (2001a)[b]	0–4.5 years	HC2	13 HR types (2)	213	0.347	0.054	0.000	∞	16.8
Kulasingam *et al.* (2002)	Nested within primary screening setting, short follow-up	HC2	13 HR types (2)	270	0.511	0.080	0.008	10.5	
Pretorius *et al.* (2002)	Short follow-up	HC2	13 HR types (2)	949	0.322	0.183	0.011	16.8	
Sherman *et al.* (2002)	RTS, short follow-up	HC2	13 HR types (2)	2198	0.540	0.104	0.005	21.1	
Lonky *et al.* (2003)	Short follow-up	HC2	13 HR types (2)	278	0.460	0.109	0.007	16.4	
Wensveen *et al.* (2003)[b]	Cross-sectional	HC2	13 HR types (2)	148	0.453	0.045	0.000	∞	8.4

ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; HR, high-risk; RTS, randomized triage study; +, positive; –, negative

[a] Relative risk corrected by adding 0.5 to each cell that contributes to the computation of the relative risk

[b] With Yates correction (+ 0.5)

Table 24. Triage of LSIL: short-term outcome of ≥ CIN3 in high-risk HPV-positive versus high-risk HPV-negative women with ASCUS

Reference	Follow-up period	HPV test method	HPV types targeted	No. of women	Test posivity rate	Proportion of ≥ CIN3		Relative risk	Relative risk[a]
						HPV+	HPV–		
Bavin *et al.* (1993)	Cross-sectional	PCR[b]	HPV 16	179	0.374	0.343	0.107	3.2	
Chesebro *et al.* (1997)	Cross-sectional	HC2	9 HR types (1)	159	0.799	0.134	0.009	15.0	
Lin *et al.* (2000)[a]	Cross-sectional	HC2	13 HR types (2)	45	0.756	0.353	0.000	∞	8.6
Zielinski *et al.* (2001a)[a]		HC2	13 HR types (2)	65	0.800	0.077	0.000	∞	2.4
Kulasingam *et al.* (2002)[a]	Nested within primary screening setting, short follow-up	HC2	13 HR types (2)	125	0.832	0.125	0.000	∞	5.7
Pretorius *et al.* (2002)	Short follow-up	HC2	13 HR types (2)	283	0.763	0.259	0.045	5.8	
Sherman *et al.* (2002)	RTS, short follow-up	HC2	13 HR types (2)	849	0.848	0.122	0.023	5.3	

ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; HR, high-risk; LSIL, low-grade squamous intraepithelial lesion; PCR, polymerase chain reaction; RTS, randomized triage study; +, positive; –, negative

[a] With Yates correction (+ 0.5)

[b] PCR targeting HPV 16 (medium/high signal)

(see Table 25). In HPV 16-positive women with ASCUS, the risk for ≥ CIN2 within 2 years was 16.1 (95% CI, 12.0–21.7) times higher than that in high-risk HPV-negative women. Positivity for other high-risk types was associated with a relative risk of 6.1 (95% CI, 4.5–8.3), which was similar to that associated with ASCUS that was unqualified by HPV. The relative risk associated with HPV positivity was lower in LSIL patients than in ASCUS patients, but was significantly higher when women were infected with HPV 16 compared with women infected with other high-risk HPV types (Castle *et al.*, 2005).

Table 25. Two-year cumulative risk for ≥ CIN2 according to initial high-risk HPV status (positivity for HPV 16 and for other high-risk HPV types) in women with ASCUS or LSIL compared with high-risk HPV-negative women

	No.	Absolute risk (%)	Relative risk	95% CI
ASCUS HC2-negative	1559	3.0	1.0	–
All ASCUS	3488	15.3	5.1	3.8–6.8
ASCUS HPV16-positive	443	48.5	16.1	12.0–21.7
ASCUS other high-risk HPV-positive, HPV16-positive	1245	18.4	6.1	4.5–8.3
LSIL HC2-negative	237	8.4	1.0	–
All LSIL	1572	25.4	3.0	2.0–4.1
LSIL HPV 16-positive	310	51.1	6.1	3.9–8.2
LSIL other high-risk HPV-positive, HPV 16-positive	931	22.7	2.7	1.7–3.7

Adapted from Castle *et al.* (2005)
ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; LSIL, low-grade squamous intraepithelial lesion

(*b*) *Prospective studies*

Since the previous review (IARC, 1995), a few large prospective studies have shown that HPV infection, as assessed by DNA testing for a group of putative high-risk types, predicted an increased risk for subsequent development of CIN3 or invasive cancer. Although such studies do not add to the assessment of type-specific carcinogenicity, they are noted for completeness.

Nobbenhuis *et al.* (1999) conducted a follow-up study of 353 women aged 18–55 years who had been referred because of cervical abnormalities for a median of 33 months (range, 2–72 months) without taking any biopsies until the clinical appearance of ≥ CIN3 or until the end of study. Two hundred and ninety-seven women (87%) had mild or moderate dyskaryosis at baseline; among them, 182 (61%) were high-risk HPV-positive (defined as harbouring one or more of the following types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,

66 or 68, using PCR with GP5+/6+ primers). Sixty-nine (31.9%) of these high-risk HPV-positive women developed ≥ CIN3 compared with only three (2.6%) of the 115 women that were high-risk HPV-negative at baseline, corresponding to a relative risk of 14.5 (95% CI, 4.7–44.8). A longer persistence of high-risk HPV-positivity was associated with an increase in the relative risk for the development of CIN3. Two of the three initially high-risk HPV-negative cases who developed CIN3 acquired HPV types during follow-up, which subsequently persisted until assessment of the outcome.

Using MY09/11 PCR with TaqGold followed by dot-blot detection of the 13 types targeted by Hybrid Capture 2, Ferreccio *et al.* (2003) examined the association of histologically confirmed CIN3 that occurred within 2 years and cancer that occurred within 7 years among 8551 women in Guanacaste, Costa Rica, who represented a mixed prevalent/incident case group. Ninety cases of CIN3 (mean age, 36.9 years) and 20 cases of invasive cancer (mean age, 43.4 years) were detected by multi-technique screening and not from symptoms. The cumulative incidence among HPV-positive women (unadjusted for loss to follow-up) was 8.6% while the comparable incidence among HPV-negative women was only 0.2%.

Sherman *et al.* (2003b) performed a 10-year follow-up of 20 810 women (mean age, 35.9 years) who were screened with a Pap smear and HPV testing from 1989 to 1999 at the Kaiser Permanente Center, Portland, USA. Among 171 women who had CIN3 or cancer diagnosed during the follow-up period, 123 (71.9%) had baseline Pap results of atypical squamous cells or worse and/or a positive HPV test, 102 (86.4%) of whom were diagnosed within the first 45 months of follow-up. During this 45-month period, the cumulative incidence of CIN3 or cancer was 4.54% among women with a Pap test result of atypical squamous cells or worse, positive HPV tests or both compared with 0.16% among women with negative Pap and HPV tests; thus, negative baseline Pap and HPV tests were associated with a lower risk for CIN3 or cancer in the subsequent 45 months.

Clavel *et al.* (2004) followed 4401 cytologically negative women for a median period of 34 months primarily to estimate the negative predictive value of Hybrid Capture 2-negativity for histologically confirmed CIN2 or CIN3 (combined). Five cases were observed and none was Hybrid Capture 2-positive at enrollment.

2.2.3 *Data on type-specific HPV*

(*a*) *Case series*

The cervix uteri of women with normal Pap smears or with mild cytological abnormalities (e.g. LSIL) harbour a broad spectrum of HPV types. Herrero *et al.* (2000) tested 3024 women in Guanacaste, Costa Rica, for 40 different HPV types and detected 34 different HPV types in women with normal cytological findings or LSIL. Franceschi *et al.* (2005) tested 1891 women in Dindigul District, India, for 44 different HPV types and detected 36 different HPV types in either single- or multiple-type infections among women with normal cytological findings or LSIL.

As the severity of cervical lesions increases, not only does the overall prevalence of HPV rise greatly, but the relative frequency of different HPV types also changes substantially. This 'enrichment' of certain HPV types, together with the depletion of others across the spectrum of cervical neoplasias, is well illustrated by the findings of three large systematic reviews carried out at the IARC on the distribution of HPV types in LSIL (8308 women from 50 studies; Clifford *et al.*, 2005), high-grade squamous intraepithelial lesions (HSIL; 4338 women from 52 studies; Clifford *et al.*, 2003a) and squamous-cell cervical carcinoma (10 058 women from 85 studies; Clifford *et al.*, 2003b).

The three IARC reviews were carried out according to the same protocol: articles that included HPV type-specific prevalence data were identified and key information (e.g. country of sample, sample size, type of cervical specimen and PCR primers used to detect HPV-positive samples) was extracted. Published findings did not generally allow the distinction of single-type from multiple-type infections and, therefore, the prevalence of each individual HPV type was evaluated independently of whether other types were detected. The three reviews were limited to studies that (*a*) included a minimum of 20 cases of LSIL, HSIL or cervical cancer and (*b*) reported type-specific prevalence of at least one HPV type other than HPV 6, 11, 16 or 18. When study methods suggested that additional type-specific data were available, these data were requested from the authors. All five continents were represented, although to varying extents. Other case series that described the distribution of HPV types in invasive cancer have been published since the IARC systematic review, including some in previously unstudied populations (Cuzick *et al.*, 2000; Bachtiary *et al.*, 2002; Dybikowska *et al.*, 2002; Mortazavi *et al.*, 2002; Nakagawa *et al.*, 2002; Pegoraro *et al.*, 2002; Alonio *et al.*, 2003; Gao *et al.*, 2003; Hwang *et al.*, 2003; Kay *et al.*, 2003; Plunkett *et al.*, 2003; Rabelo-Santos *et al.*, 2003; Stanczuk *et al.*, 2003; Tran-Thanh *et al.*, 2002; Tsuda *et al.*, 2003; Widschwendter *et al.*, 2003; Xi *et al.*, 2003; Silins *et al.*, 2004; Schellekens *et al.*, 2004).

Overall HPV prevalence was 71% among LSILs, 84% among HSILs, 88% among squamous-cell carcinomas and 77% among cervical adeno- or adenosquamous carcinomas. HPV types in Table 26 were grouped into: (*a*) HPV 16 and 18 (i.e. the types most frequently detected in cervical cancer worldwide); (*b*) other high-risk or probably high-risk types, 11 of which (i.e. HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are currently included in the Hybrid Capture 2 DNA test approved by the US Food and Drug Administration as an adjunct to primary cytological screening and for triage of women with equivocal cytology (Wright *et al.*, 2004), and five types (i.e. HPV 26, 53, 66, 73 and 82) that have been considered for inclusion in the HPV DNA test (Muñoz *et al.*, 2003); and (*c*) the three most common low-risk HPV types (i.e. HPV 6, 11 and 70).

Figure 12 gives a graphical representation of the data of Table 26.

HPV 16 was 2.5 times more prevalent in HSIL than in LSIL. Other types showed either a similar prevalence in LSIL and HSIL or a substantially higher prevalence in LSIL than in HSIL. Most importantly, HPV 16 and 18 were found three- and two times, respectively, more frequently in squamous-cell carcinoma than in LSIL, whereas HPV 26, 39, 51, 56 and 73 were at least 10-times and HPV 53 and 66 were approximately 30-times more

Table 26. Distribution of HPV types across cervical lesions of increasing severity

HPV type	LSIL		HSIL		ADC		SCC		LSIL: SCC ratio	LSIL: ADC ratio
	No.	%	No.	%	No.	%	No.	%		
16	8308	18.7	4338	45.0	1464	31.3	8594	54.3	0.5	0.8
18	8308	6.1	4338	7.1	1455	37.7	8502	12.6	0.7	0.2
Other high-risk or possibly high-risk										
31	8155	8.2	4036	8.8	1090	1.7	7204	4.2	2.7	6.8
33	8078	5.3	4302	7.2	1331	0.9	8449	4.3	1.7	8.2
35	6395	4.3	2690	4.4	985	0.8	6223	1.0	5.7	7.1
39	4301	5.8	1841	1.1	716	0.1	3899	0.4	19.0	76.0
45	4748	3.7	2214	2.3	755	5.8	5174	4.2	1.2	0.8
51	4721	8.0	2171	2.9	693	0.1	4580	0.6	17.7	106.0
52	4380	6.7	2153	5.2	757	0.5	5304	2.5	3.6	18.0
56	4431	7.2	2110	3.0	693	0.0	4493	0.7	13.6	
58	4498	6.3	2175	6.9	811	0.5	5646	3.0	2.8	16.8
59	4281	4.6	1636	1.5	681	0.7	4488	0.8	7.6	8.7
68	4292	2.5	1763	1.0	452	0.2	4148	0.5	6.6	16.5
26[a]	3506	1.0	806	0.6	362	0.0	3728	0.1	13.0	
53[a]	3358	7.6	1589	2.3	381	0.0	3053	0.2	51.0	
66[a]	4135	6.5	1778	2.1	508	0.2	4799	0.2	43.0	43.0
73[a]	3432	2.4	1364	1.0	377	0.0	2844	0.2	16.0	
82[a]	2923	1.9	812	0.5	219	0.0	2526	0.4	6.0	
Low-risk										
6	4696	6.2	3015	1.9	1049	0.1	6569	0.6	13.3	80.0
11	4525	3.2	3015	1.3	1000	0.1	6578	0.3	13.7	41.0
70	1114	2.2	1031	1.6	493	0.0	3122	0.2	17.0	

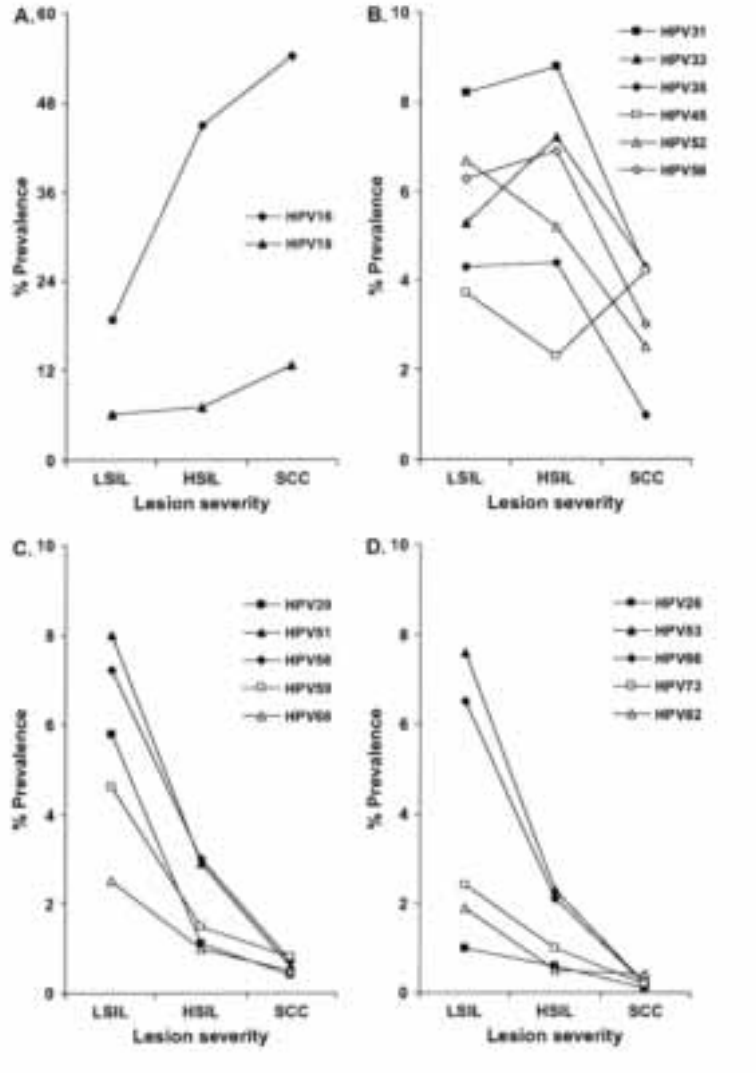
ADC, adenocarcinoma or adenosquamous carcinoma; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; SCC, squamous-cell carcinoma

[a] Not currently included in the US Food and Drug Administration-approved Hybrid Capture 2 HPV DNA test

common in LSIL than in squamous-cell carcinoma. A ratio of approximately 10 between LSIL and squamous-cell carcinoma was also found for low-risk types HPV 6, 11 and 70. The type-specific findings for adeno- or adenosquamous carcinoma were consistent with those observed for squamous-cell carcinoma except for the more marked enrichment of HPV 18 from LSIL to adeno- or adenosquamous than to squamous-cell carcinoma.

Comparisons of HPV distribution in international cross-sectional studies face several problems, including differences in the accuracy in cytological/histological classification and viral detection, as well as non-negligible heterogeneity in the distribution of HPV types across different populations.

Figure 12. Prevalence of HPV types in cervical lesions of increasing severity



Modified from Franceschi & Clifford (2005b)

Of particular note since the previous review (IARC, 1995), a high prevalence of HPV 35 has been reported in invasive cancer from previously unstudied regions in East Africa (19%) (Naucler *et al.*, 2004) and India (6%) (Castellsagué *et al.*, 2001; Franceschi & Clifford, 2005). Furthermore, a failure in the sensitivity of MY09/11 PCR primers to detect HPV 35 has also been identified, so that the prevalence of HPV 35 may have been underestimated in some of the previous case series (Iftner & Villa, 2003).

Nevertheless, the picture that emerges from the IARC systematic reviews suggests that: HPV 16 and 18 are substantially enriched in squamous-cell carcinoma compared with LSIL; some high-risk types are approximately equally represented (HPV 33 and 45) or moderately over-represented (HPV 31, 52 and 58) in LSIL than in squamous-cell carcinoma; and HPV 26, 53, 66, 73 and 82, which are not currently included in the DNA tests approved by the US Food and Drug Administration, are extremely rare in squamous-cell carcinoma, but this is also the case for some of the types that are currently included (e.g. HPV 39, 51 and 56).

In conclusion, the available evidence from cross-sectional comparisons of the distribution of HPV types in cervical lesions of increasing severity lends strong support to the notion that the risk that a woman will develop HSIL or cervical cancer varies substantially according to the specific HPV type with which she is infected.

(*b*) *Case–control studies*

Since the last review (IARC, 1995), a number of larger case–control studies have been completed that allow a more accurate evaluation of the type-specific risk of a number of additional HPV types. Only studies that reported HPV DNA results by type, as assessed by PCR, and by case and control status and included histologically confirmed end-points are reviewed and evaluated separately by disease end-point. Over the past 10 years, several specific and sensitive PCR-based methods of HPV detection have been used in epidemiological studies, and it is important to highlight that the various PCR systems differentially amplify different HPV types in disease and non-disease samples. Therefore, caution must be taken in interpreting the relative strength of the association between specific HPV types and risk for disease across studies. Due to the relative infrequency of some HPV types, smaller case–control studies have reported unstable risk estimates for certain HPV types. Greater emphasis is therefore given to larger studies and those that reported pooled data in relation to the risk associated with types other than HPV 16 and 18. As far as possible, the risk estimates presented here focus on those associated with single HPV infections only. The risk estimates published by the authors are presented where these are available by HPV type. When raw data were available from the individual publications, these were used to generate the crude odds ratio by HPV type. Finally, due to the problems of type specificity in seroepidemiological studies, those reports that only provided data on seroprevalence are not included.

In the mid-1990s, a growing interest in the risk associated with different HPV types came from examination of data from case series that indicated a relatively high prevalence of HPV types other than HPV 16 and 18 in cervical tumours (Bosch *et al.*, 1995; Huang *et al.*, 1997).

Table 27 summarizes the results of case–control studies of HPV-specific infection and pre-invasive and invasive lesions of the cervix.

In Honduras, Ferrera *et al*. (1999) conducted a population-based case–control study (149 cases of CIN3 or invasive cervical cancer and 438 controls) to investigate risk factors for cervical cancer. HPV was detected using general primer-mediated MY09/11 PCR

Table 27. Characteristics of case–control studies on HPV-specific infection and pre-invasive and invasive lesions of the cervix

Reference, study location	Study type	Methods of detection	HPV types tested	No. and type of cases	No. and type of controls
Ferrera *et al.* (1999), Honduras	Cervical screening	General primer-mediated PCR+ MY09/11 PCR sequencing	16, 18, 45, 33, 59, 31, 35, 52, 58, 56, 66, 11, 53, 70, 6, 22, 55, 62, 21	45 CIN3, 104 ICC	438 hospital-based
Hwang *et al.* (1999), Korea	Hospital-based	Consensus primer PCR; RFLP analysis	16, 18, 31, 33, 35, 52, 58	35 CIN, 41 ICC	130 healthy women
Sasagawa *et al.* (2001), Japan	Screening	LCRF1 to –4+ E7 primer; RFLP analysis	16, 18, 45, 33, 31, 35, 52, 58, 56, 51	145 LSIL, 137 HSIL, 72 SCC, 12 ADC, 16 condyloma	1562 normal cytology
Thomas *et al.* (2001b), Thailand	Hospital-based	MY09/11 PCR; generic + type-specific oligonucleotide probed for hybridization	16, 18, 31, 33, 35, 39, 45	42 ADC, 190 SCC	291 otolaryngological and general wards
Thomas *et al.* (2001c), Thailand	Hospital-based	General PCR; type-specific PCR	16, 18	190 ICC	75 CIS
Altekruse *et al.* (2003), USA	Population-based	PGMY-based 27-type reverse line blot detection	6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, 84	139 SCC, 124 ADC	307 population-based
Franceschi *et al.* (2003), India	Hospital-based	GP5+/6+ PCR; ETA with HPV specific oligoprobe cocktails	16, 18, 45, 33, 59, 31, 52, 58, 73, 56, 51, 66, 11, 70, 40, 42, 72, 81	193 ICC, 12 ADC	213

Table 27 (contd)

Reference, study location	Study type	Methods of detection	HPV types tested	No. and type of cases	No. and type of controls
Muñoz *et al.* (2003), Multicentre	Population- and hospital-based	E7 primer PCR for biopsies, for smears, MY0G/11 or GP5 +/6+ PCR; oligo-hybridization	16, 18, 26, 45, 33, 59, 31, 35, 52, 58, 66, 73, 39, 56, 26, 51, 68, 11, 53, 6, 81, 82	1918 prevalent SCC and ADC	1928
Asato *et al.* (2004), Japan	Population- and hospital-based	L1 consensus primer PCR; nucleotide sequencing	16, 18, 45, 33, 59, 31, 35, 52, 58, 73, 39, 56, 51, 66, 68, 82, 90, 91, 54, 53, 70, 6, 61, 71, 32, 42, 67, 72, 84, 86	356 SCC	3249 hospital-based
Hammouda *et al.* (2005), Algeria	Hospital-based	GP5+/6+ PCR; EIA with type-specific oligoprobe cocktail detecting 36 types and southern blot	16, 18, 45, 33, 31, 35, 52, 73, 39, 56, 51, 66, 42, 84	198 SCC	202
Herrero *et al.* (2005), Costa Rica	Population-based	MY09/11 PCR; dot blot with type-specific oligoprobes	16, 18, 45, 33, 59, 31, 35, 52, 58, 73, 39, 56, 26, 51, 66, 68, 82, 11, 54, 53, 70, 6, 61, 71, 22, 32, 40, 42, 55, 62, 67, 72, 81, 84, 74, 83, 85, 89, 21	73 CIN3, 35 SCC	8374 normal equivocal and low-grade dysplasia

See Table 7 for a description of the primers used.

ADC, adenocarcinoma; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; EIA, enzyme immunoassay; HSIL, high-grade squamous intraepithelial lesions; ICC, invasive cervical carcinoma; LSIL, low-grade squamous intraepithelial lesions; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SCC, squamous-cell carcinoma

followed by PCR-based sequencing that detected several different HPV types. However, due to the rarity of most HPV types, risk estimates for disease could only be generated for HPV types 16, 18, 31, 33, 52 and 58. The prevalence of any HPV type was 95% in cases of invasive cervical cancer, 87% in cases of CIN and invasive cervical cancer and 39% in controls. Compared with normal cytology, the odds ratio for invasive cervical cancer, associated with HPV 16-related types was 14.88 (95% CI, 5.12–43.25) and that for invasive disease associated with HPV 18-related types was 74.66 (95% CI, 7.77–717.62). Significantly elevated risks for invasive cervical cancer compared with normal cytology were also observed for HPV types 31 [odds ratio, 3.4], 33 [odds ratio, 34], 52 [odds ratio, 12.8] and 58 [odds ratio, 11.2]. In addition to the HPV types for which risk estimates could be generated, the authors observed HPV 45 infection among six cases and HPV 59 in one case, with none in the corresponding controls. A statistically significant association with HPV was observed for CIN2 and -3 and invasive cancer that showed an upward trend to more severe lesions and was more pronounced for HPV 16 and related types. A significantly elevated risk for CIN3 was observed with HPV 16 [odds ratio, 19], 18 [odds ratio, 8.9], 31 [odds ratio, 21.4], 33 [odds ratio, 71.5], 52 [odds ratio, 53.6] and 58 [odds ratio, 26.3]. HPV types 53, 66 and 70 were identified among controls but not among cases.

Hwang (1999) conducted a case–control study that included 130 healthy women, 35 patients with CIN and 41 patients with invasive cervical carcinoma in the Republic of Korea. HPV was detected by PCR followed by type-specific analyses by restriction fragment length polymorphism (RFLP). Significantly elevated risks for invasive cervical cancer were observed for HPV 16 [odds ratio, 146.3], 18 [odds ratio, 156], 52 [odds ratio, 39] and 58 [odds ratio, 78].

In Japan, Sasagawa *et al*. (2001) estimated the risk of HPV infection for biopsy-confirmed cervical malignancies by testing cell samples from 366 women with abnormal cytology and 1562 women with normal cytology for HPV with the long control region (LCR)-E7 PCR method that can amplify the E6–E7 DNA of more than 36 mucosal types of HPV. The prevalence of HPV infection was 9.7% in controls, 91% in HSIL and 93% in invasive cervical cancer. For HSIL and invasive squamous-cell carcinoma, the highest odds ratios were observed with HPV 16 (odds ratio, 43; 95% CI, 1.24–75 and 69; 95% CI, 36–131, respectively). For adenocarcinoma, the highest odds ratio was seen with HPV 18 (odds ratio, 94; 95% CI, 28–317). In addition to HPV types 16 and 18, elevated risks were observed for the association between HPV types 11, 31, 51, 52, 53 and 58 and squamous-cell carcinoma with magnitudes > 5.

Thomas *et al*. (2001b) studied women in Thailand who had been diagnosed with pre-invasive or invasive cervical cancer. PCR-based assays that used MY09/11 were carried out to determine HPV DNA in cervical scrapings from 232 diagnosed cases (190 women with squamous-cell carcinoma and 42 women with adenocarcinoma) and 291 hospitalized controls in Bangkok. HPV types 16, 18 and 45 were determined individually and a combined measurement of HPV types 31, 33, 35 and 39 was conducted. Only risk estimates for HPV 16 and 18 were reported separately. The prevalence of HPV types 16 and 18 was

72.4% in cases and 14.0% in controls. The 168 women with HPV 16- and 18-positive cervical cancers were compared with 250 HPV-negative controls. The odds ratio for HPV 16 was 83 (95% CI, 39–232) for squamous-cell cancer and 24 (95% CI, 8.7–76) for adenocarcinoma. In addition, HPV 45 was observed in one case but not in controls.

In another report by Thomas *et al*. (2001c) in Bangkok, Thailand, 190 women with invasive cervical cancer from the previous report were compared with 75 women with in-situ disease. HPV DNA testing of cervical scrapings showed high-risk types in 79% of invasive and 57% of intraepithelial tumours. The 291 hospital-based controls for invasive cervical cancer and 124 controls for carcinoma *in situ* had HPV prevalences of 6.9% and 10.4%, respectively. Types 16 and 18, but not types 31/33/35/39, were more common in invasive than in intraepithelial tumours, and untyped HPV DNA was more common in in-situ lesions. The odds ratio for invasive carcinoma *in situ* with HPV types 16 and 18 was reported to be 11.0 (95% CI, 3.9–33.0) and 10.0 (95% CI, 1.2–86.0), respectively.

In the northeastern USA, Altekruse *et al*. (2003) conducted a case–control study that included 124 women with cervical adenocarcinoma, 139 with cervical squamous-cell carcinoma and 307 control subjects to determine HPV genotypes and sexual and reproductive risk factors using a PCR-based reverse line blot detection system (MY09/11 L1 consensus primer system). Specimens were grouped hierarchically by HPV genotype: 18, 16, 18-related (39, 45, 59 and 68), other high-risk (26, 31, 33, 35, 51, 52, 55, 56 and 58) and low-risk (6, 11, 40, 42, 51, 53, 54, 57, 66, 73, 82, 83 and 84). HPV 18 was associated most strongly with adenocarcinoma (odds ratio, 11.9; 95% CI, 3.6–39.5) and HPV 16 was associated most strongly with squamous-cell carcinoma (odds ratio, 10.5; 95% CI, 5.2–21.2). The relative importance of HPV genotypes 16 and 18 and the differences in reproductive co-factors suggest distinctly separate causes for cervical adenocarcinoma and squamous-cell carcinoma.

Franceschi *et al*. (2003) evaluated the role of HPV and other risk factors in the etiology of invasive cervical carcinoma in a hospital-based case–control study in Chennai, southern India. A total of 205 cases of invasive cervical cancer (including 12 adenocarcinomas) and 213 frequency- and age-matched control women were included. HPV DNA in cervical cells was evaluated by a PCR assay (GP5+/6+). HPV infection was detected in all but one case of invasive cervical cancer and in 27.7% of control women. HPV 16 was the most common type in both cases and controls (60.2% and 17.4%, respectively), followed by HPV 18 and 33. Compared with women who were infected by HPV 16, those infected with HPV 18 showed an increased odds ratio of 3.9 (95% CI, 0.9–17.4). In this study, multiple HPV infections did not yield a higher odds ratio for invasive cervical cancer than single infections.

One of the largest studies that has contributed to an understanding of the association between infection by individual HPV types and cervical cancer is the analysis of data pooled from nine case–control studies of invasive cervical cancer conducted by the IARC in Brazil, Colombia, Mali, Morocco, Paraguay, Peru, the Philippines, Spain and Thailand, the results of which were reported by Muñoz *et al.* (2003). Detection of HPV DNA in cervical scrapings (exfoliated cells) and biopsy specimens was performed blindly in

central laboratories using PCR-based assays. PCR primers for the *L1* gene, MY09/11, were used in the Colombian and Spanish studies and the GP5+/6+ general primer system was used in the remaining studies. A total of 1918 cases and 1928 controls were included in the pooled analysis. Overall, the prevalence of HPV infections was 90.7% in cases and 13.4% in controls and the pooled odds ratio for any HPV type for cervical cancer was 158.2 (95% CI, 113.4–220.6). The authors concluded that, in addition to HPV types 16 (odds ratio, 435) and 18 (odds ratio, 248), HPV types 31 (odds ratio, 124), 33 (odds ratio, 374), 35 (odds ratio, 74), 39 (odds ratio, ∞), 45 (odds ratio, 198), 51 (odds ratio, 67), 52 (odds ratio, 200), 56 (odds ratio, 45), 58 (odds ratio, 115), 59 (odds ratio, 419), 68 (odds ratio, 54), 73 (odds ratio, 106) and 82 (odds ratio, ∞) should be considered as carcinogenic. In addition, HPV 53 was found in one case of invasive cervical cancer only. No significant associations were reported for HPV 6 and 11.

In Okinawa, Japan, Asato *et al*. (2004) conducted a case–control study to determine the association between HPV infections and invasive cervical cancer. The study included 356 women who had been newly diagnosed with squamous-cell carcinoma of the cervix and 3249 controls. Cervical swabs taken before any treatment was started were analysed using a consensus primer pair to amplify DNA from the L1 region of HPV by PCR. This method would, however, underestimate the prevalence of HPV in multiple infections. Direct sequencing of PCR products resulted in the identification of nucleotide sequences of 30 HPV DNA genotypes. Overall, 87.4% of cases and 10.2% of controls were HPV DNA-positive. Among cases, 84.5% were HPV-positive for types 16, 18, 31, 33, 35, 52 and 58. The odds ratio associated with being positive for HPV 16 was the highest (534.6). Significantly elevated risks for invasive cervical cancer were also associated with HPV 18 (odds ratio, 259), 31 (odds ratio, 137), 33 (odds ratio, 151), 35 (odds ratio, 31), 51 (odds ratio, 9), 52 (odds ratio, 36), 53 (odds ratio, 14), 54 (odds ratio, 22), 56 (odds ratio, 25), 58 (odds ratio, 180), 59 (odds ratio, 52), 66 (odds ratio, 65), 68 (odds ratio, 12), 70 (odds ratio, 32) and 82 (odds ratio, 65). HPV 45, 73 and 82 were each detected in a single case. This study is one of the first large case–control studies in which HPV genotyping was based completely on nucleotide sequencing which allowed the investigators to estimate the risks for cervical cancer associated with previously uncharacterized genotypes.

Hammouda *et al.* (2005) conducted a case–control study in Algiers, Algeria, that included a total of 198 cases of cervical carcinoma and 202 age-matched control women. HPV infection was detected in 97.7% of cases and 12.4% of controls (odds ratio, 635). HPV 16 was the most common type in both cases and controls, followed by HPV 18 and 45. Twelve types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 66 and 73) were found as single infections in cases. Significantly elevated risks for invasive cervical cancer were observed for HPV 16 [odds ratio, 503], 18 [odds ratio, 572], 31 [odds ratio, ∞], 33 [odds ratio, ∞], 35 [odds ratio, ∞], 39 [odds ratio, ∞], 51 [odds ratio, ∞], 56 [odds ratio, ∞], 66 [odds ratio, ∞], 73 [odds ratio, ∞] and 45 [odds ratio, 159]. In addition, HPV 52 was found in one case. Multiple HPV infections did not yield a higher odds ratio for cervical carcinoma than single infections. The distribution of HPV types in cases of cervical carcinoma

and controls in Algeria was observed to be more similar to that found in Europe than to that found in sub-Saharan Africa, where HPV 16 is less prevalent.

Herrero *et al.* (2005) presented the results of their prevalent case–control analysis of the enrolment visit of the cohort study in Guanacaste, Costa Rica. In this study, a population-based cohort of 8514 sexually active women was tested for individual HPV types and screened by cytology for CIN and cancer. An expert panel of pathologists histologically confirmed all the lesions detected. The overall prevalence of HPV was 26.5%, and HPV 16 was the type most commonly detected (3.6% of the population). High-risk HPV infection was strongly associated with risk for all grades of CIN and cancer. HPV 16, 58 and 18 were the most common types in women diagnosed with CIN3 and cancer. Significantly increased risks for CIN3 were found for HPV types 16 [odds ratio, 272], 31 [odds ratio, 83.3], 56 [odds ratio, 46.3], 58 [odds ratio, 73.5] and 68 [odds ratio, 96.1]. HPV types 16 [odds ratio, 504], 18 [odds ratio, 595], 45 [odds ratio, 390], 52 [odds ratio, 149], 39 [odds ratio, 202], 58 [odds ratio, 184] and 66 [odds ratio, 312] were significantly associated with an elevated risk for invasive cervical cancer. HPV types 59, 35, 56, 68, 53, 54, 26 and 73 were detected in controls only. Multiple-type infections were associated with an increased risk compared with single-type infections for all grades of CIN and cancer, except for HPV 16-positive CIN3 and cancer.

(*c*) *Cohort studies*

(i) *Prospective studies with data on DNA*

Table 28 summarizes the results of cohort studies of HPV type-specific infection and pre-invasive and invasive carcinoma.

Koutsky *et al*. (1992) conducted the first prominent cohort study of HPV infection with some type specificity and a disease end-point of CIN2 or CIN3. In the 24 months following study entry, the relative risk for women who were infected with HPV 16 or HPV 18 (combined) compared with HPV DNA-negative women was 11 (95% CI, 4.6–26).

Using the stored collection of cytological slides from Swedish women who participated in a multi-decade screening programme, Wallin *et al.* (1999) examined type-specific persistence of HPV DNA before the development of invasive cervical cancer. Using two different PCR techniques and DNA sequencing, HPV in cells scraped from cytological smears was typed. A total of 118 women in whom invasive cancer developed on average 5.6 years later (range, 0.5 months to 26.2 years) were compared with 118 women who remained healthy during a similar length of time. In addition to testing the cytological slides, the important issue was addressed of whether the HPV type in the pre-morbid cytological sample matched the diagnostic type among cases who had available histology blocks. There was a clear excess of HPV 16 persistence associated with the risk for developing cancer (16 cases, no controls). There was also a non-significant excess of HPV 18 persistence (four cases, no controls), and single cases with persistence of HPV 31, 33 and 73.

In a series of publications from the same project, Josefsson *et al.* (2000) and Ylitalo *et al.* (2000a,b) measured HPV 16 viral load by applying quantitative PCR to cell

Table 28. Cohort studies on HPV-specific infection and pre-invasive and invasive lesions of the cervix

Reference, study location	Method of detection (types included)	No. and type of cases	Odds ratio (95% CI)
Koutsky *et al*. (1992), USA	Dot filter hybridization, Virapap specific primers (16, 18, 6, 11, 31, 33, 35)	28 CIN	HPV 16/18 11 (4.6–26)
Wallin *et al.* (1999), Sweden	PCR MY09/MY1GP5+/6+ DNA sequencing	118 ICC	HPV 16/18/31/33/73 16.4 (4.4–75.1)
Josefsson *et al.* (2000); Ylitalo *et al.* (2000a,b), Sweden	Quantitative PCR (16)	478 CIS	High viral load 25 (12.4–31.8) Medium viral load 6.6 (1.7–11.2)
Woodman *et al.* (2001), United Kingdom	PCR GP5+/GP6+, MY09/11 and specific primers (16, 18, 31, 33, 52, 58)	23 CIN2/3	HPV 6/11 3.8 (1.5–9.8) HPV 16 8.5 (3.7–19·2) HPV 18 3.3 (1.4–8.1)
Zielinski *et al.* (2001a,b), The Netherlands	PCR GP5+/6+ using a cocktail of HPV type-specific oligoprobes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	57 ICC	No risk or *p*-value reported
van Duin *et al.* (2002), The Netherlands	PCR (16)	12 CIN2/3	7.7 (1.6–33)
van der Graaf *et al.* (2002), The Netherlands	Short-fragment PCR 10 general primer set (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74)	77 CIN3, SCC	HPV 16 104.8 (29.5–372.7) HPV 18, 31, 33 10.8 (4.3–27.2)
Kjaer *et al.* (2002), Denmark	PCR GP5+/6+ (high risk 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, low risk 6, 11, 40, 42, 43, 44)	112 CIN2/3	See Table 29
Xi *et al.* (2002), USA	MY09/11, HMB01, human β-globin primers (6, 11, 16, 18, 31, 33, 35, 39, 45, 56, 40, 42, 53, 54, 51, 52, 55, 58)	6 CIN 2/3	HPV 16 non-prototype-like variants 3.5 (1.0–11.8)
Schiffman *et al.* (2005), Costa Rica	PCR MY09/11 PCR (2, 6, 11, 13, 16, 18, 26, 31–35, 39, 40, 42–45, 51–59, 61, 62, 64, 66–74v, 81–85, 82v (AE2), 89, AE9, AE10)	8 ICC 61 CIN3	No risk or *p*-value reported

See Table 7 for a description of the primers used.
CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; ICC, invasive cervical carcinoma; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma

scrapings of archival cytology slides from the Swedish screening programme. A total of 2081 smears from 478 cases of carcinoma *in situ* and 1754 smears from 608 controls were tested. Elevated HPV 16 viral loads were observed among cases compared with controls starting at 13 years before diagnosis, although the smears were considered to be cytologically normal (Ylitalo *et al.*, 2000b). Thus, detection of HPV 16 DNA predicted a risk for a diagnosis of carcinoma *in situ* many years later. About 25% (95% CI, 12.4–31.8) of women infected with a high viral load before the age of 25 years developed cervical carcinoma *in situ* within 15 years. Women with a medium viral load had an absolute risk of 6.6% (95% CI, 1.7–11.2) after 15 years (Ylitalo *et al.*, 2000a). Women with low viral loads were at marginally elevated risk compared with HPV 16-negative women (Ylitalo *et al.*, 2000a). The median latency between the initial HPV 16 infection and diagnosis of carcinoma *in situ* was estimated to be between 7 and 12 years but may be up to two decades for some women (Ylitalo *et al.*, 2000b). These estimates are concordant with the time between modal ages of HPV infection and CIN3 observed in population-based cross-sectional studies.

Woodman *et al.* (2001) studied the natural history of incident cervical infection with HPV 16, 18, 31, 33, 52, 58 or 6/11 in relation to the development of CIN2 or CIN3 among 1075 British women aged 15–19 years who had recently become sexually active. The median duration of follow-up of the cohort was 29 months; thus, the few cases represented the leading edge of the incidence curve of CIN2 (14 cases) or CIN3 (14 cases). Specifically, among the 23 cases for whom HPV DNA was detected, the median time from first detection to diagnosis of CIN2 or CIN3 was 26 months (range, 0–69 months). The univariate relative risks for CIN2 or CIN3 were elevated for HPV 6/11 (3.8; 95% CI, 1.5–9.8), 16 (8.5; 95% CI, 3.7–19.2), 18 (3.3; 95% CI, 1.4–8.1), 31 (3.5; 95% CI, 1.0–11.8), 52 (2.3; 95% CI, 0.3–17.2) and 58 (2.9; 95% CI, 0.8–10.1), but not for HPV 33 (0.6; 95% CI, 0.1–4.4). The evidence of type-specific carcinogenicity afforded by these data is weakened by the short follow-up, the incomplete typing with possibility of type–type confounding and the inclusion of CIN2 in the disease group.

Zielinski *et al.* (2001b) conducted a retrospective case–control study of type-specific DNA detection (by GP5+/6+ PCR) in the last normal cervical smears archived for 57 women who developed cervical cancer approximately 8 years later compared with 114 controls matched on age and date of screening. The types found in the subsequent smears and diagnostic biopsies of the case women were the same as those detected at the baseline smear. The only statistically significant difference was for HPV 16 (29/57 cases, 2/114 controls). Other types showed non-significant excesses among cases: HPV 18 (three cases, two controls), 31 (three cases, two controls), 45 (one case, one control) and 33 (one case, no control). Most of the smears that were originally interpreted as normal were re-interpreted, blinded to other study information, as abnormal for cases but not for controls. Re-interpretation as abnormal was strongly linked to HPV DNA positivity. This study demonstrates the difficulty of determining the true cytological state at baseline in longitudinal studies.

Using quantitative PCR, van Duin *et al.* (2002) tested the viral load of archived HPV 16 DNA-positive specimens from a Dutch cohort that included 12 women who subsequently developed CIN2 or CIN3 and 47 controls who developed ≤ CIN1. All baseline smears were considered to be normal, although that interpretation does not rule out the possibility of neoplasia being missed by cytology. Over the average of almost 3 years of follow-up, an association was observed between high versus low baseline viral load of HPV 16 and risk for CIN2 or CIN3 (odds ratio, 7.7; 95% CI, 1.6–33).

van der Graaf *et al.* (2002) tested scraped cells from archived cytological smears in a nested case–control study within the Dutch (Utrecht) mass-screening programme, using a short fragment PCR 10 amplification system and typing by a reverse hybridization line probe. After exclusions for missing slides, the case group included 62 women with CIN3 and 15 with micro- or gross invasion. The 270 controls were matched to cases on age and follow-up time. During an average follow-up period of 5.6 years, 29 cases compared with three controls had slides that contained only HPV 16 (odds ratio, 104.8; 95% CI, 29.5–372.7). The presence of HPV 18, 31 and/or 33 (not distinguished individually) was associated with an odds ratio of 10.8 (95% CI, 4.3–27.2).

Kjaer *et al.* (2002) conducted a prospective cohort study among more than 10 000 women aged 20–29 years in Copenhagen, Denmark, and tested baseline specimens for type-specific DNA using GP5+/GP6+ PCR. In order to limit the study to incident cases, women with a history of cervical neoplasia, abnormal baseline cytology or abnormalities diagnosed within 9 months from baseline were excluded. A total of 112 cases of CIN2 or CIN3 were observed at the follow-up visits approximately 2 years after baseline. Because of the relatively short follow-up, some of these cases were possibly present but were missed at baseline. With this caveat, elevated univariate odds ratios were found for most putative carcinogenic HPV types, including HPV 16, 18, 31, 33, 45, 51, 52, 58 and 66 (Table 29). Increased risk estimates based on one or two cases were associated with HPV 35, 39, 56 and 59. Based on a single case each, elevated univariate risk estimates were seen for HPV 6 and 11 which are associated with condyloma acuminatum. Other identified types were rare or absent in cases and controls. However, this analysis did not take into account the possible confounding influences of multiple infections, which were found in 25% of HPV-positive cases and 12% of HPV-positive controls.

In a 5-year longitudinal cohort study among female university students in Seattle, USA, Xi *et al.* (2002) observed that incident infections with non-prototype-like HPV 16 variants conferred a 3.5 (95% CI, 1.0–11.8) increase in risk for histological CIN2 or CIN3 compared with prototype-like HPV 16 variants. Of the 48 women with incident HPV 16 prototype-like variants, six developed CIN2 or CIN3, while six of 14 women with non-prototype-like variants developed these diseases. The difference in risk was not mediated by a difference in average length of viral persistence, ethnicity or current use of oral contraceptives.

Schiffman *et al.* (2005) conducted a population-based prospective study of HPV infection and subsequent development of CIN3 and cancer in a cohort of 10 000 women in Guanacaste, Costa Rica. They tested for more than 40 types of HPV DNA using MY09/11 PCR with TaqGold polymerase, and followed more than 7000 sexually active

Table 29. Distribution of HPV types among cases and cytologically normal women who were positive for HPV at enrolment

HPV type	Cases (n = 115) of high-grade lesions (%)	Controls (n = 100) (%)	Odds ratio[a]
6	1 (0.9)	5 (0.8)	1.2
11	1 (0.9)	1 (0.2)	5.9
16	43 (37.4)	27 (4.1)	14.4
18	10 (8.9)	11 (1.7)	5.7
31	15 (13.0)	7 (1.1)	14.3
33	7 (6.1)	11(1.7)	3.9
35	1 (0.9)	1 (0.2)	5.9
39	2 (1.8)	2 (0.3)	5.9
42	0	1 (0.2)	–
44	0	1 (0.2)	–
45	5 (4.5)	1 (0.2)	30.5
51	6 (5.3)	1 (0.2)	57.0
52	5 (4.5)	2 (0.3)	15.2
56	2 (1.8)	1 (0.2)	11.9
58	5 (4.5)	5 (0.8)	6.0
59	2 (1.8)	0	–
66	4 (3.6)	1 (0.2)	24.1
X	6 (5.3)	22 (3.4)	1.6

From Kjaer *et al.* (2002)
Figures are numbers (percentages of women).
[a] Crude odds ratios calculated by the Working Group

women who had no evident prevalent CIN2, CIN3 or cancer and no hysterectomy for an average of over 5 years. Cases of incident histologically confirmed CIN3 and cancer were considered to be caused by a particular type of HPV only if that type was found both at study enrolment and at the time of diagnosis. The results confirmed the case–control literature, and were consistent with the data of Kjaer *et al.* (2002). The risks for cancer and CIN3 with HPV types were clearly associated with their phylogenetic relatedness. Compared with other types, HPV 16 was the most likely to persist for 5 years and, when persistent, to be linked to CIN3 and cancer. HPV 16-related infections also tended to lead to incident CIN3/cancer. Most of the other cancer-associated types were phylogenetically related to HPV 18. Some HPV species (clades; e.g. A3) showed virtually no association with CIN3 or cancer despite a relatively high prevalence and a tendency of some types in those clades to persist.

(ii) *Prospective studies with serological data* (Table 30)

Studies of archived sera permit the assessment of exposure to HPV before a diagnosis of CIN3 or cancer. The assays themselves tend to be type-specific, although the possibility of confounding remains because genital HPV infections are transmitted by a common sexual route and few types are assessed in any study.

Lehtinen *et al.* (1996) focused on HPV 16 serology within a cohort of Finnish women that was followed for up to 23 years. The odds ratio for HPV 16 seropositivity was 12.5 (95% CI, 2.7–57) among 72 cases (27 with cancer and 45 with carcinoma *in situ*) and 143 matched controls. The risk estimates were increased for both short and long lapses of time from sampling to diagnosis.

Dillner *et al.* (1997) compared 182 women who had developed cervical cancer with 538 controls matched on age and time of enrolment into a joint Nordic cohort. Sera were tested for antibodies to HPV 16, 18 and 33. HPV 16 seropositivity was associated prima-

Table 30. Prospective serological studies on HPV-specific infection and pre-invasive and invasive lesions of the cervix

Reference, study location	Method of detection (types included)	No. and type of cases	Odds ratio (95% CI)
Shah *et al*. (1997), USA	ELISA (16, 6, 6b)	14 ICC, 28 CIN3	HPV 16 3.9 (1.4–10.7)
Lehtinen *et al.* (1996), Finland	ELISA (16)	27 ICC, 45 CIS	12.5 (2.7–57)
Dillner *et al.* (1997) Finland, Norway, Sweden	ELISA (16, 18, 33)	182 ICC	HPV 16 3.2 (1.7–6.2)
Luostarinen *et al.* (1999), Finland, Norway, Sweden	ELISA (16, 18, 33, 6/11)	182 ICC	No signifiant association
Vonka *et al.* (1999), Czech Republic	ELISA (16, 18, 33)	43 dysplasies, 19 CIS, 5 ICC	HPV 16 3.85 (1.11–13.91)
Wallin *et al.* (2000), Sweden	ELISA (73)	41 CIN2/3	1.5 (0.35–6.65)
Hisada *et al.* (2001), USA	ELISA (16)	52 ICC, 47 CIS	2.0 (1.0–3.4)
Sigstad *et al.* (2002), Norway, Finland, Sweden	ELISA (16, 18, 33)	127 ICC	HPV 16 4.4 (2.2–8.8) HPV 18 17 (2.1–140)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; ELISA, enzyme-linked immunosorbent assay ; ICC, invasive cervical carcinoma

rily with an increased risk for squamous-cell carcinoma (odds ratio, 3.2; 95% CI, 1.7–6.2) while HPV 18 seropositivity tended to be associated with a higher risk for cervical adenocarcinomas (odds ratio, 3.4; 95% CI, 0.8–14.9). HPV 33 seropositivity was not significantly associated with either squamous-cell or adenocarcinoma (odds ratio, 1.6 and 1.7, respectively).

Shah *et al*. (1997) tested pre-diagnostic sera from 14 cases of invasive cancer and 28 cases of CIN3 and compared them with those from 83 matched controls. The odds ratio for antibodies to HPV 16 VLPs was 3.9 (95% CI, 1.4–10.7), but HPV 6 antibodies were not associated with the subsequent occurrence of cervical cancer or CIN3.

Luostarinen *et al.* (1999) further tested the same Nordic subjects followed by Dillner *et al.* (1997) for HPV 6/11 to assess the joint effect of simultaneous exposure with carcinogenic (HPV 16, 18 and 33) and non-carcinogenic HPV types on the risk for subsequent development of cancer. HPV 6/11 seropositivity was not strongly associated with risk for cancer. However, there was evidence of an antagonistic modification of effect for the combination of HPV 16 seropositivity and HPV 6/11 seropositivity (but not other combinations). The authors suggested that HPV 6/11 seropositivity might reflect cross-protective immunity.

Vonka *et al.* (1999) re-tested stored sera from the Prague cohort that was originally assembled in the 1970s to study herpes simplex virus (HSV) and cervical cancer (Vonka *et al.*, 1984) for HPV 16, 18 and 33 VLP antibodies. While the original study failed to find an association of HSV seropositivity with subsequent cervical cancer, the re-analysis of 67 prospective cases and 129 matched controls showed an elevated relative risk for all three HPV types. The case group included 43 moderate or severe dysplasias, 19 carcinomas *in situ* and five invasive carcinomas. The relative risks were 3.85 (95% CI, 1.11–13.91) for HPV 16, 2.70 (95% CI, 0.87–8.55) for HPV 18 and 1.51 (95% CI, 0.55–4.13) for HPV 33.

Wallin *et al.* (2000) explored the possible carcinogenicity of HPV 73 in a serological study of stored blood from 41 cases of CIN2–3 in northern Sweden and 82 matched controls. The odds ratio for HPV 73 antibodies preceding case diagnosis by an average of 2–3 years was 1.5 (95% CI, 0.35–6.65).

Hisada *et al.* (2001) tested precancer sera archived from pregnant women in a Californian cohort study and observed an age- and race-adjusted odds ratio of 2.0 (95% CI, 1.0–3.4) for the association of seropositivity to HPV 16 VLPs and subsequent risk for invasive (52 cases) or in-situ (47 cases) cervical cancer.

In a retrospective cohort study based on archived blood and cervical tissue, Sigstad *et al.* (2002) confirmed that pre-diagnostic HPV 16 and HPV 18 seropositivity was linked to a risk for subsequent cervical cancer and corresponded to the same HPV DNA types found in the tumours. A total of 127 cases of invasive cancer in the large Nordic Janus cohort and 376 controls matched on age, country and time of blood collection were studied. HPV 16-seropositive women had a relative risk of 4.4 (95% CI, 2.2–8.8) for developing invasive cancer containing HPV 16 DNA, but had no excess risk for developing other cancers. Similarly, HPV 18 seropositivity predicted a risk (odds ratio, 17; 95% CI, 2.1–140) only for cancers that contained HPV 18, of which 10/20 were

adenocarcinomas. The results for HPV 33 were not type-specific; tumours that contained HPV 33 were very rare and HPV 33 seropositivity was associated with the development of cancers that contained HPV 16.

In prospective studies of individual HPV types, the data from DNA-based and serological studies are concordant although absolute risks associated with DNA positivity are higher than those associated with seropositivity. HPV 16 is clearly carcinogenic and persists longer than other carcinogenic types. Because of its persistence, HPV 16 is apparently more closely linked to malignant transformation. There is also some prospective evidence for the carcinogenicity of HPV 18. The evidence for other types, although scant, is concordant with the more statistically powerful case–control literature.

2.3 Cancer at other anogenital sites

2.3.1 *Cancer of the vulva*

Vulvar cancer has two distinct histopathological types and sets of risk factors. Only a limited number of studies have characterized the prevalence of HPV DNA by histological type, but the results have been consistent. Keratinizing vulvar cancer is associated with a low prevalence of HPV (generally less than 10%), occurs in older women and is associated with lichen planus. In contrast, HPV DNA is found in a high proportion of basaloid and warty vulvar cancers (> 55%), which occur in younger women than keratinizing cancers and are associated with classical risk factors for the acquisition of HPV (Schiffman & Kjaer, 2003). These cancers are often associated with overlying vulvar intraepithelial neoplasia (VIN), which in turn has a strong association with HPV infection. The data suggest two distinct sets of cancer: one that is associated with HPV and may be preceded by VIN, and another that is not clearly associated with HPV and whose precancerous natural history is poorly understood.

(*a*) *Case series*

Table 31 presents series of more than 10 cases of cancer of the vulva or VIN3. Among the HPV DNA-positive vulvar cancers, HPV 16 is the most common type, followed by HPV 18 at a much smaller percentage. The proportion of HPV-positive vulvar cancers that contained HPV 31 or HPV 33 was variable in the small number of studies that specifically probed for these types. One study (Iwasawa *et al.*, 1997) found a 1.4% prevalence of HPV 33 but did investigate HPV 31. Another small study of 11 cases of vulvar carcinomas (Abdel-Hady *et al.*, 2001) found HPV 33 in two of three HPV-positive cases.

Similar to the data on vulvar cancer, case series on the prevalence of HPV DNA show that a high proportion (> 70%) of VIN3 are positive (Table 31) and that the most common type is HPV 16. Two studies (Junge *et al.*, 1995; Van Beurden *et al.*, 1998) also showed that a small proportion of lesions (< 11%) are DNA-positive for HPV 33. Two studies

Table 31. Prevalence of HPV DNA in case series of vulvar cancer (≥ 10 cases) and grade 3 vulvar intraepithelial neoplasia (VIN3) (≥ 9 cases)

Reference, study location	Method of detection[a] (types included)	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Vulvar cancer												
Hørding *et al.* (1994), Denmark	E6/E7 primers (6, 11, 16, 18, 33)	51 keratinizing 17 warty 10 basaloid	3.9 70.5 100	0 0 0	0 0 0	28.2	0 0 0	NR	3.8		1.3	Paraffin-embedded tissue; HPV DNA found in 81% of cancers with overlying VIN3, 9% of tissues with overlying VIN1–2 and 0% of tissues with adjacent lichen sclerosis
Trimble *et al.* (1996), USA	ISH (6/11/16/18/31/33/35/42/43/44/45/51/52/56)	21 basaloid-warty 48 keratinizing	85.7 6.3									Paraffin-embedded tissue; basaloid or warty carcinoma but not keratinizing squamous carcinoma associated with classical risk factors for cervical cancer
Iwasawa *et al.* (1997), Finland	MY09/11 type-specific (6, 11, 16, 18, 33)	74	36.5	0	0	25.7	12.2		1.4		4.0	Paraffin-embedded tissue; 65 of 74 cases were women 61 years of age or older; histological type not specified
Madeleine *et al.* (1997), USA	MY09/11 (6/11, 16, 18/45, 31/33/52) or RFLP	55	50.9	1.8		43.6	1.8[b]		3.6[c]	1.8[d]	1.8	Paraffin-embedded tissue; histological type not specified
Abdel-Hady *et al.* (2001), United Kingdom	GP5+/6+ (6/11, 16, 18, 31, 33)	11	27.3			27.3	9.0		18.1		18	Paraffin-embedded tissue; histological type not specified
Carter *et al.* (2001), USA	MY09/11 and RFLP; type-specific for 16, 18	38	79			55.3	2.6		13.2[e]	7.9[f]	0	Paraffin-embedded tissue; histological type not specified
VIN3												
Junge *et al.* (1995), Denmark	E6/E7 primers (6/11, 16, 18, 31, 33); ISH (6/11, 16/18, 31/33)	62 PCR [*n* = 55] ISH [*n* = 58]	89.1	0 0	0 0	[78.2] [36.2]	0	0	[5.2]	[10.9]	NR	Paraffin-embedded tissue; cases of severe dysplasia and carcinoma *in situ*

Table 31 (contd)

Reference, study location	Method of detection[a] (types included)	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Trimble *et al.* (1996), USA	ISH for 6/11/16/18/ 31/33/35/42/43/44 /45/51/52/56	54 basaloid or warty	88.9									Paraffin-embedded tissue; squamous hyperplasia (VIN2) and basaloid or warty VIN (VIN3) combined
Madeleine *et al.* (1997), USA	MY09/11 probes (6/11, 16, 18/45, 31/33/52) or RFLP	253	71.5	5.5		61.7	5.9[b]	5.9[c]		2.8[d]	9.5	Paraffin-embedded tissue; histological type not specified
Van Beurden *et al.* (1998), Netherlands	CPI and CPIIG and sequencing	27	100			92.6			3.7	3.7 (45)	0	Paraffin-embedded tissue; histological type not specified; patients with pre-existing, concomitant or subsequent cervical or vaginal neoplasm
Abdel-Hady *et al.* (2001), United Kingdom	GP5+/6+ and type-specific for 6/11, 16, 18, 31, 33	32	71.9	[18.8]		46.8					NR	Paraffin-embedded tissue; cases were VIN2 or VIN3, predominantly (26/32) warty or mixed warty-basaloid type.
Carter *et al.* (2001), USA	MY09/11 and RFLP; type-specific for 16, 18	181	91.2			74.6	6.6	8.8[e]		7.2[f]	6.1	Paraffin-embedded tissue; carcinoma *in situ*
Todd *et al.* (2004), United Kingdom	GP5/6 and type-specific for 16, 18, 31, 33	9	[88.8]			[66.7]	0	0	[22.2]		0	Paraffin-embedded tissue; 77.8% of cases had prior history of CIN.

See Table 7 for a description of the primers used.

CIN, cervical intraepithelial neoplasia; ISH, in-situ hybridization; NR, not reported; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

[a] Unless otherwise specified, the method is PCR; ';' denotes independent methods whereas 'and' denotes subsequent steps.

[b] 18 or 45

[c] 31, 33 or 52

[d] Unknown

[e] 31, 33, 35 or 39

[f] HPV 6, 45, 52, 54, 58, 66, 72, 73 or unknown types

(Madeleine *et al.*, 1997; Carter *et al.*, 2001) showed the presence of HPV 18 or 45 in 5.9% and that of HPV 18 in 6.6% of lesions, respectively.

(*b*) *Case–control studies*

Results from case–control studies of vulvar cancer and VIN that used serological assays as detection method are consistent with the findings on DNA.

Sun *et al*. (1996) examined HPV-specific antibodies in the sera of patients in the USA who had basaloid or warty squamous-cell vulvar cancer, keratinizing vulvar cancer and VIN to determine the association between these conditions and the presence of antibodies to HPV 16. The study included a total of 54 cases (14 basaloid or warty cancers, 18 keratinizing cancers and 22 VIN) for whom serological specimens were available and 44 controls. The prevalence of antibodies to HPV 16 VLPs was significantly higher in HPV-associated VIN (59.1%) and basaloid or warty cancers (50.0%) than in keratinizing cancers (22.2%) or controls (18.2%). The odds ratios were 5.4 (95% CI, 1.7–18) for VIN and 4.5 (95% CI, 1.2–16) for basaloid and warty cancers. For keratinizing cancers, the odds ratio was not statistically significant at 1.3 (95% CI, 0.32–4.9).

A seroepidemiological nested case–control study from Finland and Norway (Bjørge *et al.*, 1997a) showed that HPV 16 seropositivity was associated with an increased risk for vulvar and vaginal cancers combined (odds ratio, 4.5; 95% CI, 1.1–22) and a strongly increased risk for pre-invasive vulvar and vaginal lesions (odds ratio, ∞; 95% CI, 3.8–∞). Seropositivity for HPV 18 was associated with an increased risk for pre-invasive lesions (odds ratio, 12; 95% CI, 1.2–590) but not for invasive cancer (odds ratio, 1.5; 95% CI, 0.3–7.5).

Hildesheim *et al.* (1997a) studied 142 histologically confirmed cases of VIN3 and invasive vulvar cancer and 126 community controls in the USA. Sera were tested for immunoglobulin G (IgG) antibodies against HPV 16 L1/L2 VLPs. Overall, 44.4% of cases and 11.9% of controls were HPV 16-seropositive. A stronger association between HPV 16 seropositivity and disease was observed for VIN3 (odds ratio, 13.4; 95% CI, 3.9–46.5) than for invasive cancer (odds ratio, 2.9; 95% CI, 0.94–8.7), although there was a suggestion that the association was stronger among women who had been diagnosed with basaloid or warty cancer (odds ratio, 3.8; 95% CI, 0.76–18.9) than among those with keratinizing cancer (odds ratio, 1.6; 95% CI, 0.35–7.4).

Madeleine *et al*. (1997) conducted a population-based case–control study in the USA to examine the association between HPV positivity, cigarette smoking, HSV-2 infection and the risk for vulvar cancer. The study included 400 in-situ and 110 invasive tumours of the vulva diagnosed among women who lived in the Seattle area from 1980 to 1994. In most analyses, cases were compared with 1043 controls. Serum samples were analysed for antibodies against HPV 6, 16 and 18. The prevalence of seropositivity to HPV 16 was 53.3% in in-situ cases, 43.8% in invasive cases and 22.2% in controls. HPV 16 seropositivity was associated with an increased risk for in-situ and invasive vulvar cancers (odds ratio, 3.6; 95% CI, 2.6–4.8; and 2.8; 95% CI, 1.7–4.7, respectively).

Overall, the data indicate that HPV 16 is the predominant HPV type in VIN3 and vulvar cancer, particularly basaloid and warty cancer. In vulvar cancers, HPV 18, 45, 31 or 33 may play a smaller role.

2.3.2 *Cancer of the vagina*

(*a*) *Case series*

The number of case series of vaginal cancer (Table 32) has remained small since the previous review (IARC, 1995). In two studies (Carter *et al.*, 2001; Daling *et al.*, 2002), the majority of vaginal cancers were positive for HPV DNA (90.7% of 54 cases and 64.0% of 25 cases, respectively). HPV 16 was the most common type and was found in at least 70% of HPV-positive tumours. HPV 6 or 11 was found in two cases of vaginal cancer in one study. HPV 18 or 45 and HPV 31, 33, 35 or 39 were also found in a small number of cases.

Similarly to the vaginal cancers, a high proportion of grade 3 vaginal intraepithelial neoplasia (VAIN3) tissues were also positive for HPV DNA and again the most common type was HPV 16 (Table 32). HPV 6 or 11, HPV 18 or 45 and HPV 31, 33 or 35 were found in a small number of cases (Van Beurden *et al.*, 1998; Daling *et al.*, 2002).

(*b*) *Case–control studies*

As with the studies of vulvar cancer, case–control studies of VAIN and vaginal cancer that used serological assays as the detection method are consistent with the findings on HPV DNA.

Hildesheim *et al*. (1997b) conducted a case–control study of VAIN and vaginal cancer in the USA. The study included 23 histologically confirmed cases of in-situ and invasive vaginal cancer and 28 community controls. Blood samples were collected from participants and tested for the presence of antibodies to HPV 16 VLPs, HSV-2 and *C. trachomatis*. Overall, 50% of cases and 25% of controls were positive for HPV 16 VLP antibodies. Women positive for HPV 16 VLP antibodies were at a 3.5-fold increased risk for vaginal neoplasia (95% CI, 0.97–13) and those with high antibody levels (high optical density) were at a 33-fold increased risk for the disease (95% CI, 2.5–430). The risk estimate was not affected by adjustment for HSV-2 or *C. trachomatis* seropositivity. The association was stronger for in-situ neoplasia than for invasive cancer, with relative risks of 5.4 (95% CI, 0.93–31) and 1.7 (95% CI, 0.22–14), respectively.

Daling *et al.* (2002) conducted a population-based case–control study that included 156 women with in-situ or invasive vaginal cancer diagnosed between January 1981 and June 1998 and 2041 control women identified through random-digit dialling in western Washington State, USA. Antibodies to HPV-16 L1 were strongly related to risk for vaginal cancer (odds ratio, 4.3; 95% CI, 3.0–6.2). Women with vaginal cancer were more likely to have had five or more lifetime sexual partners (odds ratio, 3.1; 95% CI, 1.9–4.9), to have an early age at first intercourse (< 17 years; odds ratio, 2.0; 95% CI, 1.2–3.5) and to be current smokers at diagnosis (odds ratio, 2.1; 95% CI, 1.4–3.1) than control women.

Table 32. Prevalence of HPV DNA in case series of vaginal cancer and grade 3 vaginal intraepithelial neoplasia (VAIN3 (≥ 3 cases)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Vaginal cancer												
Carter *et al.* (2001), USA	MY09/11 and RFLP; type-specific for 16, 18	54	90.7			63.0	5.6	3.7[b]		27.8[c]	9.3	Paraffin-embedded tissue
Daling *et al.* (2002), USA	MY09/11 and probing for 6/11, 16, 18/45, 31	25	64.0		8.0	56.0	12.0[d]	0	0	0	[12.0]	Paraffin-embedded tissue
VAIN 3												
Sugase & Matsukura (1997), Japan	Southern blot with PBM-58	3	100			66.6				33.3 (51)	0	Fresh tissue
van Beurden *et al.* (1998), Netherlands	CPI and CPIIG and sequencing	8	100			75.0			12.5			Paraffin-embedded tissue; VAIN2 and 3 with simultaneous CIN lesions or invasive cervical neoplasia
Daling *et al.* (2002), USA	MY09/11 and probing for 6/11, 16, 18/45, 31	74	82.4		8.1	54.1	8.1[d]		5.4[e]			Paraffin-embedded tissue

See Table 7 for a description of the primers used.

CIN, cervical intraepithelial neoplasia; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

[a] Unless otherwise specified, the method is PCR; (;) denotes independent methods whereas 'and' denotes subsequent steps.

[b] 31, 33, 35 or 39

[c] 6, 45, 52, 54, 58, 66, 72, 73 or unknown HPV types

[d] 18 or 45

[e] 31, 33 or 35

Approximately 30% of cases had been treated for a prior anogenital tumour, most often of the cervix.

2.3.3 *Cancer of the penis*

Similarly to vulvar cancer, the prevalence of HPV DNA in penile cancer varies with histological type. Most case series do not specify the histological type (Table 33), but for those that do (Gregoire *et al.*, 1995; Bezerra *et al.*, 2001a,b; Rubin *et al.*, 2001; Ferreux *et al.*, 2003), warty and basaloid carcinomas of the penis in general had a higher prevalence of HPV infection than verrucous and keratinizing carcinoma. These studies mostly included a small number of cases and the range of HPV prevalence in the tissues was wide. Basaloid and warty carcinomas may be preceded by penile intraepithelial neoplasia (PIN), which is also associated with HPV infection (Aynaud *et al.*, 1994; Rubin *et al.*, 2001).

Among the HPV-positive penile cancers, HPV 16 was the most common type. However, the majority of studies included at least one case of cancer with HPV 6 or 11, which in several studies were more common than HPV 18 (Levi *et al.*, 1998; Rubin *et al.*, 2001). HPV 31 or 33 were detected only rarely.

Overall, similarly to other anogenital cancers, HPV 16 is the predominant HPV type and, similarly to vulvar cancer, warty and basaloid carcinomas tended to be those cancers with the highest proportion of HPV DNA positivity. However, the relationship between histopathology and HPV prevalence is not as clear as that in cancer of the vulva. Also, HPV 6 or 11 appear to play a more prominent role in penile cancers than in other cancers of the anogenital region.

2.3.4 *Cancer of the anus*

[For anal cancer in HIV-positive patients, see Section 2.8.3(*b*).]

(*a*) *Case series*

Case series of cancer of the anus are presented in Table 34. Anal cancer resembles cervical cancer more than the other anogenital cancers with respect to overall prevalence of HPV positivity. The prevalence of HPV DNA in anal cancer in different case series varies widely, but most studies that used MY09/MY11 or GP5+/GP6+ primers showed a prevalence of 80% or above. Basaloid cancers are similar to squamous-cell carcinomas with respect to prevalence of HPV DNA and are more probably a histological variant rather than a separate entity. HPV 16 is the most common type in squamous-cell cancers (76%) followed by HPV 18 with a much smaller percentage (9%). Most studies that included a broad range of HPV type-specific probes showed a low prevalence of HPV 31, 33 or 6 or 11.

Table 33. Prevalence of HPV DNA in case series of penile cancer (≥ 13 cases) and penile intraepithelial neoplasia (≥ 5 cases)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Aynaud *et al.* (1994), France	Southern blot	4 PIN3	100	0[a]		100	0		0		0	Frozen tissue
Cupp *et al.* (1995), USA	MY09/11 and type-specific for 16, 18	42 SCC 13 carcinoma *in situ*	54.8 92.3			40.5 84.6	4.8 15.4			11.9[c] 0	7.7 2.4	
Gregoire *et al.* (1995), USA and Paraguay	Type-specific for 6, 11, 16, 18 and primer for wide range including 16, 18, 31, 33, 35, 52	45 typical 12 basaloid 10 papillary 9 warty 6 verrucous 19 mixed, warty or basaloid 8 mixed, other	11.1 75.0 0 22.2 0 47.4 0	0.9	0.9	21.1				0.9[c]	0	Paraffin-embedded tissue; HPV positivity associated with aggressive, higher-grade tumours; logistic regression showed that the only association with HPV positivity was tumour histopathology.
Levi *et al.* (1998), Brazil	Type-specific for 6/11, 16, 18	64 carcinoma	28.1		4.7	14.1	3.1			9.4[c]	1.6	Paraffin-embedded tissue
	MY09/11 and probing for 6, 11, 16, 18, 31	50 carcinoma	56.0		12.0	32.0	6.0			12.0[c]	4	Frozen tissue; no comments on histopathology
Buonaguro *et al.* (2000), Uganda	Southern blot for 16; PCR for 16 and sequencing	13 SCC	38.4			38.4						Frozen tissue
Picconi *et al.* (2000), Argentina	GP5/6 and SSCP for 6, 11, 16, 18, 31, 33	34 SCC	70.6	[5.9]	0	[23.5]	[11.8]	0	0	8.8[c]	0	Paraffin-embedded tissue; histological type not specified
Bezerra *et al.* (2001a,b), Brazil	L1 consensus primers and probing for 6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/54/56/58	60 SCC 11 warty carcinoma	26.7 45.5		18.2	15 27.3	5		3.3[d]	3.3[e]	0 0	Paraffin-embedded tissue

Table 33 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Carter *et al.* (2001), USA	MY09/11 and RFLP; type-specific for 16/18	33 SCC	81.8			69.7	3	6.0[f]		12.1[c]	9.1	Paraffin-embedded tissue; no comments on histopathology
Rubin *et al.* (2001), USA and Uruguay	SPF10 and LiPA for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 and 6, 11, 34, 40, 42, 43, 44, 53, 54, 74	106 keratinizing	34.9	3.8	0	17.9	0.9	0	0	20.8[g]	NR	Paraffin-embedded tissue; no difference in prevalence between samples from Uruguay and the USA
		5 warty	100	0	0	100	0	0	0	0		
		15 basaloid	80.0	0	0	66.7	0	[6.7]	0	20.0[h]		
		30 PIN	90.0	20.0	3.3	36.7	0	30.0[i]		16.7[j]		
Ferreux *et al.* (2003), Netherlands	GP5+/6+ EIA and type-specific for 37 types; ISH	55 SCC	37.7	5.6		28.3				3.7[k]	NR	Snap-frozen samples
		48 SCC-NOS	35.4	2.1		29.2				4.2[k]		
		2 warty	100	50		50				0		
		2 sarcomatoid	0	0		0				0		
		1 verrucous	100	100		0				0		

See Table 7 for a description of the primers used.

EIA, enzyme immunoassay; ISH, in-situ hybridization; LiPA, line blot hybridization; NOS, not otherwise specified; NR, not reported; PCR, polymerase chain reaction; PIN, penile intraepithelial neoplasia; RFLP, restriction fragment length polymorphism; SCC, squamous-cell carcinoma; SSCP, single-strand conformational polymorphism

[a] 6, 11 or 42

[b] 31, 33 or 35

[c] 31, 33, 35 or 39

[d] HPV 35, 45, 52, 68, 51/70/74 and unknown types

[e] 31, 33, 39, 44, 51, 52, 58 or 66

Table 34. Prevalence of HPV DNA in case series of anal cancer (≥ 5 cases)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Noffsinger *et al.* (1995a,b), Canada, China and USA	ISH for 6/11, 16/18 and 31/33/35	54 invasive and 2 in-situ	41.1	5.6		39.3		1.8[b]			7.4	Paraffin-embedded tissue; in five samples, HPV was detected with only one of the two detection methods.
	MY09/11 and type-specific for 6, 16, 18	50	46.0[c]	6.0		38.0[c]	4.0				4.0	
Shroyer *et al.* (1995), USA	MY09/11 and probing for 6/11, 16, 18, 33	11 basaloid	90.9	0		63.6	9.1	0	0		9.1	Paraffin-embedded archival tissue
		16 non-basaloid	75.0	12.5		62.5	12.5	0	0		12.5	
	ISH for 6/11, 16/18, 31/33/35	9 basaloid	66.7	0		44.4		33.3			22.2	
		11 non-basaloid	72.7	0		72.7		0			0	
Ramanujam *et al.* (1996), USA	ISH for 6, 11, 16, 18, 31, 33, 35	53 (37 women, 16 men)	34.0									Paraffin-embedded tissue
Vincent-Salomon *et al.* (1996), France	Southern blot for 6/11/42, 16/18/33, 31/35/39	15 SCC	46.7	0	0	33.3	0	0	0	13.3[d]		Frozen tissue
		9 basaloid	55.5	0	0	44.4	0	0	0	11.1[d]		
	PCR specific for 6/11, 16, 18, 33	18 SCC	66.7	0	0	66.7	0		0	0	0	
		9 basaloid	77.8	0	0	55.5	22.2		0	0	0	
Williams *et al.* (1996), United Kingdom	ISH for 6, 11, 16, 18	35 invasive	68.6	0	0	68.6	0				0	Paraffin-embedded tissue

Table 34 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Frisch *et al.* (1997), Denmark and Sweden	GP5+/6+ and probes for HR types (16/18/31/33/35/39/45/51/52/56/58/59/66/68) and LR types (6/11/40/42/43/44); type-specific for 6, 11, 16, 18, 31, 33	388 (304 women, 84 men)	87.6	1.3	0	72.9	5.7	0.8	5.9	1.8[e]		Paraffin-embedded tissue
Unger *et al.* (1997), USA	ISH for 6/11, 16/18, 31/33/35	3 SCC from HIV-positive patient and 3 SCC from HIV-negative patient	100	16.7		83.3[f]	16.7[f]		16.7[f]		16.7[f]	Paraffin-embedded tissue
Cuesta *et al.* (1998), USA	ISH for 6/11, 16/18, 31/33/35	6 verrucous carcinomas from HIV-positive patients	83.3	66.7		33.3						Paraffin-embedded tissue
Lai *et al.* (1998), China	MY09/11 and probing for 6, 11, 16, 18, 33	19 SCC	5.3	0	0	5.3	0		0		0	Paraffin-embedded archival tissue
		8 cloacogenic cancers	0	0	0	0	0		0			
		23 adeno-carcinomas	0	0	0	0	0		0			
		6 adeno-squamous carcinomas	0	0	0	0	0		0			

Table 34 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Poletti *et al.* (1998), Switzerland	PU-1M for 16/18/31/33/52/58 and PU-31B primers for 6/11	33	39.4	3.03				36.4[g]				Paraffin-embedded tissue
Indinnimeo *et al.* (1999), Italy	Type-specific for 6/11, 16, 18, 31/33	7 squamo-cellular	71.4			28.6			42.9			Paraffin-embedded tissue
		7 cloacogenic	57.1			57.1						
Frisch *et al.* (1999), Denmark and Sweden	GP5+/6+ and probes for HR types (16/18/31/33/35/39/45/51/52/56/58/59/66/68) and LR types (6/11/40/42/43/44); type-specific for 16, 18, 31, 33	331 (253 women, 78 men)	HR: [84] Men: 63 Women: 90 LR: [4.5] Men: 6.4 Women: 4.0			[73]	[6]	[1]	[5]	[2][h]	[2.7]	Paraffin-embedded tissue
Carter *et al.* (2001), USA	MY09/11 and RFLP; specific primers for 6, 18	64 (45 women, 38 men)	93.8			79.7	9.4		6.2[i]	10.8[j]	10.9	

Table 34 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others		
Daling *et al.* (2004), USA	MY09/11 and probing for 16, 18	179 SCC	92.2			76.0	8.9					Paraffin-embedded tissue
		41 basaloid	97.6			95.1	0					
		20 adeno-carcinoma	40.0			15.0	5.0					
		Men										
		36 in-situ	94.4			80.6	5.6					
		76 invasive	81.8			66.7	7.6					
		All	92.6									
		Women										
		34 in-situ	91.2			67.7	8.8					
		112 invasive	88.4			75.9	6.3					
		All	91.8									

See Table 7 for a description of the primers used.
HIV, human immunodeficiency virus; ISH, in-situ hybridization; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SCC, squamous-cell carcinoma
[a] Unless specified otherwise, the method is PCR; ';' denotes independent methods whereas 'and' denotes subsequent steps.
[b] 31, 33 or 35
[c] The Working Group noted some discrepancies between text and table in the number of HPV-positive tumours detected by PCR: the text reported 23/50 HPV-positive tumours (46%) whereas the table presented only 22 positive cases (44%); the text reported 21 lesions positive for HPV 16 by PCR and the table reported only 19 HPV 16-positive samples.
[d] Unknown
[e] 40/42/43/44
[f] One tissue contained 16, 18 and 33.
[g] 16, 18, 31, 33, 52 or 58
[h] Untyped high risk
[i] 31, 33, 35 or 39
[j] 6, 45, 52, 54, 58, 66, 72, 73 or unknown types

(*b*) *Case–control studies*

Frisch *et al.* (1999) studied the prevalence of HPV DNA in cancers of the anal canal and of the perianal skin. Anal cancers in women and homosexual men were more frequently HPV-positive for high-risk types ($p < 0.01$) and located in the anal canal ($p \leq 0.01$) than were cancers in heterosexual men. In both women and men, cancers of the anal canal contained high-risk HPV DNA more often than perianal skin cancers and increased high-risk HPV DNA positivity was seen with higher localization in the anal canal: 95 and 83% of cancers that involved the anal canal in women and men, respectively, were HPV-positive for high-risk types versus 80 and 28% of perianal skin cancers (p for trend < 0.001). Basaloid features, adjacent and anal intraepithelial neoplasia (AIN), poor or absent keratinization and a predominance of small or medium neoplastic cells were all strongly associated with positivity for high-risk HPV types.

The relationship between HPV infection and the subsequent risk for anal and perianal skin cancer was also studied in a case–cohort study among subjects who developed anal and perianal skin cancer during follow-up (median time, 10 years). Twenty-eight cases and 1500 controls were analysed for the presence of antibodies against HPV 16, 18, 33 or 73. An increased risk for developing anal and perianal skin cancer was observed among subjects who were seropositive for HPV 16 (odds ratio, 3.0; 95% CI, 1.1–8.2) and HPV 18 (odds ratio, 4.4; 95% CI, 1.1–17). The highest risks were seen for HPV 16-seropositive patients over the age of 45 years at serum sampling and for patients with a lag time of less than 10 years (Bjørge *et al.*, 2002).

Daling *et al.* (2004) measured antibodies to HPV 16 in cases of anal cancer and controls. HPV seropositivity was found in 51% of heterosexual male cases, 49% of not exclusively heterosexual male cases, 16% of heterosexual male controls, 42% of female cases and 15% of female controls.

In summary, cancer of the anal canal resembles cervical cancer in its high prevalence of HPV 16 and, to a lesser extent, other high-risk HPV types. Among these, HPV 18 is the next most common. HPV 6 or 11, 31 and 33 are uncommon but are found in a small proportion of tumours. In contrast, cancer of the perianal skin resembles vulvar and penile cancers, with a lower prevalence of HPV DNA positivity overall. [This may in part reflect the distance of the tumour from the anal verge, representing a mixture of true anal cancers and of skin cancers that are generally negative for infection with genital HPV types (see Section 2.5).]

2.4 Cancer of the upper aerodigestive tract

2.4.1 *Cancer of the oral cavity*

Cancer of the oral cavity (including tumours of the tongue, floor of the mouth, gum, palate and other sites of the mouth) is strongly associated with tobacco smoking (IARC, 2004) or chewing (IARC, 2007) and alcoholic beverage drinking (IARC, 1988), with attributable fractions in the order of 90%. However, some tumours occur in subjects who

are not exposed to known risk factors, and only a fraction of exposed subjects develop tumours, which suggests that other exposures may be independently involved or act as co-factors. HPV is known to infect the oral cavity of healthy individuals, and several HPV-related lesions have been characterized. However, most of the epidemiology and natural history of oral HPV infection remains to be elucidated (Herrero, 2003).

(*a*) *Case series*

Numerous studies have investigated the prevalence of HPV in tumour specimens of subjects with cancer of the oral cavity. Reported estimates have ranged from 0 to 100% (reviewed by Franceschi *et al.*, 1996; Gillison & Shah, 2001; Kreimer *et al.*, 2005). Table 35 presents series that included more than 40 cases of cancer of the oral cavity and evaluated the presence of HPV using PCR methods. The prevalence of HPV in these studies ranged from 4 to 80%. For instance, in the large IARC multicentric study, the prevalence of HPV in these cancers was 3.9% (Herrero *et al.*, 2003); in a recent systematic review that pooled HPV DNA results from oral squamous-cell cancer tissue specimens (Kreimer *et al.*, 2005), the overall prevalence in 2642 cases was 23.5% (95% CI, 21.9–25.1%). The wide variation in prevalence estimates is probably related to differences in populations, HPV testing methods, prevalence of other risk factors and the combination of specific topographical locations included in the studies. In general, studies from western countries indicate lower prevalence than those conducted in India, China or Japan.

The type most commonly reported in all studies was HPV 16, which was detected in more than 60% of HPV-positive tumours in two-thirds of the studies and in more than 80% of the tumours in half of the studies (Table 35). In the meta-analysis by Kreimer *et al.* (2005), 68.2% of the positive cancers were positive for HPV 16. The second most common type was HPV 18, with occasional reports of HPV 33, 6 and 11.

Several studies have compared the prevalence of HPV DNA in cases of oral cancer and tissues from individuals without oral cancer. Ostwald *et al.* (1994) used PCR methods to detect HPV DNA in biopsies from 26 oral squamous-cell carcinomas and exfoliated cells of the buccal mucosa from 97 healthy volunteers. HPV was detected in 61.5% of cancers and 1% of volunteers. In a study in India, frozen biopsies of 83 patients with cancer of the oral cavity were compared with exfoliated cells from 102 volunteers from a dental clinic (Koppikar *et al.*, 2005). HPV was detected by PCR in 38.6% of cancer patients and 5% of the control group. HPV 8, 16 and 18 were the most common types found in these cancers, but other types, including several from the genus beta-papillomavirus, were also detected. The latter were the only types detected in normal subjects. Zhang *et al.* (2004) reported a study of 73 cases of cancer of the oral cavity and 40 specimens from patients with benign tissue biopsies in China. PCR methods were used to detect HPV DNA for types 16 and 18, which were detectable in 74% of the cases and 55% of the non-cancer patients ($p = 0.04$).

Table 35. Prevalence of HPV DNA in case series of cancer of the oral cavity (> 40 cases) detected by the polymerase chain reaction (PCR) method[a]

Reference, study location	Method of detection and types tested	Sites included	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
					6	11	16	18	31	33	Others (type)		
Balaram *et al.* (1995), India	MY09/11; GP5/6; type-specific for 6/11, 16, 18	Buccal mucosa, tongue, floor of mouth, lower alveolus, other	91	73.6	13.2	19.8	41.7	47.3				40.7	Fresh frozen and paraffin-embedded tissue
Shindoh *et al.* (1995), Japan	Type-specific for 16, 18, 33 and dot blot hybridization	Tongue, gingiva, floor of mouth, maxillary, mouth, palate, retromolar	77	31.2			31.2	1.3				1.3	Paraffin-embedded tissue
Paz *et al.* (1997), USA	MY09/11; IU/IWDO; type-specific primers for 6, 16, 18, 31/33/35/ 44/45/56	Tongue, floor of mouth, oral cavity	64	12.5	3.1		7.8				1.6[d]		Fresh frozen tissue
Wen *et al.* (1997), China	Type-specific for 16, 18 and southern blot		45	31.1			20.0	24.4				13.3	Paraffin-embedded tissue
D'Costa *et al.* (1998), India	MY09/11 and southern blot for 6, 11, 16, 18, 33	Buccal mucosa, lower alveolus, tongue, floor of mouth, hard palate, maxilla	100	15.0	0[c]	0[c]	15.0	0		0[c]			Fresh frozen tissue
Schwartz *et al.* (1998), USA	MY09/11; type-specific primers for 6, 11, 16, 18	Tongue, gum, floor of mouth, other parts of mouth, NOS	186	20.4	6*		~11*						Paraffin-embedded tissue; *read from graph

Table 35 (contd)

Reference, study location	Method of detection and types tested	Sites included	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
					6	11	16	18	31	33	Others (type)		
Gillison *et al.* (2000), USA	MY09/MY11; type-specific primers for 33 types	Oral cavity	84	11.9			~11[e]	1.2					Snap-frozen tissue
Tsuhako *et al.* (2000), Japan	Type-specific for 6, 11, 16, 18	Tongue, mouth floor, buccal mucosa, lower gum, maxilla, lip	83	57.8	15.7	1.2	33.7	37.3			1.2[d]		Paraffin-embedded tissue
Mork *et al.* (2001), Finland, Norway and Sweden	GP5+/6+; CPI/CPIIG	Tongue, floor of mouth, oral cavity NOS	59				[7]						Paraffin-embedded tissue
Premoli-de-Percoco & Ramirez (2001), Venezuela	Type-specific for 6, 11, 16, 18	Tongue, buccal mucosa, floor of the mouth, others	50	60.0	0	0	50.0	16.0	0	0			Paraffin-embedded; women only
van Houten *et al.* (2001), Netherlands	GP5+/6+	NOS	45	4.4			4.4						Snap-frozen tissue
Ringström *et al.* (2002), USA	MY09/11	NOS	41	4.9			4.9						Snap-frozen tissue
Shin *et al.* (2002), Republic of Korea	Type-specific for 16, 18, 33	Oral cavity, salivary glands	76	14.5			5.3	10.5		2.6			Tissue collection not specified

Table 35 (contd)

Reference, study location	Method of detection and types tested	Sites included	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
					6	11	16	18	31	33	Others (type)		
Chang *et al.* (2003), Taiwan, China	MY09/11; GP5+/6+ with typing by sequencing and gene chip hybridization	Tongue, mouth, gingiva, lip, palate, floor of mouth	103	49.5			28.2	26.2		1.0			Paraffin-embedded tissue
Herrero *et al.* (2003), Multicentric, 9 countries	GP5+/6+ and southern blot for 6, 11, 16, 18, 31, 33	Base of tongue, other parts of tongue, gum, floor of mouth, palate, mouth	766	3.9			~4						Snap-frozen tissue
Kansky *et al.* (2003), Slovenia	MY09/11; GP5+/6+; multiple types of primer sets and RFLP	Tongue, floor of mouth, rectomolar trigonum, buccal mucosa	55	5.5			1.8			1.8	1.8 (58)		Paraffin-embedded tissue
Kojima *et al.* (2003), USA	PCR and DNA sequencing; ISH; immunohisto-chemistry for 38	Tongue, buccal mucosa, maxillary and mandibular gingiva, hard palate, floor of mouth	53	66.0							66.0 (38)		Paraffin-embedded tissue
Ostwald *et al.* (2003), Germany	Type-specific primers for 6/11, 16, 18	Oral cavity, lip	118	43.2		4.2	29.7	13.6					Snap-frozen tissue
Sugiyama *et al.* (2003), Japan	Type-specific for 16, 18	Gingiva, tongue, oral floor, cheek, lip, palate	86	34.9			34.9	2.3				2.3	Paraffin-embedded tissue

Table 35 (contd)

Reference, study location	Method of detection and types tested	Sites included	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
					6	11	16	18	31	33	Others (type)		
Dahlgren *et al.* (2004), Sweden	GP5+/GP6+ or CPI/CIIPG and sequencing; type-specific PCR for 16, 18, 33	All tumours were from the oral tongue.	85 mobile	2.4			2.4	0		0			Paraffin-embedded tissue
			25 base	40.0			28.0	0		4.0	4.0 (35)		
Smith, E.M. *et al.* (2004a), USA	MY09/11 and dot blot and sequencing	Tongue, floor of mouth, gingiva, hard palate, lip, major salivary glands, other oral mucosa	126	10			7.9			2.3			Paraffin-embedded tissue

See Table 7 for a description of the primers used.
EIA, enzyme immunoassay; ISH, in-situ hybridization; NOS, not otherwise specified; RFLP, restriction fragment length polymorphism
[a] Only studies that used PCR as the method of detection of HPV were included.
[b] ';' denotes independent methods whereas 'and' denotes subsequent steps.
[c] Subgroup of 70 samples examined
[d] Untyped
[e] 90% of the 253 tumour specimens analysed in the study were positive for HPV 16.

(*b*) *Case–control studies*

Table 36 presents case–control studies of cancer of the oral cavity that used exfoliated cells or immunological markers to determine the presence of HPV.

The study by Schwartz *et al.* (1998) included 284 cases of cancer of the oral cavity and oropharynx combined and 477 controls. Results for an HPV 16 capsid antibody response were available for 259 cases and 446 controls. Antibodies against HPV 16 L1 VLPs were equally prevalent in tumours of the floor of the mouth and in controls (odds ratio, 1.1; 95% CI, 0.5–2.5), but were more frequent in cancers of the tongue (odds ratio, 2.4; 95% CI, 1.5–3.8) after adjustment for age, sex, tobacco smoking and alcoholic beverage drinking.

In a joint Nordic cohort, serum samples were collected from almost 900 000 individuals who were then followed through record linkage with tumour registries. Mork *et al.* (2001) evaluated the association of serum levels of antibodies against HPV 16 capsids with the occurrence of different cancers of the head and neck in a case–control study nested within this cohort. Elevated odds ratios were detected for cancers of the tongue (2.8; 95% CI, 1.2–6.6) and of the oral cavity not otherwise specified (3.6; 95% CI, 0.5–23.6). [The Working Group noted that this study adjusted for serum levels of cotinine and is important because of its prospective nature.]

In a case–control study by Dahlstrom *et al.* (2003), antibodies against HPV 16 L1 VLPs were detected at similar levels in 36 cases of squamous-cell carcinoma of the oral cavity and 120 cancer-free controls selected from a managed care organization (8.3 versus 9.2%, respectively).

Herrero *et al.* (2003) conducted a multicentre case–control study in Australia, Canada, Cuba, India, Italy, Northern Ireland, Poland, Spain and the Sudan from April 1996 to December 1999. The study included 1670 cases (1415 with cancer of the oral cavity and 255 with cancer of the oropharynx) and 1732 controls. Oral exfoliated cells and blood were obtained from all participants and fresh biopsy specimens from cases. HPV DNA was detected by PCR followed by enzyme immunoassay or southern blot hybridization. Antibodies against HPV 16 L1, E6 and E7 proteins in plasma were detected by enzyme-linked immunosorbent assay (ELISA). Detection of HPV DNA in exfoliated cells was performed in only less than 50% of samples and did not correlate with detection of HPV DNA in biopsy specimens. HPV DNA was detected in biopsy specimens of 3.9% (95% CI, 2.5–5.3%) of 766 cancers of the oral cavity with valid PCR results. Antibodies against HPV 16 L1 were associated with increased odds ratios for cancers of the oral cavity (1.5; 95% CI 1.1–2.1), as were antibodies against either HPV 16 E6 or E7 (2.7; 95% CI, 1.6–4.7) after adjusment for country, age, sex, tobacco smoking, alcoholic beverage drinking and *paan* chewing.

Van Doornum *et al.* (2003) reported the prevalence of antibodies against HPV 16 E7 to be 5.3% among 56 cases of carcinoma of the tongue and 2% among 100 non-cancer controls.

Smith, E.M. *et al.* (2004b) reported a case–control study that included 130 cases of cancer of the oral cavity and 333 control subjects, frequency-matched for age and sex.

Table 36. Case–control studies of HPV prevalence and cancer of the oral cavity

Reference, study location	Sites included	No. of cases	No. of controls	Method of detection	HPV prevalence (%)		Odds ratio (95% CI)	Comments/adjustments
					Cases	Controls		
Schwartz *et al.* (1998), USA	Oral cavity and oropharynx combined; see Table 35 for organs included and Table 38	259	446	Antibodies against HPV 16 L1 VLPs	51.4	35.0	2.3 (1.6–3.3)	Adjusted for age, sex, tobacco smoking and alcoholic beverage drinking *Read from graph
	Floor of mouth	38	446		26*	35.0	1.1 (0.5–2.5)	
	Tongue	107	446		20*	35.0	2.4 (1.5–3.8)	
		237	435 pop. controls	PCR in exfoliated cells	9.3	9.2	0.9 (0.5–1.6)	
Mork *et al.* (2001), Finland, Norway and Sweden	Tongue, floor of mouth, oral cavity NOS	19	Cohort of ~950 000 residents	Antibodies against HPV 16 L1 VLPs	11	2	3.6 (0.5–26.3)	Seropositivity for HPV 16
	Tongue	57			16	7	2.8 (1.2–6.6)	
Herrero *et al.* (2003), Multi-centric, 9 countries	Base of tongue, other parts of tongue, gum, floor of mouth, palate, mouth	511	613	PCR of exfoliated cells	4.7	6.9	0.6 (0.3–1.1)	Adjusted for country, sex, age, tobacco smoking, alcoholic beverages, *paan* chewing
		1299	1527	Antibodies against HPV 16 L1 VLPs	8.9	6.0	1.5 (1.1–2.1)	
		1319	1581	Antibodies against E6 and E7 proteins	4.2	1.5	2.7 (1.6–4.7) 4.3 (0.8–23.2) if both positive	
Smith *et al.* (2004b), USA	Oral cavity and oropharynx combined (see Table 38)	201	333	PCR and sequencing of oral exfoliated cells	28.4	18.3	1.8 (1.1–2.7)	Adjusted for age, sex, tobacco smoking, alcoholic beverage consumption
	Oral cavity: lip vermillion and inner mucosa, tongue, gingiva, floor of mouth, hard palate, other oral mucosa, parotid gland, submandibular gland	130	333		15	18.3	NR	

CI, confidence interval; NR, not reported; PCR, polymerase chain reaction; VLP, virus-like particles

[a] Detection of HPV DNA in exfoliated cells was considered to be an inadequate indication of HPV infection in this study as it did not correlate with detection of HPV DNA in tumours.

Oral exfoliated cells and tumour tissue were analysed for HPV content by PCR and dot blot hybridization and for HPV type by DNA sequencing. HPV DNA was detected in oral cells from 15% of cases of cancer of the oral cavity and 18.3% of controls. Risk estimates restricted to cancers of the oral cavity were not presented.

Studies of non-genital sites present a special challenge for the assessment of exposure to HPV because oral exfoliated cells do not appear to demonstrate infection adequately. As an alternative, serological markers of HPV exposure or expression (e.g. antibodies against HPV L1 VLPs or against E6 or E7 proteins) have been used in some recent studies (Schwartz *et al.*, 1998; Herrero *et al.*, 2003; see above).

Moreover, it is not always feasible to define clearly the precise anatomical location of the primary tumour, and multiple locations are frequent. Misclassification of the primary tumour site can introduce distortions in prevalence estimates for individual sites. For example, the base of the tongue is not classified consistently in the different studies when sites are grouped as oral cavity or oropharynx. A recent, carefully conducted study (Dahlgren *et al.*, 2004) indicated that the base of the tongue has an HPV prevalence of 40% compared with 2.3% in the oral tongue.

2.4.2 *Cancer of the oropharynx and tonsil*

Similar to cancers of the oral cavity, cancers of the oropharynx and tonsil are strongly associated with tobacco smoking and alcoholic beverage drinking, but other etiological agents may play an independent role or act as co-factors.

(*a*) *Case series*

A series of studies have reported prevalence of HPV in cancers of the oropharynx and tonsil. Those that used PCR as the method of detection and included more than 40 cases are summarized in Table 37. The prevalence of HPV in the selected studies ranged from 14 to 57%, and the largest study to date (Herrero *et al.*, 2003) reported a prevalence of 18%. In a systematic review that pooled HPV DNA results from 969 cases of squamous-cell cancer of the oropharynx (Kreimer *et al.*, 2005), the overall prevalence of HPV was 35.6% (95% CI, 32.6–38.7%). Among HPV-positive tumours, there was a marked and very consistent predominance of HPV 16. In all studies included in Table 37, HPV 16 was present in at least 78% of HPV-positive tumours and, in the review by Kreimer *et al.* (2005), the corresponding figure was 87%. HPV 18 is almost never present and other types (HPV 6, 11, 31 and 33) are only detected sporadically. In the studies that presented separate estimates for cancer of the tonsil, this anatomical site consistently showed the highest prevalence of HPV.

Using in-situ hybridization with probes for HPV 6, 11 and 16 under high stringency, Niedobitek *et al*. (1990) tested 28 tonsillar carcinomas and 30 tonsils removed because of chronic inflammation. Six of the cases and none of the controls were HPV 16-positive [$p < 0.001$].

Table 37. Prevalence of HPV DNA in case series of oropharyngeal and tonsillar cancer (> 40 cases)

Reference, study location	Method[a] of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Comment
				6	11	16	18	31	33	Others (type)	
Schwartz *et al.* (1998), USA	MY09/11; type- specific for 6, 11, 16, 18	11 oropharynx 44 tonsil	36.4 44[b]	0 10		36.4 34.1					Paraffin-embedded tissues; *read from graph
Gillison *et al.* (2000), USA	MY09/MY11/HMB01; mixture for 16/18/51/66 and probing with 33 type-specific probes	60 oropharynx 52 tonsil 8 others	56.7 62 25		1	51	1	1	3		Fresh-frozen tissues; type distribution for combination of cases of the oral cavity and oropharynx (n = 253)
Mellin *et al.* (2000), Sweden	GP5+/6+; type-specific for 16, 33	60 tonsil carcinomas	43.3			43.3			1.7		Paraffin-embedded tissues
Lindel *et al.* (2001), Switzerland	SPF 1/2 for high-risk types and sequencing	99 oropharynx 40 tonsil	14.1 15			11.1			1.0	1.0 (35) 1.0 (45)	Paraffin-embedded tissues
Strome *et al.* (2002), USA	MY09/11, others; type-specific for 16	52 tonsil carcinoma	46.2			40.4				3.8 (12)	Paraffin-embedded tissues
Herrero *et al.* (2003), Multi-centric, 9 countries	GP5+/6+ and EIA for 6, 11, 16, 18, 31, 33	142 tonsil	18.3 26			~17				[< 1]	Snap-frozen tissues
Li, W. *et al.* (2003), Australia	GP5+/6+ and other consensus primers; type-specific for 16	67 tonsil carcinoma	46.3			44.7					Paraffin-embedded tissues
China		16 tonsil carcinoma	0								Paraffin-embedded tissues
Smith *et al.* (2004a), USA	MY09/11/HMB01; dot blot and sequencing	31 tonsil 5 oropharynx	58.1 0			51.6	3.2		3.2		Paraffin-embedded tissues

See Table 7 for a description of the primers used.
EIA, enzyme immunoassay; PCR, polymerase chain reaction;
[a] Only those studies where HPV was detected by PCR were included; ';' denotes independent methods whereas 'and' denotes subsequent steps.

Snijders *et al.* (1992a,b) used PCR and southern hybridization techniques to test 10 cases of carcinoma of the tonsil and seven control patients with tonsillitis. All cases tested positive for HPV versus none of the controls [odds ratio, ∞; 95% CI, 7.5–∞]. The presence of HPV in cancer cells was confirmed by RNA in-situ hybridization.

In Japan, Watanabe *et al.* (1993) tested 12 cases of carcinoma of the oropharynx, hypopharynx and tonsil and 28 control specimens from patients with chronic tonsillitis. Three methods for HPV testing were used — dot-filter, southern hybridization and PCR. The results from PCR showed a prevalence of HPV DNA of 25% among cases and 4% among controls.

(*b*) *Case–control studies*

Table 38 presents case–control studies of cancer of the oropharynx that used exfoliated cells or immunological markers to determine the presence of HPV.

In the study by Schwartz *et al.* (1998), the odds ratio for cancer of the tonsil associated with the detection of antibodies against HPV 16 L1 was 3.9 (95% CI, 2.0–7.8) after adjustment for age, sex, tobacco smoking and alcoholic beverage drinking.

In the nested case–control study reported by Mork *et al.* (2001), the risk for developing cancer of the oropharynx associated with pre-diagnostic detection of antibodies against HPV 16 L1 VLPs was 14.4 (95% CI, 3.6–58.1).

In the serological case–control study of Dahlstrom *et al.* (2003), HPV 16 L1 VLP seropositivity was associated with a 60-fold (95% CI, 5.7–620) increase in risk for oropharyngeal squamous-cell carcinoma (70 cases) compared with 120 cancer-free, clinic-based controls, following adjustment for reported alcoholic beverage use and serum cotinine level, which is a biomarker of recent exposure to tobacco.

In the IARC multicentre study (Herrero *et al.*, 2003), there was no difference in the prevalence of HPV DNA in exfoliated cells from cancers of the oropharynx and those from controls. However, the odds ratio for antibodies against HPV 16 L1 VLPs was 3.5 (95% CI, 2.1–5.9), and that for antibodies against HPV 16 E6 or E7 proteins was 4.5 (95% CI, 2.0–10.1). When both antibodies were detected, the odds ratio was 67.1 (95% CI, 12.9–348). These estimates were adjusted for country, tobacco smoking, alcoholic beverage drinking and *paan* chewing.

In another case–control study of 48 cases of oropharyngeal carcinoma and 100 cancer-free controls, levels of HPV 16 L1 antibodies were significantly elevated in cases compared with controls (33 versus 18%; $p = 0.04$); however, seroprevalence of HPV E7 was similar (4% versus 2% for cases and controls, respectively) (Van Doornum *et al.*, 2003).

Smith, E.M. *et al.* (2004a) reported an adjusted odds ratio for oropharyngeal cancer of 3.6 (95% CI, 1.8–7.1) associated with detection of HPV in exfoliated cells.

The oropharynx, and in particular the tonsils, are the extragenital sites where the role of HPV is most clear for a defined subset of tumours. Similarly to vulvar cancer, a dual etiology has been postulated for oropharyngeal tumours (Herrero *et al.*, 2003). One subset includes smoking-related cancers, with squamous histological features and *p53* mutations.

Table 38. Case–control studies of HPV prevalence and cancers of the oropharynx and tonsil

Reference, study location	Sites included	No. of cases	No. of controls	Method of detection	HPV prevalence (%)		Odds ratio (95% CI)	Comments/adjustments
					Cases	Controls		
Schwartz *et al.* (1998), USA	Oral cavity and oropharynx combined	259	446	Antibodies against HPV 16 L1 VLPs	51.4	35.0	2.3 (1.6–3.3)	Adjusted for age, sex, tobacco smoking, alcoholic beverage drinking
	Tonsil	49	446		44.0	35.0	3.9 (2.0–7.8)	
Mork *et al.* (2001), Finland, Norway and Sweden	Oropharynx (ICD 145)	26	Cohort of ~900 000 residents	Antibodies against HPV 16 L1 VLPs	38	10	14.4 (3.6–58.1)	Seropositivity for HPV 16
Dahlstrom *et al.* (2003), USA	Base of tongue, tonsil, other oropharynx	70	120	Antibodies against HPV 16 L1 VLPs	58.6	9.2	59.5 (5.7–620)	Adjusted for cotinine, alcoholic beverages, matching variables; prevalence in tonsil cancers, 59.4%
Herrero *et al.* (2003), Multi-centric, 9 countries	Oropharynx and tonsil	90	613	PCR in oral exfoliated cells	8.9	6.9	1.0 (0.4–2.5)[a]	Adjusted for country, sex, age, tobacco smoking, alcoholic beverages, *paan* chewing
		238	1527	Antibodies against HPV 16 L1 VLPs	13.4	6.0	3.5 (2.1–5.9)	
		243	1581	Antibodies against E6 and E7 proteins	5.3	1.5	4.5 (2.0–10.1) 67.1 (12.9–348) if both positive	
Van Doornum *et al.* (2003), Netherlands	Oropharynx	48	100	Antibodies against HPV 16 L1 VLPs and E7 proteins	L1, 33 E7, 4	L1, 18 E7, 2	L1, 2.25 (1.0–4.9)	

Table 38 (contd)

Reference, study location	Sites included	No. of cases	No. of controls	Method of detection	HPV prevalence (%)		Odds ratio (95% CI)	Comments/adjustments
					Cases	Controls		
Smith *et al.* (2004b), USA	Oral cavity and oropharynx combined (see Table 36)	201	333	PCR of oral exfoliated cells and sequencing	28.4	18.3	1.8 (1.1–2.7)	
	Oropharynx: base of tongue, soft palate, uvula, palatine tonsil fossa, pillar and overlapping regions, oropharynx, oropharynx NOS	71	333		38.0	18.3	NR	

CI, confidence interval; NOS, not otherwise specified; NR, not reported; PCR, polymerase chain reaction; VLP, virus-like particles

[a] Detection of HPV DNA in exfoliated cells was considered to be an inadequate indication of HPV infection as it did not correlate with the detection of HPV DNA in tumours.

The other includes HPV-related tumours (reviewed by Gillison & Shah, 2001), which have been shown to have basaloid histological features, to occur more frequently in nonsmokers, to be less frequently associated with *p53* mutations (Braakhuis *et al.*, 2004; Dai *et al.*, 2004), to be associated with distinct patterns of genetic alterations (Braakhuis *et al.*, 2004) and possibly to have a better prognosis (Pintos *et al.*, 1999).

2.4.3 *Cancer of the oesophagus*

The possibility that HPV infection may play a role in the etiology of squamous-cell carcinoma of the oesophagus has been proposed, but the data have not been consistent (reviewed by Gillison & Shah, 2003). The epithelium of the oesophagus is similar to that of the oral cavity, and papillomas have been described at this anatomical site although they are rare, and HPV is not consistently detected therein.

(*a*) *Case series*

Table 39 presents series of cancer of the oesophagus that studied more than 40 cases; the 19 studies show great heterogeneity with regard to the prevalence of detection of HPV DNA, which ranged from 0 to 55%. Five studies from France (Benamouzig *et al.*, 1995), Italy (Talamini *et al.*, 2000), Japan (Saegusa *et al.*, 1997), the Netherlands (Kok *et al.*, 1997) and Slovenia (Poljak *et al.*, 1998), which included more than 45 cases and used PCR methods with common consensus primers, showed 0% prevalence of HPV in oesophageal cancers. In contrast, many studies from China, an area with a high incidence of cancer of the oesophagus, reported a high overall prevalence of HPV that was generally around 50%. The largest study, also from China and which included 700 cases, showed an overall HPV prevalence of 17% using in-situ hybridization (Chang *et al.*, 2000).

In oesophageal cancers, the predominance of HPV 16 is less marked than that at other sites of the head and neck, and HPV 18, 6 and 11 are detected commonly.

A recent study by de Villiers *et al.* (2004b) among patients with other head and neck cancers detected HPV DNA in 67% of the premalignant or malignant oesophageal tissue biopsies, which pointed to a particular subgroup of cancers of the oesophagus.

In the study by Benamouzig *et al.* (1992), the presence of oesophageal HPV infection was studied in endoscopic biopsies of 12 patients with oesophageal squamous-cell carcinoma, 24 control patients exposed to similar known risk factors (alcoholic beverages and tobacco) and seven non-exposed controls. Five of 12 patients with oesophageal carcinoma had HPV infection in the normal oesophagus tissue using dot blot hybridization (three of these also had HPV-positive tumour tissue). Only one of the controls had an oesophageal HPV infection ($p < 0.01$). HPV 16 and 18 were the types most frequently detected.

(*b*) *Case–control studies*

Case–control studies that assessed HPV prevalence by serology have reported contradictory results. A carefully conducted population-based case–control study in Sweden

Table 39. Prevalence of HPV DNA in case series of oesophageal carcinomas (> 40 cases)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Chang *et al.* (1992), China	Type-specific for 6, 11, 16, 18	51	49.0	5.9	13.7	17.6	13.7				2.0	Paraffin-embedded tissue
Toh *et al.* (1992), Japan	Consensus PCR for 16/18/31/33/52/58, 6/11/16/18/31/33 and type-specific primers for 16, 18	45	6.7			2.2	4.4					Fresh tissue
Togawa *et al.* (1994), International	Consensus primers and RFLP for 6, 11, 16, 18	72	23.6			12.5	1.4			9.7 (unknown)		Paraffin-embedded or frozen tissue
Benamouzig *et al.* (1995), France	MY09/11 and type-specific for 6/11, 16/18, 31/33; dot blot for 6/11, 16/18	75	0									Frozen tissue
Suzuk *et al.* (1996), China	MY09/11 Type-specific for 6, 16, 18	70 70	0 4.3	1.4		2.9						Paraffin-embedded tissue
Lam *et al.* (1997), Hong Kong, SAR	Type-specific for 16, 18 and southern blot with consensus probes	70	8.6			8.6						Snap-frozen tissue
Turner *et al.* (1997), Canada, USA	MY09/11 type-specific primers for 16, 18/33	51	2.0			2.0						Paraffin-embedded tissue
Kok *et al.* (1997), Netherlands	MY09/11, GP5+/6+, CPI/CPIIG, CPI/CPIIS	63	0									Paraffin-embedded tissue

Table 39 (contd)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Mizobuchi *et al.* (1997), Japan	Type-specific for 16, 18 and southern blot	41	7.3				7.3					Snap-frozen tissue
Saegusa *et al.* (1997), Japan	L1C1/L1C2 for 6/11/16/18/31/33/42/52/58; pU-1M/pU-2R for 16/18/31/33/52/58	92	0									Paraffin-embedded tissue
Poljak *et al.* (1998), Slovenia	MY09/11, GP5+/6+, WD; nested PCR; type-specific PCR for 6, 16, 18	120	0									Paraffin-embedded tissue
de Villiers *et al.* (1999a), China	Degenerate primer set HD; CP; GP5+/6+	70	4.3			1.4			1.4			Fresh frozen tissue
Lavergne & de Villiers (1999), China and South Africa	Degenerate PCR and sequencing	63	30.2	9.5		0	1.6			25.3 (untyped)	6.3	Snap-frozen tissue
Chang *et al.* (2000), China	ISH with broad-spectrum probe and type-specific probes for 6/11, 16, 18, 30, 53	700	16.6		1.1	3.0	1.7			12.0 (untyped)		Paraffin-embedded tissue
Talamini *et al.* (2000), Italy	MY09/11, GP5+/6+	42	0									Paraffin-embedded tissue

Table 39 (contd)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comments
				6	11	16	18	31	33	Others (type)		
Kawaguchi *et al.* (2000), China and Japan	Type-specific for 16, 18 and sequencing	75	22.7									Snap-frozen tissue
Li, L.T. *et al.* (2001, 2002), China	Type-specific for 16, 18	62	62.9									Paraffin-embedded tissue
Matsha *et al.* (2002), South Africa	MY09/11, GP5+/6+	50	46.0		22.0	4.0				14.0 (39), 2.0 (52), 4.0 (un-typed)		Paraffin-embedded tissue
Shen *et al.* (2002), China	Consensus primers PCR for 6/11, 16/18	55 44	65.5		34.1		43.2					Fresh tissue
	PCR for 16, 18	77	54.5			39.0	22.1				10.4	
Si *et al.* (2003), Hong-Kong, SAR	MY09/11 and type-specific for 16, 18	319	13.5			12.2	1.9					Paraffin-embedded and snap-frozen tissues
Lu *et al.* (2004), China	Type-specific for 16	104	52.9			52.9						Paraffin-embedded tissue
Katiyar *et al.* (2005), India	MY09/11 Type-specific for 16, 18	101 60	26.7 66.7			16.8	2.0					Snap-frozen tissue

See Table 7 for a description of the primers used.
ISH, in-situ hybridization ; PCR, polymerase chain reaction
[a] Unless otherwise specified, the method is PCR; ';' denotes independent methods whereas 'and' denotes subsequent steps.

(Lagergren *et al.*, 1999) showed no association of antibodies against HPV 16 or HPV 18 L1 VLPs with either squamous-cell or adenocarcinoma of the oesophagus after adjustment for age, sex, smoking status, alcoholic beverage intake, education and the presence of the other HPV type. Another serology-based study from the Netherlands (Van Doornum *et al.*, 2003) reported 17% HPV 16 seropositivity among cases of oesophageal carcinoma compared with 18% among cancer-free controls. No cases were seropositive for HPV 16 E7 compared with 2% of controls. In contrast, a hospital-based study in China assessed HPV16 VLPs in cases of oesophageal cancer (95% of which were squamous-cell carcinomas) and cancer-free controls and reported that higher antibody levels significantly increased the relative risk for oesophageal cancer (odds ratio, 4.5; 95% CI, 1.8–11.9), after adjustment for age and sex (Shen *et al.*, 2002a). [The Working Group noted that the study lacked the ability to adjust further for potentially important confounders, such as tobacco use, alcoholic beverage consumption, dietary patterns or sexual behaviour.]

(*c*) *Cohort studies*

Prospective seroepidemiological studies in Scandinavian cohorts point to an association between HPV and oesophageal cancer. An initial study in Finland (Dillner *et al.*, 1995b) indicated a 14-fold increase in risk for oesophageal cancer associated with prediagnostic detection of HPV 16 capsid antibodies, and another study in Norway indicated a 6.2-fold increase in risk after adjustment for cotinine levels as markers of tobacco exposure (Bjørge *et al.*, 1997b).

2.4.4 *Cancer of the larynx*

The laryngeal epithelium is known to be susceptible to HPV infection because of the well-established association of HPV types 6 and 11 with juvenile- and adult-onset laryngeal papillomatosis. A few retrospective case series have reported laryngeal squamous-cell carcinoma among patients with a history of laryngeal papillomatosis; HPV 11 DNA was most commonly detected in these cancer specimens (Shen *et al.*, 1996; Reidy *et al.*, 2004). However, laryngeal papillomatosis is not a precursor for most laryngeal cancers. Similarly to cancers of the oral cavity and pharynx, the main risk factors for laryngeal squamous-cell carcinoma are tobacco use and alcoholic beverage consumption and the attributable fraction for these exposures is large.

(*a*) *Case series*

Table 40 presents studies of at least 40 cases of squamous-cell carcinomas of the larynx that employed PCR-based detection methods. The overall HPV prevalence ranged from 7% in a study in the USA to 59% in a large study in China (Ma *et al.*, 1998). HPV 16 was the predominant type detected, and accounted for approximately all [74%] HPV-positive laryngeal squamous-cell carcinomas. HPV 18 was the second most commonly detected type; HPV 6, 11 and 33 were detected in a few cases, and no other carcinogenic HPV types were reported. [The Working Group noted that the number of specimens exa-

Table 40. Prevalence of HPV DNA in case series of laryngeal cancer (> 40 cases)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
				6	11	16	18	31	33	Others (type)		
Pérez-Ayala *et al.* (1990), Spain	Type-specific for 11 and 16 and dot blot	48	54.2		0	54.2						Snap-frozen tissues
Shidara *et al.* (1994), Japan	L1C1/L1C2 and RFLP	45	24.4			20.0	4.4					Paraffin-embedded tissues
Suzuki *et al.* (1994), Japan	L1C1/L1C2 and RFLP	41	26.8	0	0	22.0	4.9	0	0			Paraffin-embedded tissues
Fouret *et al.* (1995), France	Primers WD 72, 76, 66, 67, 154 and southern blot for 6, 11, 16, 18, 31, 33	59	5.1									Paraffin-embedded tissues
Almadori *et al.* (1996), Italy	PCR for 6, 11, 16, 18 and southern blot	45	20.0	4.4		20.0	0					Snap-frozen tissues
Paz *et al.* (1997), USA	MY09/11, IU/IWDO, type-specific for 6, 16, 18, probing for 31, 33, 35, 44, 45, 56	49	8.2	0		6.1	0			2.0 (un-known)		Snap-frozen tissues

Table 40 (contd)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
				6	11	16	18	31	33	Others (type)		
Cattani *et al.* (1998), Italy	MY09/11 and EIA for 6, 11, 16, 18, 31	75	29.3			12.0	10.7		1.3	5.3 (un-typed)	1.3	Snap-frozen tissues
Hoffmann *et al.* (1998), Germany	Type-specific primers for 6, 11, 16, 18, 33 and southern blot; consensus primers and southern blot for type-specific negative samples	29	20.7			6.8						Snap-frozen tissues
Ma *et al.* (1998), China	pU-1M/pU-2R for 6/11 and pU-31B/pU-2R for 16/18/31/33/52/58	102	58.8	25.5	2.0	29.4	21.6	0	1.0		19.6	Paraffin-embedded tissues
Mineta *et al.* (1998), Japan	Type-specific for 16, 18	42	31.0			26.2	4.8					Snap-frozen tissues
Gorgoulis *et al.* (1999), Greece	MY09/11 and GP5/6; type-specific for 6, 11, 16, 18, 31, 33, 35	91	20.9	3.3	0	14.3	3.3	0	3.3		3.3	Snap-frozen tissues
Pintos *et al.* (1999), Canada	GP5+/6+ and southern blot	52	15.4									Paraffin-embedded tissues
Gillison *et al.* (2000), USA	MY09/11/HMBO1, TS for 16/18/51/66, southern blot with 33 type-specific probes and sequencing; type-specific for 16 and 18	86	18.6			~17				1.2 (un-typed)		Fresh frozen tissues; type-specific data in combination with other sites of head and neck cancers

Table 40 (contd)

Reference, study location	Method[a] of detection and types tested	No. of cases	Overall HPV positivity (%)	Type-specific HPV positivity (%)							Multiple infections (%)	Comment
				6	11	16	18	31	33	Others (type)		
Jacob *et al.* (2002), India	Type-specific for 16 and 18	44	34.1			34.1	0					Paraffin-embedded tissues
Báez *et al.* (2004), Puerto Rico	Type-specific for 6	52	46.2			46.2						Snap-frozen tissues

See Table 7 for a description of the primers used.
EIA, enzyme immunoassay; PCR, polymerase chain reaction
[a] Unless otherwise specified, the method is PCR; ';' denotes independent methods whereas 'and' denotes subsequent steps.

mined in each study was small (< 105 cases), which may in part explain the large variability in the prevalence estimates.] A relatively low prevalence of overall HPV DNA, coupled with the high prevalence of HPV16 and the lack of other HPV types among HPV-positive laryngeal carcinomas, have been consistently reported in the more recent studies of HPV and laryngeal squamous-cell carcinoma.

Similar results were reported in a large systematic review of published studies that met the same inclusion criteria of adequate sample size and PCR-based HPV detection methods (Kreimer *et al.*, 2005). Of 1435 laryngeal and hypopharyngeal squamous-cell carcinomas, the pooled HPV DNA prevalence was 24% (95% CI, 22–26%). In the type-specific analysis among HPV-positive laryngeal squamous-cell carcinomas, HPV16 accounted for 66% of infections, and HPV18 and HPV6 accounted for 16 and 14% of infections, respectively.

(*b*) *Case–control studies*

Table 41 reports the results of case–control studies. One early study (Brandsma & Abramson, 1989) reported similarly low prevalence of HPV in both cases (5%) and controls with benign disease or structural abnormalities (4%), which suggested no association between HPV and laryngeal cancer.

Smith *et al.* (2000) investigated HPV DNA in 44 patients with laryngeal or hypopharyngeal squamous-cell carcinoma and 12 controls with benign laryngeal conditions. HPV DNA was collected from biopsy material from the cases and from upper respiratory tract brushings from the controls. HPV DNA was detected in 25.0% (11/44) of cases and in 16.7% (2/12) of controls. The prevalence of carcinogenic HPV types (HPV 16, 18, 31, 45 and 70 were detected) was similar in cases (18.2%) and controls (16.7%). However, after adjustment for tobacco, alcoholic beverage consumption and age, the presence of carcinogenic HPV DNA elevated the relative risk for laryngeal squamous-cell carcinoma threefold (odds ratio, 3.0) (although not significantly) compared with controls.

One prospective serological study that included laryngeal squamous-cell carcinoma (Mork *et al.*, 2001) investigated the seroprevalence of HPV16 L1 VLP in a nested case–control study of 76 cases of laryngeal cancer and 411 controls using several Nordic cancer registries linked with serum banks. HPV seropositivity increased the risk for developing laryngeal cancers 2.4-fold (95% CI, 1.0–5.6), following adjustment for serum cotinine levels, a biological marker for tobacco exposure. [The serological samples were collected on average 9.4 years before the diagnosis of cancer, and thereby provide evidence of exposure to HPV that preceded the development of disease. However, this study lacked additional information on potential confounding factors, such as alcoholic beverage use and sexual behaviour.]

One serological case–control study detected HPV16 L1 VLPs in 35.7% of 14 laryngeal squamous-cell carcinomas (Dahlstrom *et al.*, 2003), which was a higher prevalence than that detected in controls (9.2% of 120).

In contrast, another serological study of 127 cases of laryngeal cancer and 100 cancer-free controls reported prevalences of HPV 16 of 20 versus 18%, respectively (Van Doornum *et al.*, 2003).

Table 41. Case–control studies of prevalence of HPV and laryngeal cancer

Reference, study location	No. of cases	No. of controls	Method of detection and types tested	HPV prevalence (%)		Odds ratio (95% CI)	Comments/adjustments
				Cases	Controls		
Brandsma & Abramson (1989), USA	60 SCC	53 with benign disease or structural abnormalities	Southern blot	5	4	NR	HPV-positive tumours harboured DNA sequences related to HPV 11.
García-Milían *et al.* (1998), Cuba	33 SCC	25	PCR with MY09/11	48	16	$p < 0.05$	Matched for age
Nishioka *et al.* (1999), Japan	27	35	PCR for 16/18 and dot blot for 16 and 18	19	6	3.75 (0.72–19.67)	Matched for age and sex; adjusted for tobacco smoking
Smith *et al.* (2000), USA	44 SCC	12 patients with benign laryngeal conditions	PCR with MY09/11 or MY09/GP5+ and sequencing	18[a]	17[a]	3.0 (CI not given)	DNA from oral source, laryngeal source and biopsies; adjusted for age and alcoholic beverage and tobacco consumption
Mork *et al.* (2001), Norway, Finland and Sweden	76 SCC	411	Antibodies against HPV 16 L1 VLP	12	5	2.4 (1.0–5.6)	Adjusted for cotinine levels
Dahlstrom *et al.* (2003), USA	14 SCC	120	Antibodies against HPV 16 VLP	35.7	9.2	NR	
Van Doornum *et al.* (2003), Netherlands	127	100	Antibodies against HPV 16 L1 VLP and E7 protein	L1, 20 E7, 2	L1, 18 E7, 2	$p = 0.876$ NR	

See Table 7 for a description of the primers used.

CI, confidence interval; NR, not reported; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma; VLP, virus-like particles

[a] The high-risk types found in the study and accounted for were 16, 18, 31, 45 and 70.

Altogether, the evidence suggests that HPV may be involved in the development of some laryngeal cancers, but the associations documented to date are not as clear nor as strong as those observed at other upper aerodigestive sites, such as the tonsils and the oropharynx (Herrero, 2003). If HPV DNA causes a subset of laryngeal cancers, it is probably a smaller subset than that documented for other sites of the head and neck. However, HPV 16 predominates over other HPV types among HPV-associated laryngeal cancers. There is some evidence against an association between HPV and laryngeal squamous-cell carcinoma: (*a*) cell lines derived from laryngeal tumours contain low viral load, which probably reflects the absence of a clonal relationship (Atula *et al.*, 1999); (*b*) the presence of HPV DNA is not limited to the tumour specimen and is also found in normal surrounding tissue (Venuti *et al.*, 2000); and (*c*) HPV DNA is not consistently detected in premalignant lesions of laryngeal squamous-cell carcinoma (Fouret *et al.*, 1995; Poljak *et al.*, 1997; Smith *et al.*, 2000), which suggests a lack of continuity of HPV infection throughout the malignant process.

In the oral region, a clear lack of concordance of HPV prevalence between biopsy tissue and oral specimens has been demonstrated (Herrero, 2003). To take samples from the the larynx of healthy controls is practically impossible; therefore, new methods to assess exposure to HPV must be considered. Immunological markers (e.g. antibodies against HPV E6 and E7) may also help distinguish cancers in which HPV has played an etiological role.

2.5 Cancer of the skin and conjunctiva

2.5.1 *Cancer of the skin*

In contrast to the papillomavirus types found in the cancers of the mucosae which are classified in the genus alpha of the papillomaviruses, the papillomavirus types found in skin cancers mainly belong to the genus beta of the family also termed as epidermodysplasia verruciformis (EV)-HPV (see Section 1.1.3). This can be explained by a different tropism of the two genera, because the skin is histologically distinct from the mucosae. The pathology of skin lesions is described in Section 1.5.4.

(*a*) *Case series*

Early studies of skin cancer that used southern blot or PCR for the detection of mucosal HPV types suggested a low prevalence of HPV in skin cancers except for those that occur at periungual and palmoplantar sites. In these rare tumours (occasionally found in patients who also have HPV 16/18-positive cervical lesions), the high prevalence of HPV 16/18 DNA suggests possible transmission of HPV infection from genital sites (IARC, 1995).

Since the development of highly sensitive, partially nested PCRs designed to detect epidermodysplasia verruciformis (EV)-related and cutaneous HPV types (Berkhout *et al.*, 1995; Shamanin *et al.*, 1996; see Section 1.3.3), a much higher prevalence of HPV DNA

has been found in non-melanoma skin cancers (Tables 42 and 43). However, HPV DNA is also frequently detected in specimens of normal skin and in plucked hairs (see Section 2.5.1(*b*)). A diverse spectrum of HPV types, including HPV 20, 38, 41 and 48, have been detected, and many new partial HPV DNA sequences (350–430 nucleotides from the L1 gene) have been identified. Most of them have been assigned to genera beta (including EV-associated HPV) and gamma (see Section 1.1.3) (Berkhout *et al.*, 1995; Shamanin *et al.*, 1996; Bens *et al.*, 1998; Forslund *et al.*, 2003a).

The need for highly sensitive detection techniques can be explained by the very small amounts of HPV DNA present in skin tumours. When HPV DNA was determined by quantitative, type-specific real-time PCR in precancerous actinic keratoses and non-melanoma skin cancers that were positive in nested PCR, viral loads ranged from 1 HPV DNA copy per 14 200 cell equivalents to 50 HPV DNA copies per 1 cell equivalent (Weissenborn *et al.*, 2005). The HPV DNA load was significantly higher in actinic keratoses than in squamous-cell carcinomas and Bowen disease. In most cases, probably not every tumour cell harbours an HPV genome, which is supported by in-situ hybridization that shows only a few HPV DNA-positive cell nuclei per section.

The genus- and species-specific PCRs used differ in sensitivity towards individual types, and thus affect the spectrum of HPV types identified in cases in which DNA levels are close to the limit of detection (Pfister, 2003). When HPV 5 and 8-specific nested PCR was added to an EV-specific nested PCR with degenerate primers, for example, these types were shown to be more prevalent than had been anticipated previously (Meyer *et al.*, 2000). Using HPV 38-specific, hot-start PCR to amplify part of the E6 open-reading frame (ORF), HPV 38-related DNA was detected in 55% of basal-cell carcinomas, 46% of squamous-cell carcinomas and 10% of healthy skin samples (Caldeira *et al.*, 2003).

(i) *Squamous-cell carcinoma and keratoacanthoma*

Nearly all studies published after 1996 revealed HPV prevalences in squamous-cell carcinoma of 27–60% (Table 42). The prevalence of EV-associated beta-HPV was generally in the range of 30–50%; probably as a result of the primers employed for the PCR, it was only 12% or lower in two studies (Shamanin *et al.*, 1996; Iftner *et al.*, 2003). HPV 16 and related high-risk mucosal HPV were occasionally detected in some case series; however, the high prevalence (28%) of HPV 16 in a French study (Cairey-Remonnay *et al.*, 2002) could not be confirmed by others even when highly sensitive mucosal HPV-specific PCR was used. HPV 4 was found in one of 26 and nine of 72 squamous-cell carcinomas in two case series (Shamanin *et al.*, 1996; Iftner *et al.*, 2003) and HPV 41 in two of 10 squamous-cell carcinomas in another (Grimmel *et al.*, 1988).

The original findings of a high prevalence of HPV 16/18 in periungual squamous-cell carcinomas (Moy *et al.*, 1989; Eliezri *et al.*, 1990) were confirmed by numerous case reports and more recent surveys of digital squamous-cell carcinomas (Forslund *et al.*, 2000; Alam *et al.*, 2003; Table 44). HPV 16 and occasionally HPV 31, 35 and 73 were detected in up to 90% of these tumours (Alam *et al.*, 2003). Of 72 cases of digital squamous-cell carcinomas, 10% had an antecedent genital dysplasia or carcinoma that contained the same HPV type as

Table 42. Prevalence of HPV DNA in case series of squamous-cell carcinoma (SCC) and keratoacanthoma (KA) of the skin

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[a]		
Pfister *et al.* (1986), Germany	Southern blot and restriction digest	6 KA	33.3			25-related (2)	
Scheurlen *et al.* (1986), Germany	Dot blot hybridization and restriction digest	7 KA	14.3	14.3			Frozen tissue; HPV 9 and 37 in a single KA present at ~10 copies/cell
Grimmel *et al.* (1988), Germany	Southern blot for 41	6 KA 10 SCC	0 20.0			41 (2)	Frozen tissue. HPV 41 not found in any of 44 melanomas or 47 non-malignant skin lesions in other patients
Eliezri *et al.* (1990), USA	ISH for 6/11, 16/18, 31/33/35, 42/43/44, 45/56, 51/52	16 SCC	0				Tissue stored at –20 °C; see also Tables 43 and 44
Kawashima *et al.* (1990), Poland	Dot blot for 5/8/14, 17/20/23/24, 6/11, 16/18/33, 2/3, 1/4/7, 3/10/28; PCR and Southern blot for 11/16	33 KA 51 SCC (NOS) 25 SCC (lip)	0 2.0 4.0		 4.0*	Untyped (1)	Frozen tissue.; no HPV DNA found in any of 14 cutaneous horns in control patients (see also Tables 43 and 46) *HPV 16
Pierceall *et al.* (1991), USA	PCR for 6/11, 16, 18	21 SCC	19.0		19.0*		Fresh or frozen tissue; no HPV DNA found in the normal skin biopsies from the HPV 16- positive tumour patients; *all HPV 16
Shamanin *et al.* (1996), Germany	PCR with broad range degene-rate primers [not specific for EV]	26 SCC 4 KA	30.8 50.0	11.5	3.8	4, 32, 51 (1 each), 42 (2) 6 (1), 34 (1)	Frozen tissue

Table 42 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[a]		
Hsi *et al*. (1997), USA	Nested PCR with L1 consensus and degenerate primers and sequencing	30 KA	26.7				Paraffin-embedded tissue; in non-lesional skin samples from reduction mammoplasty specimens or uninvolved skin from melanoma patients, 1/26 was positive for HPV DNA.
Harwood *et al.* (2000), United Kingdom	PCR with 9 degenerated primers for EV, cutaneous, and mucosal HPV	22 SCC	27.2	27.2	0		Frozen tissue
Meyer *et al*. (2000), Germany	Nested PCR with mucosal-, cutaneous-, and EV-HPV-specific degenerate and type-specific (5, 8) primers, followed by RFLP analysis	10 SCC	50.0	40.0		6 (1), untyped (1)	
O'Connor *et al*. (2001a), Ireland	Nested PCR with MY09/11 and EV-HPV-specific primers and sequencing	12 SCC	83.3	58.3		Unknown (3)	Three of 20 normal skin samples from controls positive in a nested PCR with EV-HPV-specific primers
Cairey-Remonnay *et al*. (2002), France	PCR with MY09/11 and FAP-primers; hybridization for 6, 11, 16, 18, 31, 33, 35, 45, 51, 52, 58, 68	51 SCC	37.2		29.4	Untyped cutaneous (6)	Paraffin-embedded tissue
Forslund *et al.* (2003a), Norway	PCR with FAP primers	12 KA	33.3				Frozen tissue
Forslund *et al.* (2003c), Australia	PCR with FAP and HPV 38-specific primers, cloning, and sequencing	12 SCC	33.3	33.3			Tissue stored at –20°C; 92% of perilesional and 63% of buttock swabs from same patients were HPV-positive.

Table 42 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[a]		
Iftner *et al.* (2003), Germany, USA	PCR with broad range degenerate primers	72 SCC	59.7	5.6	12.5	4 (9)	Frozen tissue; other types reported in combination with basal-cell carcinomas
Meyer *et al.* (2003), Germany	(Nested) PCR with mucosal-cutaneous-, and EV-HPV-specific degenerate primers	15 SCC	46.7	46.7	6.7	6 (1)	Frozen tissue; 2/13 normal skin samples from patients with non-melanoma skin cancers (see Table 46) contained HPV DNA.
Pfister *et al.* (2003), Poland	Nested PCR with group-specific (mucosal, EV) and type-specific (HPV 8) primers and sequencing	20 SCC	45.0	45.0	5.0		Paraffin-embedded tissue

See Table 7 for a description of the primers used.
EV, epidermodysplasia verruciformis; ISH, in-situ hybridization; NOS, not otherwise specified; PCR, polymerase chain reaction
[a] Alpha 7- and alpha 9-HPV

Table 43. Prevalence of HPV DNA in case series of basal-cell carcinoma of the skin

Reference, study location	Method of detection and types tested	No. of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[a]		
Grimmel *et al.* (1988), Germany	Southern blot for 41	13	0				Frozen tissue
Eliezri *et al.* (1990), USA	In-situ hybridization (probe specificity not reported)	26	3.8				Paraffin-embedded tissue; see also Tables 42 and 44
Kawashima *et al.* (1990), Poland	Dot blot for 5/8/14, 17/20/23/24, 6/11, 16/18/33, 2/3, 1/4/7, 3/10/28; PCR and southern blot for 11/16	53	1.9	1.9			Frozen tissue; see also Tables 42 and 46
Pierceall *et al.* (1991), USA	PCR for 6/11, 16, 18	16	18.8		18.8*		Fresh or frozen tissue. No HPV DNA was found in the normal skin biopsies from the HPV-positive tumour patients; *all HPV 16
Nahass *et al.* (1992), USA	PCR [with consensus primers] and dot blot hybridization	3 scrotal	0				Fixed tissue
Zhu *et al.* (1993a,b), USA	PCR with MY09/11 and southern blot	13	0				Fresh tissue stored at 4 °C
Shamanin *et al.* (1996), Germany	PCR with broad range degenerate primers	11	36.4	27.3		4 (1), 6 (1), 7 (1)	Frozen tissue
Biliris *et al.* (2000), Greece	Multiplex, type-specific PCR for 1, 2, 5, 8, 11, 16, 18, 33	72	30.6	26.4	13.9*		Frozen tissue; *all HPV 18
Harwood *et al.* (2000), United Kingdom	PCR with EV, cutaneous and mucosal HPV-specific primers	30	36.7	33.3		Alpha 2- (1), mucosal (1)	Frozen tissue

Table 43 (contd)

Reference, study location	Method of detection and types tested	No. of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[a]		
Wieland *et al.* (2000), Germany and Poland	(Nested) PCR with genital/mucosal-, cutaneous- and EV-specific primers	69	43.4	40.6	0	6, 28, 34 (1 each)	Snap frozen; 8 of 31 (26%) perilesional skin tissues were HPV DNA-positive.
Forslund *et al.* (2003c), Australia	PCR with FAP and HPV 38-specific primers, and sequencing	19	21.1	21.1	0		Tissue stored at –20 °C; 52% of perilesional and 57% of buttock swabs from same patients were HPV-positive.
Iftner *et al.* (2003), Germany	PCR with broad range degenerate primers	18	27.8	0	16.7	27 (1), untyped (1)	Frozen tissue

See Table 7 for a description of the primers used.
EV, epidermodysplasia verruciformis; PCR, polymerase chain reaction
[a] Alpha 7- and alpha 9-HPV

Table 44. Prevalence of HPV DNA in case series of periungual and palmar squamous-cell carcinoma (SCC) of the skin

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (no. positive/ total[a])	Type-specific HPV positivity (no. positive/total)[a]		Other types detected (no.)	Comment
				6/11	16/18		
Moy *et al*. (1989), USA	Dot blot for 6/11, 16, 18	10 periungual	8/10		6/10		Frozen tissue; episomal HPV 16 found in 4/6 HPV 16-positive specimens
Ostrow *et al*. (1989a), USA	Southern blot for 2, 6, 16, 18, 31	1 digital	1/1		1/1		Episomal and integrated HPV 16 demonstrated in tumour tissue by two-dimensional gel electrophoresis
Eliezri *et al*. (1990), USA	ISH (NA)	12 periungual	9/12		7/12	Untyped (2)	Paraffin-embedded tissue; see also Tables 42 and 43
Guitart *et al*. (1990), USA	ISH for 6/11, 16/18, 31/33/35	9 nail bed SCC	1/9		1/9*		Paraffin-embedded tissue; *HPV 16; the patient also had HPV 16 in cervical tissue.
Ashinoff *et al*. (1991), USA	PCR for 16/18 and dot blot	2 periungual	2/2		2/2*		Paraffin-embedded tissue; *HPV 16 in both lesions
Moy & Quan (1991), USA	Dot blot for 6/11, 16, 18	1 digital	1/1		1/1*		Frozen tissue; *HPV 16
Sánchez-Lanier *et al*. (1994), USA	Southern blot for 6/11, 16, 18	4 digital	4/4		4/4*		Expression of unspliced E6 and spliced E6I transcripts in all patients; *all HPV 16
McHugh *et al*. (1996), USA	RT, in-situ PCR	1 metastatic digital	1/1			35 (1)	HPV 35 RNA in axillary lymph node metastases
Downs *et al*. (1997), United Kingdom	PCR with L1 consensus primers	1 digital	1/1			Untyped (1)	Subungual SCC in Darier disease
Forslund *et al*. (2000), Sweden	PCR with neighbour primers, type-specific for 16 and consensus primers	15 digital	67%		53%	73 (2)	Paraffin-embedded tissue; study investigating link with genital SCC
Alam *et al*. (2003), USA	NA	[~25] digital	~90%		[~65%]	31 (1), 35 (1), unknown (5)	History of cervical SCC in 2 HPV-positive tumour patients

ISH, in-situ hybridization; NA, not available; PCR, polymerase chain reaction; RT, reverse transcriptase

[a] Unless otherwise specified

the digital tumour (Alam *et al.*, 2003), which underlines the possibility of genital–digital spread. It should be emphasized that tumours at this site are extremely rare.

Whereas HPV was found in only a few keratoacanthomas when detected by southern blot hybridization (Pfister *et al.*, 1986; Scheurlen *et al.*, 1986), studies that used sensitive PCR reported a prevalence of about 30% in such lesions (Table 42).

(ii) *Basal-cell carcinoma*

In broad-spectrum PCR-based studies, the prevalence of HPV in basal-cell carcinoma was 21–44% in series of 11–72 cases (Table 43). EV-associated HPV usually predominated.

(iii) *Verrucous carcinoma (epithelioma cuniculatum)*

Individual reports have documented HPV 1, 6/11 and 16/18 in single cases of verrucous carcinoma (Garven *et al.*, 1991; Noel *et al.*, 1993; Sasaoka *et al.*, 1996), a rare, indolent (typically non-metastasizing) tumour that occurs at acral sites. Verrucous carcinomas also develop in the anogenital region, where they may be found in conjunction with Buschke-Löwenstein tumours, and are usually associated with HPV 6 or 11. Verrucous carcinomas may also be found in the anogenital region in the absence of Buschke-Löwenstein tumours (see Section 2.3.3). However, the relationship between HPV infection and verrucous carcinomas in the anogenital region is poorly understood. HPV was not identified in any of 11 tumours in one case series (Petersen *et al.*, 1994) (Table 45).

(iv) *Premalignant cutaneous disease (Bowen disease and actinic keratoses)*

As for squamous-cell carcinoma, early southern blot-based studies indicated a very low prevalence of HPV in Bowen disease and actinic keratoses (Table 46), except for the rarely occurring periungual and palmoplantar Bowen disease; HPV 16/18 was found in 57–70% of these lesions in several case series (Table 47). When broad-range, sensitive PCRs were used, HPV DNA was generally detected in 35–85% of actinic keratoses and Bowen disease (Table 46).

EV-associated HPV predominated in several cases series, whereas, in one study, they were found in only 7% of premalignant lesions in which a broad spectrum of cutaneous as well as high- and low-risk mucosal HPV were detected (Iftner *et al.*, 2003). In one comparative study, the prevalence of EV-HPV DNA was similar in both low- and high-grade actinic keratoses (Pfister *et al.*, 2003).

(v) *Malignant melanoma*

There is little evidence for an association between HPV and malignant melanoma. HPV 17 and HPV 38 were found in a superficial spreading malignant melanoma of an immunosuppressed patient but not in 35 other malignant melanomas (Scheurlen *et al.*, 1986). HPV DNA related to EV-associated HPV 5 and 20 was detected in two of 15 melanoma biopsies (13%) in one study in which HPV DNA was also found in seven of 20 normal skin samples (35%) from randomly selected patients who were undergoing cosmetic surgery (Astori *et al.*, 1998). In another case series, beta-HPV were identified in

Table 45. Prevalence of HPV DNA in case series of verrucous carcinoma (VC) or epithelioma cuniculatum (EC) of the skin

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (no. positive/total)	Type-specific HPV positivity (no. positive/total)		Other types detected (no.)	Comment
				6/11	16/18		
Knobler *et al.* (1989), Austria	Dot blot (6, 11, 16/18)	1 EC, lower leg	1/1	1/1			Frozen tissue; the tumour contained HPV 11.
Garven *et al.* (1991), USA	ISH for 6/11, 16, 18/31/33	1 VC, leg	1/1	1/1	1/1		Paraffin-embedded tissue; the tumour contained HPV 11 and 18.
Noel *et al.* (1993), Belgium	ISH for 1, 2, 3, 4, 11, 16, 18	1 VC, leg	1/1			1 (1)	Paraffin-embedded tissue
Petersen *et al.* (1994), Denmark	PCR with MY09/11	11 CC, site not specified	0/11				Paraffin-embedded tissue
Sasaoka *et al.* (1996), Japan	PCR with consensus primers, restriction mapping and southern blot for 16	2 VC, foot	2/2		2/2		Both tumours contained HPV 16.

CC, carcinoma cuniculatum [belongs to the group of verrucous carcinoma]; PCR, polymerase chain reaction

Table 46. Prevalence of HPV DNA in case series of Bowen disease (BD) and actinic keratoses (AK) of the skin

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (no. positive/ total)[a]	Type-specific HPV positivity (no. positive/total)[a]		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[b]		
Ikenberg *et al*. (1983), Germany	Southern blot for 16	3 BD, thumb, lower leg, dorsal hand	1/3		1/3		Frozen tissue; thumb not further defined
Pfister & Haneke (1984), Germany	Southern blot for 1, 3, 6, 8, 11, 13	1 BD, dorsal hand	1/1			2 (1)	Tissue type not reported
Kawashima *et al.* (1986), Poland	Southern blot for 1/2/4/7, 3/10/28, 5/8/14, 17/20/23/24	12 BD	0				Frozen tissue
Stone *et al.* (1987), USA	Southern blot for 1, 4, 6, 11, 16, 18	1 BD, foot	1/1		1/1		Fresh tissue
Grimmel *et al*. (1988), Germany	Southern blot for 41	6 AK	1/6			41 (1)	Frozen tissue
Guerin-Reverchon *et al*. (1990), France	ISH for 1, 2, 5, 6/11, 16/18	11 BD, leg, face, hand	5/11		2/11*	2 (1), untyped (3)	Paraffin-embedded tissue; *1 lesion with HPV 2 and 16, 1 lesion with HPV 16 and 18; no HPV DNA in 4 samples of normal skin or 6 samples of unrelated skin disease
Kawashima *et al*. (1990), Poland	Dot blot for 5/8/14, 17/20/ 23/24, 6/11, 16/18/33, 2/3, 1/4/7, 3/10/28; PCR and southern blot for 11/16	83 non-genital BD 55 AK	2/83 3/55	2/55		34 (2) Untyped (1)	Frozen tissue; see also Tables 42 and 43
Kettler *et al*. (1990), USA	ISH for 1, 6, 11, 16, 18	25 non-genital BD	6/25		[4]/25		Paraffin-embedded tissue

Table 46 (contd)

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (no. positive/ total)[a]	Type-specific HPV positivity (no. positive/total)[a]		Other types detected (no.)	Comment
				EV-, beta-HPV	16/18, related types[b]		
Inaba *et al*. (1993), Japan	ISH for 1, 6, 11, 16, 18	1 BD forearm (6-year-old boy)	1/1			1 (1)	Paraffin-embedded tissue
Harwood *et al.* (2000), United Kingdom	PCR with EV-, cutaneous- and mucosal-HPV-specific degenerate primers	11 carcinoma *in situ* (BD) and AK	6/11	3/11		3/10/28/77 (2); 6/11/16/66 (1)	Frozen tissue
Forslund *et al.* (2003c), Australia	PCR with FAP and type 38-specific primers, cloning, and sequencing	10 AK	7/10	7/10			Tissue stored at –20 °C; 80% of perilesional and 60% of buttock swabs from the same patients were HPV-positive.
Iftner *et al.* (2003), Germany	PCR with broad range degenerate primers	71 AK 20 BD	58% 70%	}7%	}11%	1 (5), 6 (3), 27 (6), 2, 3, 4, 7, 34, 57, 73 (1 each)	Frozen tissue
Meyer *et al.* (2003), Germany	(Nested) PCR with mucosal-cutaneous-, and EV-HPV-specific degenerate primers	36 AK/BD	36%	22%	8%	6 (1)	Frozen tissue; 2/13 normal skin samples from patients with non-melanoma skin cancers (see Table 42) contained HPV DNA.
Pfister *et al.* (2003), Poland	Nested group-specific (mucosal, EV) and type-specific PCR for type 8	54 AK 60 AK 18 BD	85% 67% 33%	80% 40% 33%	0% 0% 11%		Frozen tissue Paraffin-embedded tissue Paraffin-embedded tissue

See Table 7 for a description of the primers used.
EV, epidermodysplasia verruciformis; ISH, in-situ hybridization; NA, not available; PCR, polymerase chain reaction
[a] Unless otherwise specified
[b] Alpha 7- and alpha 9-HPV

Table 47. Prevalence of HPV DNA in case series of periungual and palmoplantar Bowen disease of the skin

Reference, study location	Method of detection and types tested	No. and type of lesions	Overall HPV positivity (no. positive/ total)	Type-specific HPV positivity (no. positive/ total)		Other types detected (no.)	Comments
				6/11	16/18		
Kawashima *et al*. (1986), Poland	Southern blot hybridization	1 periungual	1/1			34 (1)	Frozen tissue
Rüdlinger *et al*. (1989), Switzerland	Southern blot hybridization for 1–5, 7, 10, 27, 30, 31, 33–38	1 periungual	1/1			35 (1)	Bowenoid lesion on vulva also contained HPV 35.
Kettler *et al*. (1990), USA	ISH for 1, 6, 11, 16, 18	5 palmoplantar	4/5		4/5		Paraffin-embedded tissue; 3/4 contained HPV 16, 1/4 contained HPV 16-related type.
Ashinoff *et al*. (1991), USA	PCR for 16/18 and dot-blot	5 periungual	3/5		3/5		Fixed tissue; all HPV 16
McGrae *et al.* (1993), USA	PCR and dot blot for 6, 11, 16, 18, 31, 33, 39, 45	3 periungual (1 patient)	3/3	?	3/3		All contained HPV 16; the patient had HPV 6-containing condylomata of the penis.
Nordin *et al*. (1994), Sweden	PCR for 16	1 digital	1/1		1/1		HPV 16 also found in vulvar and cervical dysplastic tissue from this patient
Sau *et al*. (1994), USA	ISH for 6/11, 16/18, 31/33/ 51	7 nail bed	4/7		4/7		Paraffin-embedded tissue

ISH, in-situ hybridization; PCR, polymerase chain reaction

four of 54 malignant melanomas (7%). Samples of normal skin of the patients were not available (Miracco *et al.*, 2001).

(*b*) *Case–control studies*

A few studies compared the prevalence of HPV DNA in skin cancer tissue with that in healthy skin samples from control subjects or in swabs from perilesional skin. Depending on the sensitivity of the detection systems, prevalence rates of 22 and 86% were found in cancer tissue from immunocompetent patients compared with 8 and 22% in uninvolved skin, respectively (Stark *et al.*, 1994; O'Connor *et al.*, 2001a). In a study that compared the prevalence of EV-HPV DNA in 91 cases of solar keratosis or Bowen disease, 72 squamous-cell carcinomas and 106 normal skin samples, adjusted odds ratios for the presence of EV-HPV DNA were 9.2 (95% CI, 1.0–80) for solar keratosis and Bowen disease and 9.6 (95% CI, 0.9–100) for squamous-cell carcinoma (Iftner *et al.*, 2003). In another study in Poland, very little difference between the positivity rates of basal-cell carcinomas and paired healthy skin (32% and 26%) was observed (Wieland *et al.*, 2000). The prevalence of HPV DNA was much higher in perilesional swabs (92% and 52%, respectively) than in squamous-cell carcinomas (33%) and basal-cell carcinomas (21%) of patients in Australia (Forslund *et al.*, 2003a). The high prevalence in swabs may reflect contamination of the skin surface by HPV rather than infection (Forslund *et al.*, 2004).

A significant association between the presence of EV-HPV DNA and skin cancer was observed in punch biopsies from the clinically normal skin of 38 immunosuppressed renal transplant recipients and 39 immunocompetent individuals (odds ratio for both groups combined, 6.41; 95% CI, 1.79–22.9) (Harwood *et al.*, 2004). Conversely, no association was found between the presence of cutaneous or mucosal HPV types and skin cancer.

A slightly positive, non-significant association was found between EV-HPV DNA in plucked eyebrow hairs and squamous-cell carcinoma in a population in subtropical Australia (odds ratio, 2.00; 95% CI, 0.50–8.0) (Boxman *et al.*, 2000). However, a strong association was observed between EV-HPV DNA and solar keratoses among men (odds ratio, 3.40; 95% CI, 1.77–6.53) but not among women (Boxman *et al.*, 2001). In a Dutch population, HPV DNA in eyebrow hairs (all EV-HPV types except for HPV 2 in one individual) was associated with a history of squamous-cell carcinoma (odds ratio, 1.7; 95% CI, 1.1–2.7) (Struijk *et al.*, 2003). Positive associations were observed for the individual EV-HPV types 5, 8, 15, 20, 24 and 38 and the adjusted odds ratios for HPV 5, 15 and 20 were statistically significant.

Two seroepidemiological case–control studies evaluated HPV infection as a risk factor for cutaneous squamous-cell carcinoma in immunocompetent individuals (Feltkamp *et al.*, 2003; Masini *et al.*, 2003). Infection with EV-related HPV types (5, 8, 15, 20, 23, 24, 36 and 38) was assessed by serology using a L1-VLP ELISA method. In Italy (Masini *et al.*, 2003), positive serology for HPV 8 was associated with an odds ratio for cutaneous squamous-cell cancer of 3.2 (95% CI, 1.3–7.9). Other variables significantly associated with this tumour were family history of non-melanoma skin cancer, high professional or recreational exposure to the sun, light eye colour, large number of solar

keratoses and seborrheic keratoses on the body surface and residence in buildings that emit radon. In the Netherlands (Feltkamp *et al.*, 2003), the estimated relative risk for squamous-cell carcinoma was significantly increased in HPV 8- and HPV 38-seropositive subjects after adjusting for age and sex (odds ratio, 14.7; 95% CI, 1.6–135; and 3.0; 95% CI, 1.1–8.4, respectively). The estimated relative risk for nodular and superficial multifocal basal-cell carcinoma was also significantly increased in HPV 8-positive subjects (odds ratio, 9.2; 95% CI, 1.1–78.2; and 17.3; 95% CI, 2.1–143, respectively) and to a lesser extent in HPV 20-seropositive subjects (odds ratio, 3.2 and 3.4, respectively). No associations were found for HPV 16. The relative risk for developing malignant melanoma was not increased among HPV-seropositive individuals.

Available studies do not allow an evaluation of whether exposure to the sun confounds or modifies the effect of HPV on skin cancer.

2.5.2 *Cancer of the eye and conjunctiva*

Early case reports and case series on the prevalence of HPV in eye lesions have been reviewed previously (IARC, 1995). HPV (mostly type 16) was found by PCR in both intraepithelial neoplasia of the conjunctiva (80–100%) and in nearly all invasive carcinomas of the conjunctiva, eyelid and lacrimal sac that were tested. In studies published since that time, the detection rate of HPV has varied widely.

In a study in subtropical Tanzania, most cases of conjunctival epithelial dysplasia and epithelial neoplasms were found to be HPV-positive by in-situ hybridization (Moubayed *et al.*, 2004).

Using PCR-RFLP and in-situ hybridization methods, Saegusa *et al.* (1995) detected HPV 16 in two of four dysplasias (50%) and one of four squamous-cell carcinomas (25%) but in no basal-cell epithelioma of the conjunctiva. No other HPV types were found. Nakamura *et al.* (1997a) detected HPV 16 in two of four dysplasias and HPV 18 in one case of severe dysplasia and in one of four carcinomas of the conjunctiva. Eng *et al.* (2002) failed to detect DNA of HPV types 6, 11, 16 or 18 in any of 20 formalin-fixed, paraffin-embedded malignant epithelial tumours of the conjunctiva.

HPV DNA was detected in about 30% of non-familial sporadic retinoblastoma in two studies (Orjuela *et al.*, 2000; Palazzi *et al.*, 2003). In spite of a similar overall prevalence of HPV, the spectrum of types differed between the two studies, e.g. for HPV 16 (10% versus 23%) and for HPV 18 (28% versus 0%).

It has been proposed that HPV infection represents an alternative carcinogenic mechanism to retinoblastoma gene inactivation but there was no significant correlation between the detection of HPV DNA and immunohistochemical detection of the retinoblastoma protein (Orjuela *et al.*, 2000).

HPV 16 DNA and E6-specific mRNA were detected by in-situ hybridization and reverse transcriptase in-situ PCR, respectively, in five of 10 conjunctival intraepithelial neoplasias; HPV 18 DNA and mRNA were present in the other five specimens (Scott *et al.*, 2002b). Neither HPV DNA nor mRNA were detected in clinically uninvolved con-

junctival specimens from the same patients or from five age-matched control subjects ($p < 0.001$). In contrast to these findings, other studies detected HPV not only in epithelial neoplasms but also in non-neoplastic lesions as well as in apparently healthy conjunctiva. In one study, HPV 16 DNA was found by PCR with consensus primers and dot blot hybridization using 28 type-specific probes, in two of 10 invasive cancers of the conjunctiva, and in the normal muscosa of one of 30 age- and sex-matched controls (Palazzi *et al.*, 2000). In another study, HPV 16 infection was found by PCR in seven of 20 samples from carcinomas (35%) and in two of six samples from conjunctivitis (Waddell *et al.*, 1996). Karcioglu and Issa (1997) identified HPV 16 and 18 DNA by PCR in eight of 14 (57%) in-situ squamous-cell carcinomas, in 17 of 31 (55%) invasive squamous-cell carcinomas, in four of 20 (20%) samples of climatic droplet keratopathy, in 11 of 31 (35%) samples of scarred corneas and in six of 19 (32%) samples of normal conjunctival tissue obtained during routine cataract extractions. HPV DNA was not detected by PCR with MY09/11 primers in any of 28 pathological specimens that ranged from intraepithelial neoplasia to invasive squamous-cell carcinoma or in 23 disease-free, age- and sex-matched patients (Tulvatana *et al.*, 2003).

The weak association between infections with genital HPV types and carcinoma of the conjunctiva is supplemented by the lack of a statistically significant association between anti-HPV 16 antibody status and the risk for conjunctival neoplasia (Newton *et al.*, 2002; Waddell *et al.*, 2003).

In a pilot study of 21 squamous-cell carcinomas of the conjunctiva and 22 conjunctival samples of control subjects from Uganda, broad-spectrum and EV-specific PCR-based assays detected EV-HPV types in 86% of the cases and in 36% of controls (odds ratio after adjustment for exposure to the sun, 22.7; 95% CI, 1.7–312) (Ateenyi-Agaba *et al.*, 2004). No mucosal HPV types were found in either cases or controls by genus alpha- and type 16-, 18-, and 45-specific PCR. As human immunodeficiency virus (HIV) serology was not available for the study patients, the strong association between EV-HPV and carcinoma of the conjunctiva could not be adjusted for a possible immunosuppression due to HIV infection, which appeared to be strongly associated with conjunctival cancer in Uganda (Newton *et al.*, 2002).

2.6 Cancer at other sites

2.6.1 *Cancer of the nose and nasal sinuses*

Inverted papillomas are rare tumours of the nasal cavity and paranasal sinuses. Although commonly benign, they frequently reveal signs of invasive growth and convert into malignant tumours in up to 13% of cases (Bernauer *et al.*, 1997). They are therefore discussed jointly with carcinomas of the nasal cavity.

The presence of HPV infection in an extensive squamous-cell papilloma of the nasal cavity was first detected by immunoperoxidase staining of group-specific antigens (Syrjänen *et al*., 1983). A further study analysed 14 patients with 13 inverted papillomas

and three squamous-cell carcinomas that extended to several sites of the nasal cavity and paranasal sinuses by indirect immunoperoxidase staining (Syrjänen *et al*., 1987b). Seven of the 25 papilloma biopsies analysed expressed HPV antigens. By in-situ hybridization with probes for HPV 6, 11 and 16, nine lesions in seven patients were shown to contain HPV 11 DNA. The three carcinomas tested were positive for HPV 16 DNA. In another study that used only in-situ hybridization to detect HPV 6 and 11, a high prevalence of both HPV 6 and 11 was noted in the 21 inverted papillomas analysed (Weber *et al*., 1988). A new virus type, HPV 57, was subsequently isolated from an invasively growing inverted papilloma of the maxillary sinus (de Villiers *et al.*, 1989). When type-specific primers were used, this virus was subsequently identified in six of eight inverted nasal papillomas, in one of three inverted papillomas with dysplasia and in two of four inverted papillomas with carcinoma (Wu *et al.*, 1993). HPV 57 was also detected in a further case of inverted papilloma (Ogura *et al.*, 1996).

Most other studies that used type-specific primers detected HPV 11 and HPV 6 in inverted papillomas and in some malignant tumours, and a limited number of cases were reported to be positive for HPV 18 and 16 DNA. Two inverted nasal papillomas contained HPV 11 DNA, one of which had a 500-base-pair insertion (Respler *et al.*, 1987). In another study, HPV 6 DNA was identified by southern blot in one of seven inverted papillomas (Ishibashi *et al*., 1990). Kashima *et al*. (1992b) found HPV 11 in five of 29 inverted papillomas; two other papillomas contained HPV 6 DNA, and one of 24 squamous carcinomas contained HPV 18 DNA. A specific search for HPV 16 and 18 sequences by PCR in nasal carcinomas found that six of 49 cases were positive for HPV 16 and one for HPV 18 DNA (Furuta *et al*., 1992). Bernauer *et al.* (1997) detected HPV DNA in seven of 21 inverted papillomas; one lesion, which was associated with a squamous-cell carcinoma, was positive for HPV 18. One of two carcinomas that occurred within papillomas of the nasal septum contained HPV 6/11 DNA and the other contained HPV 18 DNA (Buchwald *et al.*, 1997). Expression of E6/E7 genes of HPV 6 was detected in an inverted papilloma (Harris *et al.*, 1998a). HPV 11 was found by in-situ hybridization and by PCR in one inverted papilloma (Kraft *et al.*, 2001). Among 28 cases of squamous-cell carcinoma associated with inverted papillomas, four were HPV-positive: one for HPV 6/11, one for HPV 16/18, one for HPV 6/11 and 16 and one for HPV 18 (Buchwald *et al*., 2001).

In summary, it appears that inverted papillomas are frequently positive for HPV 11, 6 and 57 DNA, whereas a small percentage (~5–15%) of carcinomas arising at the same sites contain HPV 18, 16, 11, 6 and 57 DNA at decreasing frequency.

2.6.2 *Cancer of the lung*

A number of studies have investigated the prevalence of HPV DNA in lung cancer tissues in patients with juvenile-onset recurrent respiratory papillomatosis and in women with a history of CIN3. [It must be noted that almost all studies are case series and laboratory personnel were not blinded as to the nature of the specimens; other aspects of proper epidemiological design were also lacking.]

Table 48 summarizes the prevalence of HPV detected by PCR in studies of lung cancer that involved at least 20 subjects. HPV DNA prevalence in lung tumours ranged from zero (Shamanin *et al.*, 1994a; Szabó *et al.*, 1994; Welt *et al.*, 1997; Wistuba *et al.*, 1998; Gorgoulis *et al.*, 1999) to very high levels, especially in studies conducted in Asia. Many of the positive reports that come from Asia are from Taiwan, China (Cheng *et al.*, 2001, 2004; Wu *et al.*, 2005) and Okinawa, Japan (Hirayasu *et al.*, 1996; Tsuhako *et al.*, 1998; Iwamasa *et al.*, 2000; Miyagi *et al.*, 2000, 2001).

A recent study in Taiwan, China, that tested non-cancer lung specimens as controls (Cheng *et al.*, 2001) found that 77 (55%) of 141 lung cancers were positive for HPV 16/18 by nested PCR and in-situ hybridization, and that the detection of HPV 16 and HPV 18 was more common in tumour specimens from women than in those from men ($p < 0.0001$), in those from nonsmokers than in those from smokers ($p < 0.001$) and in adenocarcinomas than in squamous-cell carcinomas ($p < 0.03$). Furthermore, specimens from cases were significantly more likely to be HPV DNA-positive than lung tissue from non-cancer patients (55% versus 27%; $p < 0.001$). From these results, it was suggested that HPV infection may play a role in lung carcinogenesis among Taiwanese nonsmoking women: only 10% of lung cancer cases in women occur in patients with a history of cigarette smoking, and adenocarcinomas constitute 59% of lung cancers among women compared with 31% among men (Chen *et al.*, 2004). In two other studies that involved many of the same patients, HPV 11 was also reported in 13% of lung specimens from non-cancer patients (Cheng *et al.*, 2004) and HPV 16 was reported in 13% of peripheral blood specimens from non-cancer patients (Chiou *et al.*, 2003). [Although the high prevalence of HPV DNA in normal control specimens could reflect false-positive results, a high concordance with in-situ hybridization was demonstrated, and the prevalence of HPV was higher in specimens obtained from lung cancer patients than in those from non-cancer patients in each of these studies.]

Similarly, in tumours collected during 1993 in Okinawa, Japan, 34 (79%) of 43 squamous-cell carcinomas tested were positive for HPV DNA when type-specific E6/E7 PCR for HPV 16, 18, 6 and 11 was used (Hirayasu *et al.*, 1996). Subsequently, it was reported that the prevalence of HPV-positive squamous-cell carcinomas steadily decreased in specimens obtained after 1995 (Miyagi *et al.*, 2000): 68% were HPV DNA-positive in 1995, 35% in 1996, 23% in 1997 and 24% in 1998. The decreasing prevalence of HPV-positive specimens correlated with a marked fall in the number of squamous-cell carcinomas of the lung in Okinawa during the same time frame, and a concordance of nearly 100% between PCR and in-situ hybridization results was shown. A separate evaluation of adenocarcinomas showed that 78% of 23 case specimens contained HPV DNA (Tsuhako *et al.*, 1998). Cases of HPV DNA-positive squamous-cell carcinoma that had high infiltration with Langerhans cells were found to have a better prognosis (Miyagi *et al.*, 2001). Normal control tissue specimens were not tested.

In spite of the above results, several large case series failed to detect HPV DNA in any lung cancer specimens tested or detected it in a very small percentage among a total of 290 cases (Shamanin *et al.*, 1994a; Hiroshima *et al.*, 1999; Clavel *et al.*, 2000).

Table 48. Prevalence of HPV DNA in case series of lung cancer detected by the polymerase chain reaction (PCR) method

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)					Multiple infections (%)	Comment
					6	11	16	18	Others (type)		
Ogura *et al.* (1993), Japan	PCR, southern blot for 16, 18	29	SCC	10.3			10.3	0			Snap-frozen tissue
Liu *et al.* (1994), China	PCR for 11, 16; ISH for 11, 16	49	SCC	14.3		12.2	4.1			2.0	Paraffin-embedded archival tissue
Shamanin *et al.* (1994a,b), Germany	L1 consensus primers and 4/60/65 + southern blot	85	SCC (40%), ADC (15%), others	0							Frozen samples
Szabo *et al.* (1994), Japan	PCR	47	SCC (85%), large cell carcinoma	0							Paraffin-embedded tissue
Xing *et al.* (1994), China [cited in Syrjänen (2002)]	PCR, ISH	49	SCC	14.2		8.2	8.2	2			
Al-Ghamdi *et al.* (1995), United Kingdom	E1 consensus primers and type-specific for 6, 7, 11, 16, 18	66	SCC (50%), others	9.1	1.5	4.5	1.5			1	Paraffin-embedded tissue
Kinoshita *et al.* (1995), Japan	PCR for 16, 18, 33 and southern blot for 18; ISH for 18	36	ADC (61%), SCC (28%), small cell carc.	8.3				36.1			Frozen (PCR, southern blot) or fixed (ISH) samples
Li *et al.* (1995), China	Dot blot for 16, 18; PCR for 16, 18	50	SCC (54%), ADC (32%), small cell (4%)	32.0			44.0	12.0		2.0	Paraffin-embedded (90%) or frozen tissue (10%)
Nuorva *et al.* (1995), Finland	MY09/11 and nested PCR for 6, 11, 16, 18, 31, 33; ISH for 6, 11, 16, 18, 31, 33	22	Bronchioalveolar carcinoma	36.4	9.1	13.6	4.5	4.5	22.7 (31); 22.7 (33)	31.8	Paraffin-embedded tissue
Zhang, Z.F. *et al.* (1995), China	PCR for 6/11, 16, 18	34	[SCC]	11.8							
Da *et al.* (1996), China	Consensus primer; ISH for 16/18	40	SCC (40%), ADC (30%); small-cell carcinoma (23%)	PCR, 55.0 ISH, 25.0							

Table 48 (contd)

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)					Multiple infections (%)	Comment
					6	11	16	18	Others (type)		
Hirayasu *et al.* (1996), Japan	PCR for 6, 11, 16, 18 and southrn blot	73	SCC, ADC, small-cell carcinoma, large-cell carcinoma	58.9	15.1	0	37.0	43.8		30.1	Paraffin-embedded tissue
	ISH for 6/11, 16/18, 31/33/51	94		28.7	9.6		28.7			7.4	
Noutsou *et al.* (1996), Greece	Consensus and mixed type-specific primers for 11/16/18/33, and RFLP	99	SCC (41%), ADC (41%)	15.2		3.0	4.0	8.1	2.0		Paraffin-embedded tissue
Soini *et al.* (1996), Finland	MY09/11 and nested PCR for 6, 11, 16, 18, 31, 33; ISH for 6, 11, 16, 18, 31, 33	43	SCC (65%), ADC (26%), small-cell carcinoma (7%)	30.2	14.0	14.0	18.6	16.3	20.9 (31); 20.9 (33)	25.6	Paraffin-embedded tissue
Welt *et al.* (1997), Germany	ISH for 6, 11, 16, 18; PCR with MY09/11 and nested with CN3/MY09	32	SCC	0							Paraffin-embedded tissue
Bohlmeyer *et al.* (1998), USA	MY09/11, southern blot, and dot blot for 6, 11, 16, 18, 33	34	SCC	5.9				5.9			Paraffin-embedded tissue
Papadopoulou *et al.* (1998), Greece	MY09/11 and Southern blot for 6/11, 16/18	52	SCC	69.2	11.5		21.2		13.5	5.8	Paraffin-embedded tissue
Tsuhako *et al.* (1998), Japan	ISH; PCR for 6, 11, 16, 18, 31/33/51	23	Adenosquamous carcinoma	78.3	13.0	13.0	52.2	30.4		30.4	Paraffin-embedded tissue
Wistuba *et al.* (1998)	PCR for 16, 18, 31, 33	35	Small cell (40%), SCC (31%), ADC (29%)	0							Paraffin-embedded archival tissue
Gorgoulis *et al.* (1999), Greece	MY09/11, nested GP5/6 and type-specific for 6, 11, 16, 18, 31, 33, 35 and dot-blot	68	SCC (46%), ADC (47%), large cell carcinoma	0							Frozen and paraffin-embedded tissue
Hennig *et al.* (1999a), Norway	GP5+/6+ and probing for 6, 11, 16, 18; ISH for 6, 11, 16, 18	75	ADC (37%), SCC (24%), small-cell carcinoma, others	49.3	17.3	1.3	33.3	1.3		9.3	Paraffin-embedded tissue; patients with history of CIN3

Table 48 (contd)

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)					Multiple infections (%)	Comment
					6	11	16	18	Others (type)		
Hiroshima *et al.* (1999), Japan	PCR for 16, 18, 33 and southern blot	285	AdC	0.4			0.4				Paraffin-embedded tissue
Clavel *et al.* (2000), France	Hybrid Capture II	185	SCC (55%), AdC (32%)	2.7					2.7*		Snap-frozen tissue; *oncogenic types in Hybrid Capture II assay
Iwamasa *et al.* (2000), Japan	PCR for 6, 11, 16, 18 and southern blot										Storage of tissue not reported; *read from graph
1993		43	SCC	80*							
1998		21	SCC	24*							
Miyagi *et al.* (2000), Japan	PCR for 6, 11, 16, 18 and southern blot; ISH for 6, 11, 16, 18	157	SCC	51.0	14.0	8.9	24.2	25.5		18.5	Paraffin-embedded tissue
Cheng *et al.* (2001), Taiwan, China	MY09/11 and type-specific for 16, 18; ISH for 16, 18	141	SCC (41%), AdC (59%)	54.6			35.5	41.1			Tissue section
		60	Normal biopsies	26.7			15.0	11.7			
Miyagi *et al.* (2001), Japan	PCR for 6, 11, 16, 18 and southern blot; ISH for 6, 11, 16, 18	59	SCC	49.2	10.2		18.6	20.3			Paraffin-embedded tissue
		62	AdC	19.4	1.6		6.4	11.3			
Cheng *et al.* (2004), Taiwan, China	MY09/11 and type-specific for 6, 11; ISH for 6, 11	141	SCC (41%), AdC (59%)	38.3	28.4	9.9					Tissue section
		60	Normal biopsies	15.0	1.7	13.3					
Zafer *et al.* (2004), Turkey	MY09/11 and RFLP for 16, 18	40	SCC (63%), ADC (33%)	5.0				5.0			Tissue stored at –20 °C
Brouchet *et al.* (2005), France	ISH; immuno-histochemistry with VP1 antibody for 6/11/16/18/31/33/42/51/52/56/58	122	SCC (33%), ADC (25%)	0							Paraffin-embedded tissue
Wu *et al.* (2005), Taiwan, China	Not reported	166	SCC (43%), ADC (57%)	54.8							Frozen tissue

See Table 7 for a description of the primers used.
ADC, adenocarcinoma; CIN, cervical intraepithelial neoplasia; ISH, in-situ hybridization; RFLP, restricted fragment length polymorphism; SCC, squamous-cell carcinoma
* Three additional cases with HPV-31/33/35
[a] ';' denotes independent methods whereas 'and' denotes subsequent steps.

At multiple locations in the respiratory tract, including the bronchial spurs, squamo-columnar junctions are found, which are types of tissue that may be particularly prone to HPV-associated tumorigenesis (Syrjänen, 2002). Juvenile-onset recurrent respiratory papillomatosis, an HPV-associated lesion that predominantly contains HPV 6 and 11, may spread to the trachea and bronchi, and solitary squamous-cell papillomas of the bronchi have been reported (Syrjänen, 2002). Some of these papillomas have been found to contain HPV, mostly types 6 and 11 (Flieder *et al.*, 1998; Syrjänen, 2002). Although HPV 6 and 11 are thought to cause primarily benign lesions in anogenital epithelium, a recent study detected HPV 11 DNA in three lung cancers in patients with juvenile-onset respiratory papillomatosis; HPV 6, 16 or 18 were not detected. In one tumour specimen for which adequate material was available for testing, the HPV 11 genome was found to be integrated into the host genome (Reidy *et al.*, 2004). Several other studies of lung cancer in patients with respiratory papillomatosis also detected HPV 11 in tumour specimens (Byrne *et al.*, 1987; Guillou *et al.*, 1991; Rady *et al.*, 1998; Cook *et al.*, 2000). In one case, HPV 6 was found (DiLorenzo *et al.*, 1992). Together, these data suggest that, within the setting of juvenile-onset recurrent respiratory papillomatosis, HPV 11 and, to a lesser extent HPV 6, may on rare occasions (estimated to occur in less than 1% of patients) be associated with the development of lung cancer (Cook *et al.*, 2000; Reidy *et al.*, 2004).

An increased risk for lung cancer has been reported in women who have been diagnosed with anogenital cancer or CIN3 (Frisch & Melbye, 1995). It was hypothesized that the relationship was due to a mutual association of lung cancer and anogenital tumours with tobacco smoking, but a possible connection with HPV infection has also been suggested (Hennig *et al.*, 1999a). There is at least one well-documented case of an HPV 16-positive anaplastic lung carcinoma in a woman who had had a cervical cancer 9 years previously, although the possibility of a late metastasis could not be fully excluded (Stremlau *et al.*, 1985). Furthermore, in 75 women with bronchopulmonary cancer following a diagnosis of CIN3 (with no radiotherapy), 37 (49%) were shown to be HPV-positive by PCR with GP5+/GP6+ primers (18 for HPV 16, 12 for HPV 6, five for HPV 16/6, one for HPV 16/11 and one for HPV 16/18), and the overall concordance between the HPV types in the lung tumours and in CIN3 specimens was greater than 60% (Hennig *et al.*, 1999a). [It was not stated whether this was more than a chance finding, in view of the high prevalence of HPV 16 in lung tumours and the predominance of HPV 16 in CIN3.] Of 22 cases of bronchopulmonary cancers who did not have a history of CIN (controls), three were HPV 6-positive and none contained HPV 16, 11 or 18. In contrast, no HPV-positive bronchopulmonary carcinomas were detected when tested by in-situ hybridization.

In two studies summarized earlier in this section (Chiou *et al.*, 2003; Cheng *et al.*, 2004), it was speculated that haematogenous spread of HPV 16 and 18 from the cervix may explain some HPV-associated cancer in the lung. In this connection, another study reported the detection of HPV DNA sequences in 52% of peripheral blood mononuclear cells from patients with genital HPV infections, but not in the 19 control subjects with no HPV infection (Pao *et al.*, 1991).

In summary, with the exception of the studies in Taiwan, China, there is a paucity of data in non-cancer lung specimens, which greatly limits the interpretation of the large number of studies that have been reported to date. In those studies that did test non-cancer lung specimens, the high prevalence of HPV DNA reported was unexpected. [Simultaneous testing of normal human tissues, for which there is broad agreement that the prevalence of HPV is very low (in addition to cancer and non-cancer lung specimens), is necessary before the specificity of the assay results reported can be accepted entirely.]

2.6.3 *Cancer of the colon and the rectum*

Cancers of the colon and the rectum are biologically distinct from cancer of the anus. Thus, data that combine anal and rectal cancers should be interpreted with caution and the term anorectal cancers should be avoided. Whereas anal cancer has a strong association with HPV (see Section 2.3), the relationship between HPV and cancers of the colon and rectum has not been established.

Most studies that analysed a possible role of HPV in cancer of the colon were carried out in the early 1990s. A study performed in former Czechoslovakia that tested 13 adenocarcinomas and 10 adenomas of the colon for HPV 2, 6, 16 and 18 by southern blot hybridization failed to find any evidence of HPV DNA (Boguszakova *et al.*, 1988). Similarly, Gilbert *et al.* (1991) failed to find HPV 16 DNA in eight adenocarcinomas of the anus, rectum or sigmoid colon in patients with Crohn disease. Shroyer *et al.* (1992) analysed 22 colon adenocarcinomas by PCR for HPV 6, 11, 16, 18 and 33 and by in-situ hybridization for HPV types 6/11, 16/18 and 31/33/35. None of the colon cancers revealed a positive reaction. Similar results were reported by Shah *et al*. (1992) who found no HPV DNA in 19 primary tumours of the colon using PCR with the MY09/MY11 consensus primers and southern blot hybridization with a generic probe for HPV 16/18. A more recent study (Audeau *et al.*, 2002) also reported negative results after testing 20 colorectal cancers by immunohistochemistry staining with a monoclonal antibody that reacts with HPV 6, 11, 16 and 18.

The number of positive reports is small and some were based mainly on immunohistochemistry. Kirgan *et al.* (1990) detected the presence of HPV antigen in 29/30 (97%) invasive carcinomas, in 18 of 30 adenomas and in seven of 30 biopsies (23%) of normal mucosa of the colon. Two years later, it was reported that 13 of 38 (32%) carcinomas, 8/21 (38%) adenomas and two of 24 (8%) normal biopsies of the colon contained the HPV L1 region as demonstrated by PCR amplification (McGregor *et al*., 1993). A study from Taiwan, China, reported the presence of both HPV 16 and HPV 18 DNA in three cell lines derived from colorectal adenocarcinomas (Cheng *et al*., 1991); subsequently, it was found that NIH3T3 cells transformed with colonic cancer cells contained HPV 16 DNA (Cheng *et al*., 1993). A study from Turkey reported HPV 18 and 33 infections in 39 and 30, respectively, of 51 colon cancers using PCR and direct sequencing (Sayhan *et al*., 2001).

In a retrospective case–control study of 55 cases of colorectal cancer, the same number of tissues adjacent to the tumour and 10 control specimens were tested by nested

PCR and in-situ PCR for the presence of HPV DNA (Bodaghi *et al.*, 2005). In this series, 23 of 55 (42%) samples of colorectal cancer tissue and 15 of 52 (29%) samples of tissues adjacent to the tumour were positive for HPV DNA; 31 had HPV 16, five were positive for HPV 18 and two contained HPV 45 DNA. Ten samples contained HPV DNA in both the tumour and adjacent tissues and five contained HPV DNA only in the tissues adjacent to the tumour. None of the control tissues was HPV-positive. The findings were confirmed by in-situ hybridization, although the HPV DNA copy number was generally low.

In view of the limitations of the techniques used in most of the studies and in the absence of larger studies that include more case–control analyses, the observed positive reports require a cautious interpretation.

2.6.4 *Cancer of the breast*

Data on HPV in malignant tumours of the breast are controversial. In a series of 15 breast cancers analysed by low-stringency filter hybridization, Ostrow *et al.* (1987) failed to find evidence of HPV DNA. In studies that used HPV 16- and 18-specific primers, Wrede *et al.* (1992) and Gopalkrishna *et al.* (1996) also failed to find HPV DNA in 80 and 30 breast cancer biopsies, respectively. Similarly, Bratthauer *et al.* (1992) were unable to detect HPV 6, 11, 16 or 18 in 15 intraductal papillomas, 15 papillary carcinomas and 13 infiltrating ductal carcinomas of the breast. The analysis of paraffin sections from 20 nipples with Paget's disease (10 central intraductal and 10 invasive carcinomas) by PCR with MY09/MY11 consensus primers and by dot blot hybridization for HPV 6/11, 16/18 or 31/33/35 provided no evidence of HPV DNA (Czerwenka *et al.*, 1996).

These negative findings contrasted with several positive reports: Di Lonardo *et al.* (1992) detected HPV 16 DNA sequences by PCR and southern blot hybridization using specific primers in ten of 40 breast carcinomas and some axillary lymph node metastases. HPV 11 and 18 were not detected in any sample. More recently, Hennig *et al.* (1999b) examined 41 breast carcinomas from 38 patients with a history of CIN3 by PCR with specific primers and detected HPV 16 DNA in 19 cases (46%). Only one of these tumours was also positive by in-situ hybridization. A study conducted in China reported the presence of HPV 33 DNA in 14/32 cases of invasive ductal carcinomas of the breast, detected by PCR and Southern blot hybridization (Yu *et al.*, 2000). Liu *et al.* (2001) examined 17 breast cancer samples using broad spectrum PCR, cloning and sequencing and by Southern blot hybridization for HPV 16, 18 and 31. Six (35%) of the biopsies were positive for HPV types 16, 18 and 31, and viral DNA was largely episomal. Another report from China found 19 of 28 (68%) breast carcinoma samples to be HPV-positive (Li, T. *et al.*, 2002).

In a study that compared cancer tissue with normal biopsies, Damin *et al.* (2004) analysed 20 specimens of reduction mammoplasty, 21 fibroadenomas and 101 breast carcinomas using specific primer sets that target the E6 region of HPV 16 or 18. Twenty-five (24.7%) of the carcinomas, but none of the other biopsies, were found to be HPV 16- (15 cases) or HPV 18- (11 cases) positive. One specimen contained sequences of both virus types. Recently, de Villiers *et al.* (2005) cloned several HPV types from 25 of 29 (86%)

breast carcinomas and 20 of 29 (69%) corresponding samples of the mamilla from the same patient. Many ductal areas of the mamillae revealed condyloma-like histological patterns. The most prevalent HPV type in carcinomas and nipples was HPV 11, followed by HPV 6. A number of additional types were found, including those commonly detected in mucosal and cutaneous lesions, such as HPV 16, 23, 27 and 57 (nipples and carcinomas), 20, 21, 32, 37, 38, 66 and GA3-1 (nipples only) and 3, 15, 24, 87 and DL473 (carcinomas only). [It is plausible that surface areas may be infected frequently by a variety of different HPV types (see Section 2.5); thus the significance of these findings is currently difficult to assess.]

2.6.5 *Cancer of the ovary*

HPV was not detected in eight case series of cancer of the ovary from North America and Europe (de Villiers *et al.*, 1986b; Ostrow *et al.*, 1987; Leake *et al.*, 1989; McLellan *et al.*, 1990; Beckmann *et al.*, 1991; Trottier *et al.*, 1995; Anttila *et al.*, 1999; Chen *et al.*, 1999). Initial results that showed the presence of HPV 6 in 10/12 samples of epithelial ovarian carcinomas using in-situ hybridization (Kaufman *et al*., 1987) could not be reproduced in later analyses by either in-situ or southern blot hybridization or by PCR of the same and additional ovarian carcinoma specimens (Kaufman *et al*., 1990).

In two studies of ovarian cancer from Taiwan, China, HPV 16 DNA was found in 50 and 8% and HPV 18 DNA in 17 and 2% of 18 and 60 cases, respectively, using PCR (Lai *et al.*, 1994; Ip *et al.*, 2002). Quantitative real-time PCR of the samples from the latter study revealed a prevalence of HPV 16 in 18 of 56 cases and between less than one and four HPV 16 DNA copies per cellular genome (Yang, H.J. *et al*., 2003). Only one ovarian cancer contained several copies per genome.

One study from China compared the prevalence of HPV 16 detected by in-situ hybridization with an E6-specific probe in 50 ovarian epithelial cancers and in 30 non-malignant ovarian tissues, most of which had been removed for uterine pathology (Wu, Q.-J. *et al.*, 2003). Twenty-six (52%) of the cancers were positive compared with two (7%) of the controls (odds ratio, 16.7; 95% CI, 3.2–71.4).

In rare cases, squamous-cell carcinoma of the ovary may originate from cervical squamous-cell carcinoma *in situ*. Pins *et al.* (1997) described a case of CIN3 with contiguous upward spread to the endometrium, fallopian tubes and ovaries, focal invasion and HPV 16 DNA in all tumours detected by PCR. Upward spread of HPV 16-positive CIN may also explain the HPV 16-positivity of primary squamous-cell carcinoma of the ovary in two further cases (Mai *et al.*, 1996; Manolitsas *et al.*, 1998).

2.6.6 *Cancer of the prostate*

A possible association of prostatic cancer with sexual behaviour and exposure to sexually transmitted infection has been reported (Hayes *et al.*, 2000; Rosenblatt *et al.*, 2001; Strickler & Goedert, 2001). Furthermore, men with anal cancer, a disease that has

been associated with HPV, have an increased risk for developing subsequent prostatic cancer (Rabkin *et al.*, 1992).

Table 49 presents case series of cancer of the prostate and benign prostatic hypertrophy in association with HPV prevalence. A few studies found an association of HPV with prostatic cancer (McNicol & Dodd, 1990a; Anwar *et al.*, 1992a; Serth *et al.*, 1999; Carozzi *et al.*, 2004). In three of these studies, specimens of non-cancerous prostate were also found to have a substantial, although lower, prevalence or copy number of HPV DNA (McNicol & Dodd, 1990a; Serth *et al.*, 1999; Carozzi *et al.*, 2004). Other studies reported that HPV DNA was equally prevalent in cancers, benign prostatic hypertrophy and normal prostatic tissue (McNicol & Dodd, 1990b, 1991; Ibrahim *et al.*, 1992; Dodd *et al.*, 1993; Wideroff *et al.*, 1996b). The majority of studies did not detect HPV in prostatic cancer specimens (Masood *et al.*, 1991; Effert *et al.*, 1992; Serfling *et al.*, 1992; Anderson *et al.*, 1997; Gherdovich *et al.*, 1997; Noda *et al.*, 1998; Strickler *et al.*, 1998a; Saad *et al.*, 1999). A few studies have reported the detection of HPV DNA in specimens of benign hypertrophic prostate tissue and prostate cancer using non-amplification methods that are less prone to false-positive test results than PCR (McNicol & Dodd, 1990a).

Seroepidemiological studies of exposure to HPV have similarly reported conflicting findings. Four cross-sectional studies failed to detect a relationship between the presence of HPV antibodies and prostatic cancer (Strickler *et al.*, 1998a,b; Hayes *et al.*, 2000; Rosenblatt *et al.*, 2003), and another cross-sectional study found a borderline association of prostatic cancer with antibodies to HPV 33 (odds ratio, 1.6; 95% CI, 1.0–2.7) but not to HPV 16 or 18 (Adami *et al.*, 2003). In contrast, two nested case–control studies reported odds ratios for the association between HPV 16 antibodies and prostatic cancer greater than 2.5 (Dillner *et al.*, 1998; Hisada *et al.*, 2000). These latter findings have been interpreted by some authors as evidence that prospective HPV serological data reflect the strong association of HPV antibodies with sexual behaviour and the relationship of sexual behaviour with the risk for prostatic cancer. Such an association is not found in cross-sectional studies because, by the time prostatic cancer occurs, men have aged sufficiently that some have lost detectable levels of HPV antibody (Strickler & Goedert, 2001).

Overall, the failure of most studies that used sensitive PCR methods to detect HPV in prostatic cancer or, when detected, to find similar or higher prevalence of HPV DNA in non-cancer than in cancer tissues does not support a role of HPV in prostate carcinogenesis.

2.6.7 *Cancer of the urinary bladder and urethra*

The majority of bladder cancers that occur in the developed world are transitional-cell carcinomas (approximately 90%), and the proportion of squamous-cell carcinomas ranges from 3 to 10%. In contrast, in countries where schistosomes are endemic, the majority of bladder cancers are squamous-cell cancers (60–80%) (Cooper *et al.*, 1997).

The prevalence of HPV DNA in case series of cancers of the urinary bladder is summarized in Table 50. [For these tumours, contamination from the lower genital tract during acquisition of tissues is a particular concern.] In studies of transitional-cell carcinomas, or

Table 49. Prevalence of HPV DNA in case series of prostate cancer and benign prostatic hypertrophy

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)			Comment
					6	16	18	
McNicol & Dodd (1990a), Canada	Southern blot for 16/18	4 12	Cancer Benign hypertrophy	75.0 33.3				Mostly TURP, also SPP; frozen tissue
McNicol & Dodd (1990b), Canada	PCR for E6 HPV 16, 18	4 15 5	Cancer Benign hypertrophy Normal autopsies	100 93.3 20.0		100 93.3 20.0	 20.0	Mostly TURP, also SPP and autopsy; frozen samples
Masood *et al.* (1991), USA	ISH for 6/11, 16, 18/31, 33/35	20 20	Cancer Benign hypertrophy	0 0				Biopsies and TURP; paraffin-embedded tissue
McNicol & Dodd (1991), Canada	PCR for E6 HPV 16, 18	27 56	Cancer Benign hypertrophy	51.9 62.5		51.9 60.7	3.7 5.4	Mostly TURP, also SPP; frozen samples
Anwar *et al.* (1992a), Japan	PCR for E6 HPV 16, 18, 33	68 10 10	Cancer Benign hypertrophy Normal autopsies	41.2 0 0		16.2	25.0	TURP, SPP and autopsy; paraffin-embedded tissue
Effert *et al.* (1992), USA	'Differential' PCR for 16, 18 and southern blot	30	Cancer	0				Collection not specified; frozen tissue
Ibrahim *et al.* (1992), USA	PCR and dot-blot for 6, 11, 16, 18, 31, 33, 35, 39, 45	40 12 17	Cancer Benign hypertrophy Normal tissue	15.0 0 11.8		15.0 11.8		Biopsies/TURP/SPP; paraffin-embedded and frozen samples
Rotola *et al.* (1992), Italy	PCR for E6 HPV 6/11, 16	8 17	Cancer Benign hypertrophy	NS [> 75] NS [≥ 82]	50.0[b] 64.7[b]	75.0 82.3		Collection not specified; frozen samples
Serfling *et al.* (1992), USA	PCR with L1 consensus primer and Southern blot	30	Cancer	0				Collection not specified; frozen samples
Dodd *et al.* (1993)[c], Canada	RT-PCR for E6/E7 mRNA of HPV 16	7 10	Cancer Benign hypertrophy	42.9 50.0		42.9 50.0		Collection not specified; frozen samples

Table 49 (contd)

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)			Comment
					6	16	18	
Sarkar *et al.* (1993), USA	PCR for E6/E7 of 6/11/16/18; Southern blot for 16	23	Cancer and intraepithelial neoplasia	13.0[d]		13.0		Surgical, not TURP; paraffin-embedded tissue
Tu *et al.* (1994), USA	PCR with L1 consensus primer and Southern blot for 16, 18	43 17 1	Cancer Metastases in lymph nodes Normal tissue	2.3 5.9 0		2.3	 5.9	Surgical not TURP; tumours, paraffin embedded; metastases, frozen samples
Moyret-Lalle *et al.* (1995), France	PCR for E6 of 16, 18 and hybridization	17 22	Carcinoma Adenoma	52.9 31.8		52.9 31.8	0 0	Collection not specified; frozen samples
Suzuki *et al.* (1996), Japan	PCR with L1 consensus primer and RFLP	51	Cancer	15.7		15.7		Surgery or autopsy; frozen tissue
Wideroff *et al.* (1996b), USA	PCR with L1 and E6 consensus primers and dot blot for 6, 11, 16, 18, 31, 33, 45	56 42	Cancer Benign hypertrophy	L1, 12.5 L1, 9.5	0 0	0 0	0 0	TURP, surgery and biopsy; paraffin-embedded tissue
Anderson *et al.* (1997), United Kingdom	PCR with E2- and E6-specific primers for 16, and E1 consensus primer	14 10	Cancer Benign hypertrophy	0 0				TURP; frozen tissue
Gherdovich *et al.* (1997), Italy	PCR with MY09/11; nested PCR	5 60	Cancer Benign hypertrophy	0 0				Surgery; frozen tissue

Table 49 (contd)

Reference, study location	Method of detection and types tested	No. of cases	Type of lesion	Overall HPV positivity (%)	Type-specific HPV positivity (%)			Comment
					6	16	18	
Terris & Peehl (1997), USA	PCR for E6 HPV 16 (2 probes) and for L1 HPV 6/11/16/18/33	53	Cancer	$E6_a$, 3.8 $E6_b$, 18.9 L1, 0		3.8 18.9		Radical retropubic resection of the prostate; paraffin-embedded tissues from 41 patients
		21	Benign hypertrophy	$E6_a$, 9.5 $E6_b$, 33.3 L1, 0		9.5 33.3		
		37	Normal tissue	$E6_a$, 2.7 $E6_b$, 13.5 L1, 0		2.7 13.5		
Noda *et al.* (1998), Japan	Nested PCR with consensus primer and RFLP for 16, 18, 31, 33, 35, 52, 58	38 71	Cancer Benign hypertrophy	0 4.2		 4.2		Surgical and TURP; paraffin-embedded tissue
Strickler *et al.* (1998a), Italy and USA	PCR with MY09/11 and GP5+/6+	63 61	Cancer Benign hypertrophy	0 0				Mostly TURP, also SPP; frozen samples
Saad *et al.* (1999), Canada	PCR with MY09/11 and southern blot	40	Cancer	0				Collection not specified; fresh samples
Carozzi *et al.* (2004), Italy	PCR with consensus primer and primer for 16/18/31/33/35/45/52/58, and typing for 6, 11, 16, 18, 31, 33, 35, 45, 52, 58	26 25	Cancer Benign hypertrophy	65.4 48.0				Transperineal biopsy fixed in 10% formalin

See Table 7 for a description of the primers used.
ISH, in-situ hybridization; NS, not specified; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RT, reverse transcriptase; SPP, suprapubic resection of the prostate; TURP, transurethral resection of the prostate
[a] ';' denotes independent methods whereas 'and' denotes subsequent steps.
[b] 6 or 11
[c] All samples also reported in study by McNicol & Dodd (1991)
[d] Samples positive only after Southern blot analysis
[e] HPV positivity determined in the preservation fluid after 1–2 h of storage of the biopsies

Table 50. Prevalence of HPV DNA in case series of cancer of the urinary bladder

Reference, study location, year(s) of study	Method[a] of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)				Other types (n)	Multiple infections (%)	Tissue collection; storage
				6	11	16	18			
Bryant *et al.* (1991), United Kingdom, NR	ISH for 6/11, 16/18	76 TCC 3 SCC	15.8 0			15.8				Transurethral resection; paraffin-embedded specimens
Kerley *et al.* (1991), USA, NR	PCR for 6, 11, 16, 18 and restriction digest	18 TCC 4 SCC 5 controls	0 25.0 0		25.0					Paraffin-embedded specimens
Anwar *et al.* (1992b), Japan, NR	PCR for 6, 11, 16, 18, 33 and dot-blot	46 TCC 2 SCC 21 controls	82.6 50.0 33.3		47.8[b] 19.0	28.3 50.0[c] 9.5	38	33 (14) 33 (2)	60 5	Paraffin-embedded archival specimens; type-specific positivity combines TCC and SCC.
Knowles (1992), United Kingdom, NR	PCR with GP5/6 and Southern blot	100 TCC	0							Cystoscopic biopsies; frozen samples
Furihata *et al.* (1993), Japan, 1981–92	ISH for 16, 18, 33	90 TCC	31.1			21.1	18.9	33 (16)	19	Cystectomy; paraffin-embedded specimens
Chang *et al.* (1994), Finland, 1966–87	PCR with MY09/11	108 TCC	0							Paraffin-embedded archival specimens
Maloney *et al.* (1994), USA, 1979–92	PCR with GP5/6 and type-specific for 16, 18	20 TCC 22 SCC	0 4.5				4.5			Cystectomy; paraffin-embedded specimens
Kamel *et al.* (1995), Finland, 1987–92	ISH for 6, 11, 16, 18, 31, 33	40 TCC 7 SCC	60.0 42.9	30.0 14.3	25.0	20.0 28.6	40.0	31 (16); 33 (12) 31 (3); 33 (1)	45 29	Paraffin-embedded archival specimens

Table 50 (contd)

Reference, study location, year(s) of study	Method[a] of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)				Other types (n)	Multiple infections (%)	Tissue collection; storage
				6	11	16	18			
LaRue *et al.* (1995), Canada, NR	PCR and southern blot with L1 consensus primer and probe; typing for 6, 11, 16, 18, 33 by dot blot and sequencing	71 TCC 8 controls	39.4 0		1.4	38.0				Transurethral resection or cystectomy; frozen specimens
Lopez-Beltran & Muñoz (1995), Spain, NR	PCR for 6, 11, 16, 18; ISH for 6/11, 16/18, 31/33/35	76 TCC	PCR, 9.2 ISH, 5.3	1.3		9.2 5.3			1.3	Transurethral resection; paraffin-embedded specimens
Sano *et al.* (1995), Japan, 1989–93	PCR with pU-31B/2R, pU-1M/2R, L1C1/L1C2 and type-specific for 16, 18	80 TCC 11 SCC	0 0							Paraffin-embedded archival specimens
Smetana *et al.* (1995), Israel, 1986–90	ISH for 6/11, 16/18, 31/33/35; PCR with E1 consensus primer and southern blot for 6/11, 16/18	110 TCC 41 controls	25.5 4.9		16.4 2.4	9.1	2.4			Paraffin-embedded specimens and control biopsies
Boucher *et al.* (1996), United Kingdom, NR	Southern blot for 6/11, 16	54 TCC, 1 SCC	0							Radical cystectomy or transurethral resection; formalin-fixed specimens?
Mvula *et al.* (1996), Japan, NR	PCR with L1 consensus primer and type-specific for 16, 18	34 TCC 2 SCC	8.8 0			2.9				Paraffin-embedded archival specimens
Tenti *et al.* (1996), Italy, NR	PCR with MY09/11 and type-specific primers and southern blot for 6/11, 16, 18, 33	79 TCC	32.9			29.1	12.7		8.9	Transurhetral resection or cystectomy; paraffin-embedded specimens

Table 50 (contd)

Reference, study location, year(s) of study	Method[a] of detection and types tested	No. and type of lesions	Overall HPV positivity (%)	Type-specific HPV positivity (%)				Other types (n)	Multiple infections (%)	Tissue collection; storage
				6	11	16	18			
Cooper *et al.* (1997), South Africa, NR	ISH for 6, 11, 16, 18, 31, 33; PCR with 5 E6 consensus primers	25 SCC	0							Paraffin-embedded archival specimens from patients infested with *Schistosoma haematobium*
Lu *et al.* (1997), United Kingdom, 1987–94	ISH for 16, 18	22 TCC 5 SCC	0 0							Paraffin-embedded archival specimens
Aynaud *et al.* (1998), France, NR	PCR with MY09/11 and type-specific for 6, 11, 16, 18, 33	58 TCC	0							Transurethral excision; frozen specimens
Simoneau *et al.* (1999), Canada, 1990–92	PCR with MY09/11 and dot-blot for 6, 11, 16, 18, 33; PCR for 16	187 TCC	9	2.1	0.5	4.8	2.1		1.1	Transurethral resection; frozen specimens
Sur *et al.* (2001), South Africa, 1994–96	PCR with GP5+/6+	64 TCC	1.6							Paraffin-embedded archival specimens
Westenend *et al.* (2001), Netherlands, NR	ISH for 6/11, 16/18 31/33/51	16 SCC	0							Biopsy or cystectomy; paraffin-embedded archival specimens

See Table 7 for a description of the primers used.
ISH, in-situ hybridization; NR, not reported; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma; TCC, transitional-cell carcinoma
[a] ';' denotes independent methods whereas 'and' denotes subsequent steps.
[b] Not clear from article if 22 or 28 HPV 6/11 positive samples (47.8 and 60.9%, respectively)
[c] 16, 18 or 33

in a few studies in which the type of bladder cancer was not specified and that were conducted in countries where schistosomes are not endemic, no HPV DNA was found in a total of 430 bladder cancers using PCR (Kerley *et al.*, 1991; Knowles, 1992; Saltzstein *et al.*, 1993; Sinclair *et al.*, 1993; Chang *et al.*, 1994; Maloney *et al.*, 1994; Sano *et al.*, 1995; Aynaud *et al.*, 1998) or in a total of 91 bladder cancers using in-situ hybridization or Southern blot technique (Ostrow *et al*., 1987; Boucher *et al.*, 1996; Lu *et al*., 1997).

In other PCR-based studies, HPV was detected in 3–80% of the samples of transitional-cell carcinomas: in about 3% in the studies by Chetsanga *et al.* (1992; 1/44), Mvula *et al.* (1996; 1/34), Tekin *et al.* (1999; 2/42), Sur *et al.* (2001; 1/91) and Fioriti *et al.* (2003; 1/32); in 9% in the studies by Lopez-Beltran and Muñoz (1995; 7/76) and Simoneau *et al.* (1999; 16/187); in 20% in the study by Gopalkrishna *et al.* (1995; 2/10); in about 30% in the studies by Smetana *et al.* (1995; 20/59), Tenti *et al.* (1996; 26/79), Chan *et al.* (1997; 6/20) and Gazzaniga *et al.* (1998; 11/35); and in 39%, 50% and 83% in the studies by LaRue *et al.* (1995; 28/71), Aglianò *et al.* (1994; 23/46) and Anwar *et al.* (1992b; 38/46), respectively.

Widely variable prevalences of HPV positivity were also determined using less sensitive methods such as in-situ or southern blot hybridization: 5% (Lopez-Beltran & Muñoz, 1995; 4/76), 16% (Bryant *et al.*, 1991; 12/76), 20% (Shibutani *et al.*, 1992; 4/20), 31% (Furihata *et al.*, 1993; 28/90), 40% (de Gaetani *et al.*, 1999; 17/43) and 60% (Kamel *et al.*, 1995; 24/40).

Only genital (low- and high-risk) HPV types have been assessed in these studies and HPV 16 and 18 were detected most frequently.

Relatively few studies addressed HPV positivity in squamous-cell carcinoma of the bladder using PCR (Kerley *et al*., 1991; Anwar *et al*., 1992b; Maloney *et al.*, 1994; Sano *et al*., 1995; Mvula *et al*., 1996) or in-situ or southern hybridization (Bryant *et al.*, 1991; Kamel *et al.*, 1995; Boucher *et al.*, 1996; Lu *et al*., 1997; Westenend *et al*., 2001) (see Table 50). From these studies, six of 73 (8%) squamous-cell carcinomas were found to be positive for HPV 6, HPV 11 or high-risk HPV. HPV 6 was reported in another squamous-cell carcinoma of the bladder and was identified by southern blot, PCR and in-situ hybridization (Wilczynski *et al*., 1993). No HPV DNA was detectable by PCR in 25 schistosomiasis-associated bladder cancers (Cooper *et al.*, 1997).

In carcinomas of the urethra, HPV 6 or 11 has been detected in two individual cases and in three of a series of four cases (Grussendorf-Conen *et al.*, 1987; Mevorach *et al.*, 1990; Alonso *et al.*, 1997). HPV 16 has been detected in eight of 17 invasive cancers (47%) and in four of six metastases in women with urethral carcinoma (Wiener & Walther, 1994), as well as in four of 14 squamous-cell cancers of the urethra (29%) in men (Wiener *et al.*, 1992). In a study that investigated the location within the male urethra, HPV 16 DNA was found in six squamous-cell carcinomas of the pendulous urethra, but in none of the primary cancers of the bulbous (six) and posterior (two) urethra (Cupp *et al.*, 1996).

2.7 Co-factors of HPV in cervical cancer

It is now clear that HPV infection is causally related to cervical cancer and its precursor lesions (IARC, 1995). However, because of the high prevalence of HPV infection in the general female population and the relatively low rate of cervical cancer, environmental and host genetic factors may influence the progression from infection to cancer (Santos *et al.*, 2001; Castellsagué & Muñoz, 2003; Wang & Hildesheim, 2003). To evaluate the co-factors of HPV, it is important to note the complex and multifactorial origin of cancer. One or more co-factors could substantially contribute to HPV-initiated cervical carcinogenesis depending on its (their) prevalence in the population studied. For example, in female populations whose use of tobacco use is low to non-existent, significant associations between tobacco smoking and disease are unlikely to be detected. Similarly, variations in the prevalence of hormonal contraceptive use in populations by region and country result in the differences in risk estimates for this factor that are observed across countries. These population differences need to be taken into account when interpreting the strength of the association observed between any putative HPV co-factor and cervical cancer.

Evidence for co-factors of HPV derives from two types of study design: prospective cohort studies in which exposure is assessed before the occurrence of disease and case–control studies in which exposure is ascertained after the diagnosis of disease. Moreover, several different analytical approaches may be used in the statistical analyses of these data. Since cervical cancer only develops in women who are infected with HPV, the most appropriate analytical approach is to restrict all statistical analyses to HPV-positive women; that is, women who are at risk for development of the disease. The ideal controls or comparison group would be women who were infected with HPV at the same time as the case women, but who did not develop the disease. Methodologically, this is difficult to achieve; therefore, one approach has been simply to restrict analyses to both cases and controls who were infected with either any HPV type or carcinogenic HPV types. It is important to note that the use of this method of restriction involves a potential for disease misclassification that would bias the risk estimate observed towards the null. Therefore, the studies summarized below may have reported underestimates of the true association between, for example, tobacco smoking and disease.

2.7.1 *Non-infectious co-factors for cervical cancer*

A complete summary of possible co-factors for cervical cancer and its precursor lesions has not been attempted here because many potential co-factors have not been evaluated rigorously in epidemiological studies, and the focus has therefore been placed on tobacco smoking, hormonal contraceptive use, parity and nutritional and genetic factors.

(*a*) *Tobacco smoking*

The role of tobacco smoking in the etiology of cervical cancer has been a topic of debate for many years (Layde, 1989). In an extensive review of the literature through to 1985, Winkelstein (1986) stated that 15 of 18 studies reported a significantly increased risk for cervical cancer in tobacco smokers. In another review of the literature that covered the years 1986–89 (Winkelstein, 1990), 11 of 15 studies reported significant positive associations of different magnitude between tobacco smoking and risk for cervical cancer. Despite this evidence, there have still been doubts as to whether tobacco smoking is truly associated with the risk for cervical cancer. The discrepancy is based on the problem of misclassification with respect to exposure to HPV. Moreover, most studies conducted before 1990 did not measure HPV status or control for sexual activity, which is known to differ according to smoking status. Since 1990, most studies have shown a significant association between tobacco smoking and cervical cancer and its precursor lesions after either adjustment for HPV infection in the analyses or restriction of the analyses to HPV-positive women. Evidence for the association between tobacco smoking and cervical cancer reported here derives from the most rigorously designed studies that restricted statistical analyses to HPV-positive women.

(i) *Case–control studies*

Over the past few decades, numerous case–control studies have been conducted worldwide to quantify the association between tobacco smoking and the risk for cervical cancer (reviewed by Haverkos *et al*., 2003). Muñoz *et al*. (1993) first reported an analysis of the association between tobacco smoking and the risk for CIN3/carcinoma *in situ* that was restricted to 218 HPV-positive women who participated in a case–control study in Colombia and Spain. No significant association was observed, but the study was hampered by low statistical power.

Studies on tobacco smoking and cervical cancer that have been published subsequently are summarized in Table 51.

In a case–control study conducted in Denmark (Kjaer *et al*., 1996) that included 141 prevalent cases, current and former smokers had a twofold increased risk for SIL or ASCUS compared with those who had never smoked. In an update of the same study, the risk appeared to be higher (Kruger-Kjaer *et al*., 1998).

In a comparison of cases of CIN3 and CIN1 in the USA, Ho *et al*. (1998b) observed a significant dose–response in the increase in risk among women who smoked 10 or more cigarettes per day and had a smoking history of more than five pack–years compared with those who had never smoked or former smokers. No association with CIN2 was observed.

Among HPV 16-positive women, Olsen *et al*. (1998a) observed an increase in risk of 4.6 for CIN2/3 among those who had ever smoked tobacco in a population-based case–control study conducted in Norway.

Ylitalo *et al*. (1999) conducted a case–control study among women who were selected from those registered in the Uppsala county cervical cytology programme in Sweden. Cases and controls were matched by HPV status and selected on the basis of cytology

Table 51. Case–control studies of tobacco smoking and pre-invasive and invasive cervical cancer restricted to HPV-positive women

Reference, study location	No. and type of cases	No. and type of controls	Smoking status[a]	Odds ratio (95% CI)	Intensity/duration of smoking	Odds ratio (95% CI)	Detection method and comments
Kjaer *et al.* (1996), Denmark	141 ASCUS and SIL combined	153 normal cytology	Former Current	2.3 (0.8–6.6) 1.9 (1.2–3.2)	–	–	PCR GP5+/6+; adjusted for age
Ho *et al.* (1998b), USA	44 CIN3	163 CIN1	Former Current	1.8 (0.64–5.22) 2.1 (1.09–5.15)	≤ 10 cigarettes/day* > 10 cigarettes/day* ≤ 5 pack–years* > 5 pack–years*	1.49 (0.61–3.67) 3.35 (1.22–9.15) *p* trend = 0.018 1.75 (0.71–4.31) 2.66 (1.15–6.15) *p*-trend = 0.019	PCR and southern blot; no association with CIN2 (n = 52); adjusted for age, education, ethnicity, no. of Pap smears in past 3 years, high-risk versus low-risk HPV infection; *reference category is never or former smokers.
Kruger-Kjaer *et al.* (1998), Denmark	82 ASCUS 86 LSIL 71 HSIL	155 normal cytology	ASCUS LSIL HSIL *Current versus former* ASCUS LSIL HSIL	4.2 (1.4–12.6) 2.5 (0.8–8.0) 3.2 (0.9–11.4) 1.9 (1.0–3.4) 1.5 (0.8–2.7) 1.9 (1.0–3.8)	–	–	PCR GP5+/6+; adjusted for age, years of sex life without barrier contraceptive, partner's education, marital status [follow-up of Kjaer *et al.* (1996)]
Olsen *et al.* (1998a), Norway	60 CIN2/3, histologically confirmed	14 with no dysplasia	Ever versus never smoker	4.6 (0.9–22.9)	Former smoker 1–10 cigarettes/day > 10 cigarettes/day	4.2 (0.5–37.9) 3.3 (0.5–20.8) 5.9 (1.0–35.6)	PCR; population-based controls; adjusted for age
Ylitalo *et al.* (1999), Sweden	178 CIS HPV 16/18-positive	178 HPV16/18-positive; no history of in-situ or ICC and no hysterectomy	Former Current	2.1 (1.0–4.3) 2.3 (1.3–4.3)	1–9 years 10–19 years ≥ 20 years 0.15–3.95 pack–years 4.00–7.95 pack–years ≥ 8.00 pack–years	2.3 (1.1–5.2) 2.5 (1.3–4.7) 1.8 (0.8–4.1) *p* trend = 0.62 2.3 (1.1–4.8) 2.4 (1.6–7.3) 1.6 (0.8–3.2) *p*-trend = 0.17	PCR; adjusted for marital status, OC use, age at sexual debut, no. of sexual partners, age at menarche, parity, years in school
Kjellberg *et al.* (2000), Sweden	122 CIN2/3 histologically confirmed	346 cytologically normal	Never and party smokers Former Current	1.0 2.3 (1.0–5.6) 2.6 (1.2–5.6)	Never and party smokers 1–4 cigarettes/day 5–14 cigarettes/day ≥ 15 cigarettes/day	1.0 0.5 (0.1–1.9) 3.2 (1.2–8.4) 5.8 (1.7–19.4) *p*-trend < 0.001	PCR; adjusted for HPV DNA, age

Table 51 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Smoking status[a]	Odds ratio (95% CI)	Intensity/duration of smoking	Odds ratio (95% CI)	Detection method and comments
Hildesheim *et al.* (2001), Costa Rica	136 HSIL/cancer high-risk HPV-infected, histologically confirmed	624 high-risk HPV	Former Current	2.4 (1.2–5.1) 2.3 (1.3–4.3)	1–5 cigarettes/day ≥ 6 cigarettes/day < 10 years ≥ 10 years	2.3 (1.3–3.9) 2.7 (1.1–6.7) 2.6 (1.2–5.3) 2.2 (1.2–4.2) *p*-trend = 0.003	HC2 + PCR; no association with passive exposure; adjusted for age, no. of pregnancies, HPV types
Lacey *et al.* (2001), USA	58 ADC	49 healthy community members	Ever Former Current	0.7 (0.3–1.5) 1.0 (0.4–2.5) 0.5 (0.2–1.1)	< 1 pack/day ≥ 1 pack/day ≤ 10 years 11–20 years > 20 years	1.2 (0.5–2.9) 0.4 (0.2–1.0) *p*-trend = 0.10 0.9 (0.4–4.0) 1.3 (0.4–2.1) 0.4 (0.1–4.0) *p*-trend = 0.25	PCR MY09/11; adjusted for age, ethnicity
	70 SCC		Ever Former Current	1.5 (0.7–3.0) 1.2 (0.5–3.1) 1.6 (0.7–3.5)	< 1 pack/day ≥ 1 pack/day ≤ 10 years 11–20 years > 20 years	1.6 (0.6–3.9) 1.3 (0.6–3.0) *p*-trend = 0.49 1.2 (0.5–3.0) 3.2 (1.0–9.7) 0.8 (0.3–2.7) *p*-trend = 0.57	
Plummer *et al.* (2003), 4 continents	1463 squamous ICC 211 CIS	254 hospital- and population-based	Ever Former Current	2.08 (1.33–3.27) 1.80 (0.95–3.44) 2.30 (1.31–4.04)	≤ 5 cigarettes/day > 6 cigarettes/day < 20 years ≥ 20 years	1.89 (1.05–3.41) 2.23 (1.18–4.20) 2.36 (1.30–4.29) 1.85 (0.97–3.51)	PCR MY09/11; pooled analysis of 10 case–control studies; adjusted for age, centre, education, no. of sexual partners, age at first intercourse, OC use, parity, screening
Giuliano *et al.* (2004), Mexico border	35 ASCUS/AGUS 25 LSIL 19 HSIL cytology-based diagnosis	201 normal cytology	*ASCUS/AGUS* Former Current *LSIL* Former Current *HSIL* Former Current	 1.57 (0.52–4.76) 0.75 (0.29–1.92) 0.44 (0.05–3.78) 2.19 (0.82–5.85) 0.43 (0.05–3.63) 0.61 (0.16–2.30)	–	–	PCR MY09/11; adjusted for country, parity, *C. trachomatis* infection, Pap smear in the past 3 years, age

Table 51 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Smoking status[a]	Odds ratio (95% CI)	Intensity/duration of smoking	Odds ratio (95% CI)	Detection method and comments
Harris *et al.* (2004), USA	High-risk HPV-positive 137 CIN 1 143 CIN 2/3 histologically confirmed	181 ≤ ASCUS and high-risk HPV-positive	*CIN1* Former Current *CIN2/3* Former Current	 1.7 (0.8–3.6) 1.8 (1.1–3.1) 2.0 (0.9–4.1) 1.6 (1.0–2.7)	 1–10 cigarettes/day > 10 cigarettes/day 0.1–5 pack–years > 5 pack–years 1–10 cigarettes/day > 10 cigarettes/day 0.1–5 pack–years > 5 pack–years	 1.4 (0.9–2.5) 2.5 (1.2–5.3) 1.7 (1.0–2.8) 2.1 (1.0–4.5) 1.4 (0.8–2.4) 2.6 (1.3–5.5) 1.4 (0.8–2.4) 2.6 (1.3–5.2)	PCR MY09/11; adjusted for age, no. of HPV types

ADC, adenocarcinoma; AGUS, atypical glandular cells of undetermined significance; ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; HC2, Hybrid Capture 2; HSIL, high-grade intraepithelial lesion; ICC, invasive cervical cancer; LSIL, low-grade squamous intraepithelial lesion; OC, oral contraceptive; Pap, Papanicolaou test; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma; SIL, squamous intraepithelial cells
The reference category is never smokers, if not otherwise specified.

from the most recent Pap smear. A significant approximately twofold increase in risk for carcinoma *in situ* was observed for both former and current smokers compared with those who had never smoked.

Among women who participated in a study in Västerbotten County, Sweden, ever having used tobacco was associated with a strong dose–response in the increase in risk for CIN2/3 (Kjellberg *et al*., 2000).

Among women with high-risk HPV infection, Hildesheim *et al*. (2001) observed a significant approximately twofold increase in risk for HSIL and cervical cancer among former and current smokers.

In a multicentric case–control study among women in the USA, Lacey *et al*. (2001) did not observe a significant increase in risk for adenocarcinoma or squamous-cell carcinoma of the cervix with smoking.

Plummer *et al*. (2003) pooled data from eight IARC case–control studies of invasive cervical cancer and two studies of carcinoma *in situ*, including the study of Muñoz *et al*. (1993). More than 1600 cases were included in the pooled analysis. Ever and current smokers had a twofold higher risk for cervical cancer compared with those who had never smoked. Increasing intensity and duration of smoking did not confer substantial additional risk.

In a study conducted at the USA–Mexico border among Hispanic women, Giuliano *et al*. (2004) did not observe a significant association between tobacco smoking and the risk for LSIL or HSIL.

Harris *et al*. (2004) reported an increase in risk for ≥ CIN1 among women in the USA who were positive for high-risk HPV-type infections and who were current smokers. A dose–response in the increase in risk for ≥ CIN1 was observed for intensity of smoking.

(ii) *Prospective studies*

Several large prospective cohort studies have published risk estimates for the association between tobacco smoking and risk for cervical cancer (Table 52).

Deacon *et al*. (2000) reported a significant increase in risk among participants in a cohort study of 61 570 women in Manchester, United Kingdom. In this nested case–control study, 199 histologically confirmed incident cases of CIN3 were compared with 181 HPV-positive controls (women with normal to CIN2 lesions). In adjusted analyses, ever use of tobacco was associated with a significant more than twofold increase in risk, as was smoking one or more packs of cigarettes per day.

Among participants of the Kaiser Permanente Cohort in Portland, Oregon (USA), Castle *et al*. (2002b) reported a significant increase in risk of at least twofold for incident CIN3 among former smokers and current smokers compared with nonsmokers, regardless of the intensity of smoking and analytical method used to assess the association (Kaplan Meier estimates versus logistic regression) among women infected with high-risk HPV types.

Other prospective studies supported a role for tobacco smoking in the modulation of the natural history of HPV infections. In a prospective study in the USA, Giuliano *et al*.

Table 52. Prospective studies of tobacco use and pre-invasive and invasive cervical cancer restricted to HPV-positive women

Reference, study location	Parent cohort	No. and type of cases	No. and type of controls	Smoking status	Odds ratio (95% CI)	Intensity/ duration of smoking	Odds ratio (95% CI)	Detection method and comments
Deacon *et al.* (2000), United Kingdom	61 570	199 incident CIN3, histologically confirmed	181 normal and < CIN3	Ever Former	2.20 (1.44–3.35) 1.69 (0.76–3.75)	< 1 pack/day ≥ 1 pack/day	1.48 (0.79–2.76) 2.57 (1.49–4.45) *p* trend < 0.001	PCR MY09/11; adjusted for age at first intercourse, total no. of sex partners, years since start of last regular relationship, history of spontaneous abortion
Castle *et al.* (2002b), USA	20 759 women of whom 1812 high-risk HPV-positive	58 incident CIN3 10 incident cancers (high-risk HPV-positive only)	1790 with normal cytology	Former Former	2.1 (1.1–3.9)[a] 3.3 (1.6–6.7)[b]	< 1 pack/day ≥ 1 pack/day < 1 pack/day ≥ 1 pack/day	2.2 (1.2–4.2)[a] 2.9 (1.5–5.6)[a] 2.9 (1.4–6.1)[b] 4.3 (2.0–9.3)[b]	HC2; 10 years follow-up; matched by cytologic interpretation of baseline Pap smears, age, screening behaviour [a] Kaplan Meier analysis [b] Logistic model

See Table 7 for a description of the primers used.
CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; Pap, Papanicolaou test; PCR, polymerase chain reaction

(2002a) observed a longer duration of high-risk HPV infections and a lower probability of clearing these infections among women who had ever smoked. Similarly, in a cohort of HIV-positive and -negative women, Minkoff *et al*. (2004) observed a significant increase in the incidence of persistent HPV infections among current smokers.

(*b*) *Hormonal contraceptive use*

The use of hormonal contraceptives, most commonly combined oral contraceptive formulations of estrogen and progesterone, has been hypothesized to be associated with development of pre-invasive and invasive cervical lesions (Castellsagué & Muñoz, 2003). In addition to the limitations in design of research conducted without taking HPV status appropriately into account, the investigation of hormonal contraceptives relative to the risk for cervical cancer has been limited by confounding with Pap smear history, and formulation and dose of hormonal contraceptives.

(i) *Case–control studies* (Table 53)

Several case–control studies evaluated the risk for pre-invasive and invasive cervical cancer associated with the use of exogenous hormones, either for contraception or control of menopausal symptoms, among HPV-positive women (Kjaer *et al.*, 1996; Kruger-Kjaer *et al.*, 1998; Lacey, J.V. *et al.*, 1999; Ylitalo *et al.*, 1999; Lacey *et al.*, 2000; Hildesheim *et al.*, 2001; Moreno *et al.*, 2002; Berrington *et al.*, 2002; Smith *et al.*, 2003; Giuliano *et al.*, 2004; Shields *et al.*, 2004). Of these, four observed significant associations with invasive lesions (Ylitalo *et al.*, 1999; Berrington *et al.*, 2002; Moreno *et al.*, 2002; Smith *et al.*, 2003) and one with adenocarcinoma *in situ* (Lacey *et al.*, 1999).

In a case–control study in a Danish population reported by Kjaer *et al*. (1996) and updated by Kruger-Kjaer *et al*. (1998), no association with preneoplastic disease was observed for current users of oral contraceptives or current users with a long duration of use. In contrast, Ylitalo *et al*. (1999) reported a significantly increased cervical cancer risk of 2.65 for current oral contraceptive use in Sweden. The risk was significant after 2 years of use. Studies conducted among populations in the USA have failed to detect associations with either pre-invasive or invasive squamous-cell carcinoma despite adjustment for Pap smear screening history (Lacey, J.V. *et al*., 1999; Giuliano *et al*., 2004). However, Lacey, J.V. *et al*. (1999) detected a significant positive association between current oral contraceptive use and adenocarcinoma *in situ*. In the same study population, no association between non-contraceptive hormonal use and either adenocarcinoma or squamous-cell carcinoma of the cervix was detected (Lacey *et al.*, 2000). In a prevalent case–control study conducted by Hildesheim *et al*. (2001) in Costa Rica, a significant increase in risk for HSIL/cancer was observed only among oral contraceptive users with a duration of use of 5 years or more who had had three or fewer pregnancies. In a meta-analysis of studies restricted to HPV-positive women, Smith *et al.* (2003) observed a significantly increased risk (odds ratio, 2.5; 95% CI, 1.6–3.9) for CIN3 and invasive cervical cancer only for long-term oral contraceptive use (≥ 10 years). Shields *et al*. (2004) found no significant association between endogenous

Table 53. Case–control studies of oral contraceptive (OC) use and pre-invasive and invasive cervical cancer restricted to HPV-positive women

Reference, study location	No. and type of cases	No. and type of controls	OC use status	Odds ratio (95% CI)	Intensity/ duration of use	Odds ratio (95% CI)	Detection method and comments
Kjaer *et al.* (1996), Denmark	141 HSIL and SIL combined	153 cytologically normal	Never users ≥ 20 years 17–19 years ≤ 16 years	 0.5 (0.2–1.4) 0.6 (0.3–1.3) 0.8 (0.4–1.7)	–	–	PCR GP5+/6+; adjusted for age
Kruger-Kjaer *et al.* (1998), Denmark	82 ASCUS 86 LSIL 71 HSIL	155 cytologically normal	No association reported	NR	–	–	PCR GP5+/6+; adjusted for age, years of sex life without barrier contraceptive, partner's education, marital status; [update of the study by Kjaer *et al.* (1990)]
Lacey, J.V. (1999), USA	48 squamous CIS 91 squamous ICC 33 ADC *in situ* 91 ADC	48 healthy population-based	*ADC* in situ Ever Former Current	 5.4 (0.7–43.4) 3.1 (0.4–27.5) 17.1 (1.5–188.2)	 ≤ 2 years 2–6 years > 6 years	 4.0 (0.4–44.3) 4.8 (0.4–51.9) 6.2 (0.7–52.7) *p*-trend = 0.12	PCR MY09/11; multicentre study; adjusted for age, ethnicity, income, lifetime no. of sexual partners, no. of Pap smears; current use is defined as use 12 months before diagnosis for cases and at reference date for controls.
			Invasive ADC Ever Former Current	 1.3 (0.4–4.4) 1.3 (0.4–4.1) 2.1 (0.4–11.9)	 ≤ 2 years 2–6 years > 6 years	 1.5 (0.3–6.6) 1.1 (0.2–5.2) 1.0 (0.2–4.2) *p*-trend = 0.88	
			Squamous-cell CIS Ever Former Current	 1.7 (0.5–6.2) 1.8 (0.5–6.7) 1.6 (0.3–8.5)	 ≤ 2 years 2–6 years > 6 years	 1.4 (0.3–7.2) 3.8 (0.7–19.3) 1.1 (0.3–5.0) *p*-trend = 0.85	
			Squamous ICC Ever Former Current	 1.2 (0.4–3.8) 1.0 (0.3–3.2) 0.7(0.1–3.6)	 ≤ 2 years 2–6 years > 6 years	 1.1 (0.3–4.2) 1.9 (0.4–8.4) 0.9 (0.2–3.7) *p* trend = 0.99	

Table 53 (contd)

Reference, study location	No. and type of cases	No. and type of controls	OC use status	Odds ratio (95% CI)	Intensity/ duration of use	Odds ratio (95% CI)	Detection method and comments
Ylitalo *et al.* (1999), Sweden	178 CIS	178 HPV16/18-positive	Former Current	1.54 (0.76–3.12) 2.65 (1.06–6.67)	< 2 years 2–< 10 years ≥ 10 years	1.55 (0.65–3.70) 2.23 (1.02–4.86) 2.79 (1.14–6.87)	PCR; adjusted for marital status, smoking, age at sexual debut, no. of sexual partners, age at menarche, parity, years in school
Lacey *et al.* (2000), USA	139 SCC 124 ADC	49 healthy community members matched by age, ethnicity, residence	Ever use ADC SCC	 1.1 (0.31–3.9) 0.49 (0.13–1.9)	 < 3 months ≥ 3 months < 3 months ≥ 3 months	 1.4 (0.23–8.4) 0.9 (0.17–4.7) 0.8 (0.13–5.1) 0.3 (0.04–1.9)	PCR MY09/11; multicentric study; non-contraceptive hormone use; no association with either age at first use or whether estrogen was opposed or unopposed.
Hildesheim *et al.* (2001), Costa Rica	136 HSIL/cancer high-risk HPV-postive histo-logically confirmed	624 high-risk HPV	Former Current	0.93 (0.55–1.6) 1.5 (0.83–2.8)	< 5 years ≥ 5 years	0.99 (0.58–1.7) 1.30 (0.70–2.3)	HC2 + PCR; adjusted for age, no. of pregnancies, cigarettes/day; no statistical interaction with parity; however, significantly elevated risk observed among women with OC use ≥ 5 years and having < 3 pregnancies (odds ratio, 3.1; 95% CI, 1.1–1,9)
Berrington *et al.* (2002), United Kingdom	221 ICC	393 from general practitioners' records	–	–	0 year 1–4 years 5–9 years ≥ 10 years	1.00 (0.1–7.8) 1.74 (0.8–3.8) 0.76 (0.3–2.1) 3.92 (1.1–14.1)	Serology; odds ratio calculated as floating absoluted risk with floating CI
Moreno *et al.* (2002), 4 continents	1676 squamous ICC and CIS	255 population- or hospital-based	Ever	1.42 (0.99–2.04)	1 year 2–4 years 5–9 years ≥ 10 years	0.67 (0.41–1.08) 0.80 (0.51–1.24) 2.82 (1.46–5.42) 4.03 (2.09–7.79)	PCR MY09/11; pool of 10 case–control studies (8 ICC and 2 CIS); adjusted for age, centre, education, no. of sex partners, age at first intercourse, parity, no. of Pap smears in life

Table 53 (contd)

Reference, study location	No. and type of cases	No. and type of controls	OC use status	Odds ratio (95% CI)	Intensity/ duration of use	Odds ratio (95% CI)	Detection method and comments
Shapiro *et al.* (2003), South Africa	484 SCC 40 ADC	254	*Injectable (progesterone)* Ever	0.9 (0.6–1.5)	< 1 year 1–4 years 5–9 years 10–14 years ≥ 15 years	0.9 (0.5–1.6) 0.9 (0.6–1.6) 0.8 (0.5–1.4) 1.1 (0.6–2.2) 0.8 (0.4–1.7)	Study population had high exposure to hormonal contraceptives and high rate of disease; adjusted for age, ethnicity, age at first intercourse, lifetime no. of sex partners, education, smoking, rural/urban residence, no. of previous Pap smears
			Combined OC use Ever	0.9 (0.7–1.3)	< 1 year 1–4 years ≥ 5 years	0.8 (0.5–1.2) 0.9 (0.6–1.6) 1.3 (0.6–2.7)	
Smith *et al.* (2003), 4 continents	1279 cases CIN 3 and ICC	265	NR		< 5 years 5–9 years ≥ 10 years	0.9 (0.7–1.2) 1.3 (1.0–1.9) 2.5 (1.6–3.9)	Meta-analysis of 5 case–control studies including Lacey *et al.* (1999), Deacon *et al.* (2000), Hildesheim *et al.* (2001), Berrington *et al.* (2002) and Moreno *et al.* (2002). Each study adjusted for different potential confounding factor.
Giuliano *et al.* (2004), USA–Mexico Border	35 ASCUS/AGUS 25 LSIL 19 HSIL cytology–based diagnosis	201	HSIL *OC use* Former Current *Injectable use* Former Current ASCUS/AGUS *OC use* Former Current *Injectable use* Former Current LSIL Former Current *Injectable use* Former Current	 0.50 (0.15–1.66) 0.66 (0.17–2.59) 0.63 (0.16–2.54) 1.75 (0.40–7.65) 0.87 (0.53–1.42) 1.38 (0.81–2.36) 0.86 (0.49–1.52) 0.90 (0.41–2.00) 0.59 (0.28–1.24) 0.35 (0.11–1.11) 0.86 (0.33–2.22) 1.61 (0.54–4.82)			PCR PGMY09/11; adjusted for country, parity, *C. trachomatis* infection, Pap smears in the past 3 years, age

Table 53 (contd)

Reference, study location	No. and type of cases	No. and type of controls	OC use status	Odds ratio (95% CI)	Intensity/ duration of use	Odds ratio (95% CI)	Detection method and comments
Shields *et al*. (2004), Costa Rica	67 ≥ CIN2 pre-menopausal 43 ≥ CIN2 post-menopausal	134 pre-menopausal 86 post-menopausal		No association			No associations between sex hormone binding globulins, estradiol, free estradiol, estrone, estrone sulfate, or dehydroepiandrosterone and disease regardless of menopausal status; adjusted for menopausal status, age, days since last menses or years since menopause

See Table 7 for a description of the primers used.
ADC, adenocarcinoma; AGUS, atypical glandular cells of undetermined significance; ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; HC2, Hybrid Capture 2; HSIL, high-grade intraepithelial lesion; ICC, invasive cervical cancer; LSIL, low-grade squamous intraepithelial lesion; NR, not reported; OC, oral contraceptive; Pap, Papanicolaou test; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma; SIL, squamous intraepithelial cells

circulating hormone concentrations and risk for HSIL among the same Costa Rican study population.

In summary, no study reported a significant increased risk for invasive cervical cancer in ever versus never users of oral contraceptives. When data were pooled across the studies, a significant elevation in risk was only observed among women who had used oral contraceptives for 5 or more years and the risk increased further among those with a duration of use of 10 or more years (Smith *et al.*, 2003). Among a South African population that has a high burden of cervical cancer, a high prevalence of hormonal contraceptive use and a low prevalence of Pap smear screening, Shapiro *et al*. (2003) observed no significant associations with hormonal contraceptive use regardless of the formulation (combined oral estrogen and progesterone or injected progesterone) or duration of use.

(ii) *Prospective studies* (Table 54)

Two prospective studies evaluated the association between hormonal contraceptive use and incidence of CIN among HPV-positive women. Deacon *et al*. (2000) reported no significant increase in risk among current users of oral contraceptive in a study conducted in the United Kingdom. Similarly, Castle *et al*. (2002b) reported no association with current oral contraceptive use among women aged 16 and older in the USA.

(*c*) *Parity*

(i) *Case–control studies* (Table 55)

For several decades, high parity has been suspected to increase the risk for in-situ carcinoma and cancer of the cervix. Unfortunately, many of the studies on cervical cancer either did not measure HPV or did not control for HPV infection or other variables in sexual history that are potential confounders of the association between parity and risk for cervical cancer. The few studies that restricted their statistical analyses to HPV-positive women are reviewed below.

In Denmark, Kjaer *et al*. (1996) observed an increased risk of borderline significance of 1.9 between one or more live births and the risk for ASCUS and SIL combined compared with women who reported no previous pregnancies. However, in a later update of the study by Kruger-Kjaer *et al*. (1998), no significant associations were observed when the cytological categories ASCUS, LSIL and HSIL were examined separately.

Hildesheim *et al*. (2001) observed a significant elevation in risk for HSIL/cancer among women who had had four to five live births among participants in Costa Rica. However, a linear increase in risk with increasing parity was not observed.

The strongest evidence for an association between parity and risk for cervical cancer is from the pooled analysis conducted at IARC on 10 case–control studies by Muñoz *et al*. (2002). Although only two of the eight individual studies of invasive cervical cancer and one of the two studies of carcinoma *in situ* observed significant associations with parity, when the studies were pooled, an odds ratio of 1.81 was observed for women who reported one to two full-term pregnancies compared with none. The risk estimate increased to 3.82 among women who had had seven or more full-term pregnancies. While no statistically

Table 54. Prospective studies of oral contraceptive (OC) use restricted to HPV-positive women

Reference, study location	Parent cohort	No. and type of cases	No. and type of controls	OC use status	Odds ratio (95% CI)	Intensity/ duration of OC use	Odds ratio (95% CI)	Detection method and comments
Deacon *et al*. (2000), United Kingdom	61 570	199 incident CIN3 histologically confirmed	181 < CIN3	Former Current	1.15 (0.63–2.10) 1.28 (0.66–2.50)	1–47 months 48–95 months ≥ 96 months	1.19 (0.58–2.43) 0.76 (0.38–1.53) 1.52 (0.80–2.88)	PCR MY09/MY11; no age restriction
Castle *et al*. (2002b), USA	20 759 women of whom 1812 high-risk HPV-positive	58 incident CIN3 10 incident cancers (high-risk HPV-positive only)	1790 with normal cytology	Current	0.84 (0.49–1.5)[a] 0.61 (0.32–1.1)[b]			HC2; 10 years follow-up; no adjustment [a] Kaplan Meier [b] Conditional logistic model

See Table 7 for a description of the primers used.
CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; PCR, polymerase chain reaction

Table 55. Case–control studies of parity and pre-invasive and invasive cervical cancer restricted to HPV-positive women

Reference, study location	No. and type of cases	No. and type of controls	No. of full term pregnancies	Odd ratio (95% CI)	Detection method and comments
Kjaer *et al*. (1996), Denmark	141 ASCUS and SIL combined	153 cytologically normal	0 ≥ 1	0.9 (0.5–1.5) 1.9 (1.0–4.4)	PCR GP5+/6+; adjusted for age
Kruger–Kajer *et al*. (1998), Denmark	82 ASCUS 86 LSIL 71 HSIL	155 cytologically normal	*HSIL* 0 ≥ 1	 0.8 (0.4–1.7) 1.8 (0.3–2.3)	PCR GP5+/6+; adjusted for age, years of sex life without barrier contraceptive, partner's education, marital status; no association with either ASCUS or LSIL; follow-up of the study by Kjaer *et al.* (1996)
Hildesheim *et al*. (2001), Costa Rica	136 HSIL/cancer high-risk HPV-positive, histologically confirmed	624 high-risk HPV	0–1 2 3 4–5 6–8 ≥ 9	1.0 1.0 (0.48–2.2) 1.5 (0.73–3.2) 3.5 (1.7–7.2) 2.2 (0.98–5.0) 1.4 (0.56–3.4) *p* trend = 0.04	HC2 + PCR; no association with passive exposure; adjusted for age, no. of pregnancies, cigarettes/day

Table 55 (contd)

Reference, study location	No. and type of cases	No. and type of controls	No. of full term pregnancies	Odd ratio (95% CI)	Detection method and comments
Muñoz *et al.* (2002), 4 continents	1676 squamous CIS and ICC 124 adeno CIS and ICC	255	*Squamous CIS/ ICC* 0 1–2 3–4 5–6 ≥ 7 *Adeno CIS/ICC* 0 1–2 3–4 5–6 ≥ 7	 1.00 (0.55–1.81) 1.81 (1.31–2.52) 2.55 (1.95–3.34) 2.83 (2.02–3.96) 3.82 (2.66–5.48) *p* trend = 0.0002 1.00 (0.21–4.86) 3.47 (1.80–6.70) 2.90 (1.77–4.75) 2.47 (1.44–4.23) 2.92 (1.55–5.50)	PCR MY09/11; pool of 10 case–control studies (8 ICC and 2 CIS); adjusted for age, centre, education, no. of sex partners, age at first intercourse, OC use, smoking, history of Pap smears; CIs were estimated by treating the relative risk as a floating absolute risk.
Giuliano *et al.* (2004), USA–Mexico Border	35 ASCUS/AGUS 25 LSIL 19 HSIL cytology-based diagnosis	201	0 1–2 3–4 ≥ 5	1.0 0.38 (0.08–1.88) 2.14 (0.60–7.64) 0.81 (0.06–10.65)	PGMY 09/11; adjusted for country, parity, *C. trachomatis* infection, Pap smears in the past 3 years, age

See Table 7 for a description of the primers used.

AGUS, atypical glandular cells of undetermined significance; ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CIS, carcinoma *in situ* HC2, Hybrid Capture 2; HSIL, high-grade intraepithelial lesion; ICC, invasive cervical cancer; LSIL, low-grade squamous intraepithelial lesion; OC, oral contraceptive; Pap, Papanicolaou test; PCR, polymerase chain reaction; SIL, squamous intraepithelial cells

significant interaction was detected, there appeared to be a higher risk among women with high parity and young age at first full-term pregnancy, and high parity and 5 or more years of oral contraceptive use. A significant increase in risk for adenocarcinoma was also detected among women with one to two full-term pregnancies, although this did not increase linearly with increasing parity.

In a study of women residing along the USA–Mexico border by Giuliano *et al*. (2004), no significant association between SIL and parity was observed.

(ii) *Prospective studies* (Table 56)

The number of prospective studies that have evaluated the association between parity and risk for cervical cancer among HPV-positive women is limited. Deacon *et al*. (2000) observed no increase in risk with increasing parity among women participating in a cohort study in Manchester, United Kingdom. Similarly, Castle *et al*. (2002b) found no increase in risk for CIN3 or cancer among participants in the cohort study in Guanacaste, Costa Rica. However, this study is limited by the fact that parity was assessed only at baseline with no further assessment throughout the 10-year follow-up period.

(*d*) *Nutrients*

Over the past few decades, numerous studies have examined the association between risk for cervical cancer and dietary intake or serological measures of nutrient concentrations. However, most of these studies have methodological limitations that include lack of measurement or adequate consideration of HPV infection in the analyses. Of the studies that did measure HPV infection, very few restricted their analyses to HPV-positive women. Therefore, only a small proportion of all studies are reviewed here.

In addition to the problems of assessment of HPV infection, a drawback that is common to all studies that attempt to examine associations between nutrients and disease is the incomplete consideration of confounding factors, such as tobacco smoking or oral contraceptive use, which are associated with both cervical cancer and nutritional status. In addition, most studies were conducted before the availability of reliable laboratory methods for separating and quantifying the major carotenoids and their geometric isomers in serum. Finally, due to the significant disparity in the content of nutrients in foods by region and variety, the data on carotenoid, selenium and folate content in the food supply are liable to be imprecise. The net result of these limitations is a significant exposure misclassification that results in attenuation of the true association.

(i) *Case–control studies* (Table 57)

Of the studies that restricted analyses to HPV-positive women, only two assessed the association between dietary intake of nutrients and the risk for cervical cancer or CIN (Wideroff *et al.*, 1998; Rajkumar, 2003). Wideroff *et al*. (1998) examined the association between vitamins A, C and E, β-carotene, folate and zinc and the risk for incident HSIL among women resident in Portland, Oregon (USA). Although the risk appeared to be lower among those who consumed higher concentrations of β-carotene, folate and zinc, none of

Table 56. Prospective studies of parity and pre-invasive cervical cancer restricted to HPV-positive women

Reference, study location	Parent cohort	No. and type of cases	No. and type of controls	Number of full term pregnancies	Odds ratio (95% CI)	Detection method and comments
Deacon *et al*. (2000), United Kingdom	61 570	199 incident CIN3 histologically confirmed	181 (includes < CIN 3) stratified random sampling	0 1 2 ≥ 3	1.0 1.57 (0.88–2.77) 1.13 (0.64–1.99) 1.90 (0.94–3.85)	PCR MY09/MY11
Castle *et al*. (2002b), USA	20 759 women of whom 1812 high-risk HPV-positive women	58 incident CIN3 10 incident cancers (high-risk HPV-positive only)	1790 with normal cytology	0 1–2 ≥ 3 0 1–2 ≥ 3	1.0 1.1 (0.64–1.7)[a] 0.7 (0.31–1.6)[a] 1.0 1.2 (0.67–2.1)[b] 0.7 (0.24–1.9)[b]	HC2; parity assessed at enrolment only, not throughout the 10 year follow–up period [a]Kaplan Meier analysis [b]Logistic regression models

See Table 7 for a description of the primers used.
CI, confidence interval; CIN, cervical intraepithelial neoplasia; HC2, Hybrid Capture 2; PCR, polymerase chain reaction

Table 57. Case–control studies of nutrients and pre-invasive and invasive cervical cancer restricted to HPV-positive women

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio for serum/plasma levels (95% CI)	Detection method and comments
Ho *et al.* (1998b), USA	44 CIN3	163 CIN1		*CIN3 versus CIN1* Vitamin C 2.86 (0.61–13.52) Log α-toc. 0.63 (0.04–9.01) Log β-car. 0.49 (0.13–1.82) Retinol 1.01 (1.00–1.03)	PCR MY09/11 ; adjusted for age, education, ethnicity, no. of Pap smears in past 3 years, high-risk versus low-risk HPV, smoking status; odds ratio for a unit increase in micronutrient level
Ho *et al.* (1998c), USA	262 histo-logically confirmed ≥ CIN1	80 normal cytology with no history of abnormal cytology		*CIN1–3 versus normal* Vitamin C 0.34 (0.13–1.00) Log α-toc 0.25 (0.04–1.66) Log β-car. 0.88 (0.33–2.33) Retinol 0.99 (0.98–1.00) Vitamin C < 0.8 mg/dL 1.00 ≥ 0.8 mg/dL 0.41 (0.19–0.89)	PCR MY09/11; adjusted for age, ethnicity, income, smoking status; odds ratio for a unit increase in micro-nutrient level
Wideroff *et al.* (1998), USA	68 high-risk HPV-positive HSIL	69 high-risk HPV-positive	**Diet + supplements** *Vitamin A quartiles* 1 1.0 2 1.9 (0.6–5.5) 3 1.0 (0.3–2.8) 4 1.4 (0.5–4.2) *β-Carotene quartiles* 1 1.0 2 0.6 (0.2–2.0) 3 0.8 (0.2–2.3) 4 0.6 (0.2–2.0)		PCR and dot blot hybri-dization; adjusted for age

Table 57 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio for serum/plasma levels (95% CI)	Detection method and comments
Wideroff *et al.* (1998) (contd)			*Vitamin C quartiles* 1 1.0 2 1.9 (0.7–5.6) 3 1.0 (0.4–2.8) 4 1.3 (0.4–3.6) *Vitamin E quartiles* 1 1.0 2 0.8 (0.2–2.2) 3 0.6 (0.2–1.7) 4 1.0 (0.4–2.6) *Folate quartiles* 1 1.0 2 0.7 (0.2–1.9) 3 0.7 (0.2–2.2) 4 0.7 (0.3–2.1) *Zinc quartiles* 1 1.0 2 1.0 (0.3–2.9) 3 1.0 (0.4–2.9) 4 0.8 (0.3–2.2)		
French *et al.* (2000), USA	208 HIV-infected SIL	673 HIV-infected normal cytology		*Retinol* ≥ 1.1 µmol/L 1.0 < 1.1 µmol/L 1.8 (1.1–1.3)	PCR MY09/11; analyses adjusted for age, race/ethnicity, CD4+ cell count, HIV type 1 RNA, body mass index, serum albumin
Goodman *et al.* (2001), USA	150 histologically confirmed SIL	179 normal cytology		*MTHFR variants* CC 1.0 CT 2.0 (1.1–3.7) TT 2.9 (1.0–8.8) *p* trend = 0.02	PCR MY09/11 and dot-blot hybridization; adjusted for age, ethnicity, tobacco, alcohol, no. of sex partners before age 20 years, HPV infection

Table 57 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio for serum/plasma levels (95% CI)	Detection method and comments
Weinstein *et al.* (2001a), USA	75 (serum)/63 (RBC folate) histologically confirmed ICC	27 (serum)/23 (RBC folate) HPV 16-seropositive		*Serum folate* High 1.0 Low 2.4 (0.8–7.4) *RBC folate* High 1.0 Low 1.4 (0.5–4.8)	Serology ELISA; adjusted for ethnicity, study site
Weinstein *et al.* (2001b) USA	183 ICC	79 HPV 16-seropositive		*Homocysteine quartiles* 1 1.00 2 2.45 (0.9-7.1) 3 3.81 (1.3-11.2) 4 1.93 (0.6-5.9) *p* trend = 0.42	Serology only; adjusted for age, ethnicity, study site, no. of sex partners, age at first intercourse, years since last Pap smear, no. of pregnancies, smoking, OC use, education, income
Rajkumar *et al.* (2003), Chennai, India	190 ICC	51 cyto-logically normal	*Vegetable and fruit intake (servings/week)* < 6 1.00 6 0.88 (0.26–3.03) ≥ 7 0.37 (0.11–1.22) *p* trend = 0.08		PCR GP5+/6+; adjusted for age, area of residence, occupation, marital status, age at first marriage, no. of pregnancies, husband's extramarital affairs, body mass index, chewing habit

See Table 7 for a description of the primers used.

β-car., β-carotene; CI, confidence interval; CIN, cervical intraepithelial neoplasia; ELISA, enzyme-linked immunosorbent assay; HSIL, high-grade intraepithelial lesion; ICC, invasive cervical cancer; MTHFR, methylene tetrahydrofolate reductase; OC, oral contraceptive; Pap, Papanicolaou test; PCR, polymerase chain reaction; RBC, red blood cell; α-toc., α-tocopherol

these associations reached statistical significance. Among women resident in Chennai, India, Rajkumar *et al*. (2003) observed a non-significant inverse association between consumption of vegetables and fruit and the risk for invasive cervical cancer.

Serum retinol has been examined in two studies that restricted their analyses to HPV-positive women. Ho *et al*. (1998b,c) did not observe significant associations between serum retinol and CIN1–3 among women in the USA. This was the only study that examined the association between serum carotenoids, α-tocopherols and vitamin C concentrations and risk for CIN1–3 combined or considered separately. Only serum vitamin C was significantly associated with a reduced risk for disease (odds ratio, 0.41 for ≥ 0.8 mg/dL versus < 0.8 mg/dL), and the association was limited to the comparison between women with CIN1–3 and those with normal cytology. Among women infected with HIV in the USA, French *et al*. (2000) observed significant associations between higher serum retinol concentrations and the risk for SIL.

Two studies examined the concentration in serum or red blood cells of nutrients that are involved in one-carbon methyl transfer reactions, such as folate and vitamin B12, or the accumulation in serum of homocysteine, a biomarker of insufficient one-carbon nutrient status (Goodman *et al.*, 2001; Weinstein *et al.*, 2001a,b). Weinstein *et al*. (2001a,b) observed elevated risks for invasive cervical cancer among women in the USA who had low serum and red blood cell concentrations of folate, although these associations did not reach statistical significance. In the same study, an increase in risk for invasive cervical cancer with elevated levels of serum homocysteine was observed, but no significant trend in risk. Goodman *et al*. (2001) observed a significant increase in risk for SIL among women with single nucleotide variants in the methylenetetrahydrofolate reductase gene, which is involved in the methylation of homocysteine to methionine. The analysis was not restricted to HPV-positive women but HPV status was controlled for in the statistical analysis.

(ii) *Prospective studies* (Table 58)

Of the prospective studies that restricted analyses to HPV-positive women, only one examined risk for invasive cervical cancer (Lehtinen *et al.*, 1999) and the others examined the risk for persistent HPV infection (Giuliano *et al.*, 1997; Sedjo *et al.*, 2002a,b; Giuliano *et al.*, 2003; Sedjo *et al.*, 2003a,b).

Lehtinen *et al*. (1999) examined the association between serum retinol and α-tocopherol and the risk for invasive cervical cancer among women who were resident in Finland and Sweden. No significant associations were observed, although retinol levels appeared to interact with HPV status.

Giuliano *et al.* (1997) observed significant associations among Hispanic women resident in the USA between serum β-carotene, β-cryptoxanthin, lutein and α-tocopherol when those who were transiently infected with HPV were compared with those who were persistently infected with high-risk HPV over a 3-month period.

The Young Women's Health Study reported a decreased risk for HPV persistence for the highest versus the lowest tertile of serum levels of vitamin B12 (odds ratio, 0.40) and *cis*-

Table 58. Prospective studies of nutrients restricted to HPV-positive women

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio/*p* value for serum/ plasma levels (95% CI)	Detection method and comments
Giuliano *et al.* (1997), USA	33 persistently HPV-positive women	32 intermittently HPV-positive women		α-Carotene 0.028 β-Carotene 0.179 Lycopene 0.351 β-cryptoxanthin 0.010 Lutein 0.034 α-Tocopherol 0.001 Vitamin C 0.556	HC 1 high-risk probe; *p*-value for difference in adjusted mean (μM) value; Mexican American women; adjusted for age, age at first intercourse, no. of pregnancies, duration of OC use
Lehtinen *et al.* (1999), Finland and Sweden	34 ICC	105 matched controls		*Retinol* Low 1.0 High 1.19 (0.55–2.58) *α-Tocopherol* Low 1.0 High 1.52 (0.70–3.30)	HPV 16-, 18-, 33-seropositive
Sedjo *et al.* (2002a), USA	131 persistent HPV (diet) 109 persistent HPV (nutrient biomarkers)	70 intermittent HPV (diet) 60 intermittent HPV (biomarkers)	*Folate tertiles* 1 1.00 2 0.63 (0.29–1.38) 3 0.52 (0.23–1.18) *p* trend = 0.109 *Vitamin B12 tertiles* 1 1.00 2 1.47 (0.68–3.19) 3 0.68 (0.30–1.54) *p* trend = 0.439 *Vitamin B6 tertiles* 1 1.0 2 0.92 (0.43–2.00) 3 0.61 (0.27–1.39) *p* trend = 0.254	*Folate tertiles* 1 1.00 2 0.95 (0.39–2.29) 3 1.20 (0.51–2.78) p-trend = 0.662 *Vitamin B12 tertiles* 1 1.00 2 0.57 (0.24–1.32) 3 0.40 (0.17–0.96) *p* trend = 0.037	HC 2 Probe B; risk of persistent infection; adjusted for age, age at first intercourse, marital status, smoking, race, body mass index

Table 58 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio/*p* value for serum/ plasma levels (95% CI)	Detection method and comments
Sedjo *et al.* (2002b), USA	131 persistent HPV (diet) 101 persistent HPV (nutrient biomarkers)	69 intermittent HPV (diet) 58 intermittent HPV (bio-marker)	*Fruit tertiles* 1 (low) 1.00 2 0.89 (0.42–1.87) 3 (high) 0.59 (0.27–1.30) *p* trend = 0.206 *Vegetable tertiles* 1 (low) 1.00 2 0.38 (0.17–0.84) 3 (high) 0.46 (0.21–0.97) *p* trend = 0.033 *Lutein tertiles* 1 (low) 1.00 2 0.37 (0.13-0.82) 3 (high) 0.50 (0.24–1.07) *p* trend = 0.054	trans-*Lycopene tertiles* 1 (low) 1.00 2 0.49 (0.21–1.14) 3 (high) 0.78 (0.35–1.78) *p* trend = 0.496 cis-*Lycopene tertiles* 1 (low) 1.00 2 0.57 (0.25–1.29) 3 (high) 0.44 (0.19–1.01) *p* trend = 0.046	HC 2 Probe B; adjusted for age, race, smoking, body mass index; risk of persistent infection; no significant association detected with plasma vitamin A, α- and β-carotene, β-cryptoxanthin, lutein
Sedjo *et al.* (2003a), USA	84 high-risk HPV-positive with persistent infection			trans-*Lycopene tertiles* 1 (low) 1.00 2 3.03 (1.02–7.65) 3 (high) 2.79 (1.17–6.66) *p* trend = 0.025 cis-*Lycopene tertiles* 1 (low) 1.00 2 3.50 (1.51–8.08) 3 (high) 2.92 (1.28–6.63) *p* trend = 0.010	PCR PGMY 09/11; Cox proportional hazard model adjusted for age, ethnicity, no. of new male partners, marital status; hazard ratios were estimated for the association of HPV clearance to each nutrient tertile; no significant association detected with α- and β-carotene, β-cryptoxanthin, lutein/zeaxanthin, tocopherols, folate, vitamin B12

Table 58 (contd)

Reference, study location	No. and type of cases	No. and type of controls	Odds ratio for diet (95% CI)	Odds ratio/*p* value for serum/ plasma levels (95% CI)	Detection method and comments
Giuliano *et al.* (2003a), Brazil	185 HPV type-specific persistent infection	248 transiently HPV-infected	*β-Cryptoxanthin quartiles* 1 (low) 1.00 2 0.60 (0.33–1.09) 3 0.48 (0.27–0.87) 4 0.47 (0.26–0.85) *p* trend = 0.007 *Lutein/zeaxanthin quartiles* 1 (low) 11.00 2 20.58 (0.32–1.05) 3 30.44 (0.24–0.78) 4 40.49 (0.27–0.87) *p* trend = 0.066 Vitamin C quartiles 1 1.00 2 0.63 (0.35–1.15) 3 0.84 (0.47–1.48) 4 0.50 (0.27–0.92) *p* trend = 0.66		PCR MY09/11; risk of persistent infection; adjusted for kcal, income, education, no. of persons in household, no. of sex partners during past 5 years, total no. of pregnancies; significant inverse association also detected with papaya and orange consumption; no significant association detected with α- and β-carotene

See Table 7 for a description of the primers used.
CI, confidence interval; HC, Hybrid Capture; OC, oral contraceptive; PCR, polymerase chain reaction

lycopene (odds ratio, 0.44) (Sedjo *et al.*, 2002a,b). In the same study, the authors reported an approximately threefold higher probability of oncogenic HPV clearance among women in the highest compared tertile of both *trans*- and *cis*-lycopene concentrations (Sedjo *et al.*, 2003a).

Increasing levels of dietary vegetables decreased the risk for persistent HPV infection (Sedjo *et al.*, 2002b). Giuliano *et al*. (2003) assessed the association between dietary nutrient intake and risk for HPV persistence among women who participated in the Ludwig-McGill HPV Natural History Study in Sao Paolo, Brazil. Dietary intakes of β-cryptoxanthin, lutein/zeaxanthin and vitamin C were significantly inversely associated with risk for persistent type-specific HPV infection. In addition, consumption of papaya was inversely associated with persistent HPV infection in this population.

(iii) *Clinical trials* (Table 59)

In this section, all nutrient-based clinical trials are evaluated, although several did not measure HPV infection and none limited their analyses to HPV-positive women.

Randomization assures that the proportion of HPV-positive women is comparable between treatments and that the majority of women who had cervical preneoplastic lesions were HPV-positive. Although randomized clinical trials are considered to be the gold standard to demonstrate the efficacy of a chemopreventive agent, intervention studies of nutrients and risk for cervical cancer present unique problems. First, the widespread adoption of the use of supplemental vitamins and minerals in the USA, where most chemoprevention studies have been carried out to date, can adversely affect the feasibility of testing the efficacy of a single nutrient to prevent cervical cancer. Unlike pharmaceutical agents that proceed through phase I and II trials to determine the safe and optimal dose, a 'best-guess' estimate has been used to choose doses for phase III nutrient chemoprevention trials. Information on duration of treatment and length of follow-up that is needed to demonstrate an effect has also been lacking. Finally, the statistical power of a study to assess efficacy is based on the accumulation of an adequate number of events (e.g. regression or progression of CIN). Non-compliance to the study regimen and high rates of spontaneous regression of lesions such as CIN1 (> 80% spontaneous regression) combine to reduce the power of any study to detect differences between treatment and control groups. As a consequence of the above limitations, only one of 10 clinical trials conducted has shown significant protective effects of the nutrient that was tested, which was topical all-*trans*-retinoic acid (Meyskens *et al*., 1994).

Folate trials

Two phase II trials of folic acid for the prevention of cervical cancer have been completed (Butterworth *et al.*, 1992; Childers *et al.*, 1995). Both found no significant effect of treatment on the regression or progression of lesions. In Alabama, USA, Butterworth *et al*. (1992) found no protective effect of a daily dose of 10 mg folate on either regression or progression of cervical lesions after 6 months of treatment. However, the majority of participants entered the trial with CIN1 lesions that have a high rate of spontaneous

Table 59. Clinical trials of nutrients and pre-invasive cervical lesions

Reference, study location	No. of subjects enrolled	No. of subjects completing trial	Entry diagnosis	End-point	Detection method of primary end-point	Dose	Duration	Results
Folic acid								
Butterworth *et al.* (1992), USA	235	199	CIN1/2	Lesion regression and HPV DNA	Biopsy and PCR HPV 16	10 mg/day	6 months	No effect
Childers *et al.* (1995), USA	331	262	89% KA/CIN1 11% CIN2	Lesion regression	Colposcopy	5 mg/day	6 months	No effect
β-Carotene								
De Vet *et al.* (1991), Netherlands	278	278	28% CIN1 42% CIN2 30% CIN3	Lesion regression	Cytology, colposcopy	10 mg/day	3 months	No effect
Fairley *et al.* (1996), Australia	114	111	5% Atypia 62% HPV 23% CIN1 10% CIN2	Lesion regression and HPV DNA	Cytology and PCR and HC HPV DNA	30 mg/day	12 months	No effect on either lesion regression or HPV positivity
Romney *et al.* (1997), USA	98	69	51% CIN1 46% CIN2 39% CIN3	Lesion regression and HPV 16	Biopsy and PCR HPV 16	30 mg/day	9 months	Decreased regression in β-carotene arm
Mackerras *et al.* (1999), Australia	147	141	100% minor atypia or CIN1	Lesion regression	Cytology	30 mg/day β-carotene 500 mg/day vitamin C	24 months	No effect of either β-carotene or vitamin C
Keefe *et al.* (2001), USA	103	78	43% CIN2 57% CIN3	Lesion regression and HPV	Biopsy and PCR HPV DNA	30 mg/day	24 months	No effect on regression

Table 59 (contd)

Reference, study location	No. of subjects enrolled	No. of subjects completing trial	Entry diagnosis	End-point	Detection method of primary end-point	Dose	Duration	Results
Retinoic acid								
Meyskens *et al.* (1994), USA	301	232	50% CIN2 50% CIN3	Lesion regression	Biopsy	1 mL 0.375% topical all *trans*-retinoic cream	Treated at 1, 3, 6 months; end-point assessed at 15 months	Increased regression of CIN2 at 15 months
Follen *et al.* (2001), USA	39	36 at 6 months 30 at 12 months	33% CIN2 67% CIN3	Lesion regression and HPV DNA	Biopsy and HC	200 mg/day oral *N*-(4-hydroxyohenyl) retinamide	6 months	No effect
Alvarez *et al.* (2003), USA	114	104	39% CIN2 61% CIN3	Lesion regression	Cytology	25 or 50 mg aliretinoin or placebo daily	3 months	No effect

CIN, cervical intraepithelial neoplasia; HC, Hybrid Capture; KA, koilocytic atypia; PCR, polymerase chain reaction

regression. In Arizona, USA, Childers *et al*. (1995) similarly found that 5 mg folic acid per day had no significant effect on cervical lesions after 6 months of treatment. Again, the majority of participants entered the study with CIN1 lesions.

β-Carotene trials

Five phase II/IIII trials of β-carotene supplements have been conducted, none of which demonstrated an increase in regression or a decrease in progression of any preneoplastic lesion. De Vet *et al*. (1991) found no effect of treatment with 10 mg per day β-carotene for 3 months among women in The Netherlands. A longer duration of treatment (9–24 months) with higher doses (30 mg) was also ineffective in altering rates of regression of lesions in studies conducted by Fairley *et al*. (1996) in Australia, Romney *et al*. (1997) in the USA, Mackerras *et al*. (1999) in Australia and Keefe *et al.* (2001) in the USA.

Retinoic acid trials

Three trials tested different formulations of retinoic acid (Meyskens *et al.*, 1994; Follen *et al.*, 2001; Alvarez *et al.*, 2003). In the only placebo-controlled trial of a topical retinoid (all-*trans*-retinoic acid), Meyskens *et al*. (1994) found a significant effect of three administrations of the compound on the regression of CIN2 lesions after 15 months. In comparison, two studies of oral doses of retinoids failed to demonstrate an effect (Follen *et al.*, 2001; Alvarez *et al.*, 2003). In a small trial conducted by Follen *et al*. (2001), daily oral doses of 200 mg *N*-(4-hydroxyohenyl) retinamide had no effect on regression of CIN2/3 lesions after 6 months of treatment. Similarly, daily administration of 25 or 50 mg aliretinoin had no effect on the regression of CIN2/3-lesions after 3 months of treatment (Alvarez *et al*., 2003).

Most of the studies were small and high rates of spontaneous regression of lesions were observed. As a result, these studies had insufficient power to test adequately the efficacy of the selected agents. Since effective ablative treatments are available for CIN2/3, it is questionable whether continued efforts should be made to find chemopreventive alternatives to the current standard of care. Future efforts in this area will require multisite collaboration to ensure that an adequate sample size of women with histologically confirmed CIN2/3 lesions are enrolled and complete the treatment protocol.

(*e*) *Genetic factors*

Familial clustering of cervical cancer has been explored as a potential marker of inherited genetic susceptibility.

Population-based studies that used routinely collected data and record linkage in Sweden, Iceland and America consistently found moderately increased risk estimates for carcinoma *in situ* and invasive carcinoma among women who had a first- or a second-degree relative with cervical cancer (Amundadottir *et al.*, 2004; de Zelmanowicz *et al.*, 2005; Couto & Hemminki, 2006). However, these investigations did not fully take into account relevant covariates such as HPV status or screening practices.

Heritability estimates from the Swedish Cancer Registry suggest that genes are responsible for less than 30% of cervical tumours (Hemminki *et al.*, 1999; Magnusson *et al.*, 2000; Couto & Hemminki, 2006).

In a multicentric case–control study in Latin America that included 481 patients with invasive cervical cancer and 801 population controls, Brinton *et al.* (1987) found a familial tendency for all cell types of cervical cancer. Women who had a family history of cervical cancer had a 2.49-fold higher risk for adenocarcinoma (based on one case), a 9.93-fold higher risk for adenosquamous carcinoma (based on 2 cases) and a 3.11-fold higher risk for squamous cell carcinoma (based on 13 cases).

In the Republic of Korea, Yoo *et al.* (1997) conducted a case–control study that included 203 cases of invasive cervical cancer and reported a 2.20-fold (95% CI, 1.21–4.01) increase in risk associated with a family history of cervical cancer. Cusimano *et al.* (1989) reported an odds ratio of 2.87 (95% CI, 1.05–7.83) for a family history of cervical cancer in Sicily. In a case–control study in the USA, Hildesheim *et al.* (1999) reported a significantly increased risk for rapid onset of cervical cancer among young women whose mothers had a history of cervical cancer.

Familial studies are limited in their capacity to separate fully the effects that can be attributed to genetic susceptibility from those that are related to common environmental and behavioural traits. However, reports have consistently suggested that a familial risk exists and further studies aimed at identifying relevant biomarkers would be pertinent.

(*f*) *Human leukocyte antigen (HLA) polymorphisms and risk for cervical cancer*

Major histocompatibility gene products that are complexed with peptides derived from viral antigens can induce T-cell responses on the surface of antigen-presenting cells that clear viral infections. HLA class II genes (*DR*, *DQ* and *DP*) are expressed on B cells and macrophages, where they present antigen fragments to $CD4^+$ T cells. Although they are not usually expressed on epithelial cells, expression of HLA class II genes is increased in cervical cancer cells (Glew *et al.*, 1992). $CD4^+$ T cells have been reported to have killer activity and could thus potentially kill cervical cancer cells directly (de Jong *et al.*, 2004; Steele *et al.*, 2005); however, $CD4^+$ T cells more commonly provide helper functions that assist the maturation of $CD8^+$ T cells. $CD8^+$ T cells recognize peptides in conjunction with the more ubiquitously expressed HLA class I genes. These genes (*A*, *B* and *C*) are highly polymorphic and present antigens on most cells, including cervical epithelial cells. Although class I and II gene products were initially defined using serological reagents, it has more recently been possible to characterize the HLA genotype using PCR-based methods with specific primers. Several studies have examined the association between HLA genotypes and the risk for cervical cancer. Most of these focused on class II alleles because the methods for genotyping these genes were developed earlier than those for the detection of class I alleles.

Class II *DR* and *DQ* alleles are co-dominantly expressed. The large number of polymorphisms of these alleles lead to variations in the antigen recognition site on the cell

surface, which may confer susceptibility or resistance to HPV infection and neoplastic progression. Malignant transformation and regression of cottontail rabbit papillomavirus-induced lesions were clearly shown to be associated with class II DR and DQ genes (Han *et al.*, 1992).

The results of several selected studies are summarized in Table 60.

The first associations between HLA class II genes and cervical cancer were reported with *DR5*, *DR6* and *DQ3* (Wank & Thomssen, 1991). A second report on the same samples employed HLA typing using DNA-based methods and assigned the increase in risk to *DQB1*0301/0303* (Wank *et al.*, 1993). A number of studies that used different ethnic populations confirmed this association (Helland *et al.*, 1992; Gregoire *et al.*, 1994; Nawa *et al.*, 1995; Duggan-Keen *et al.*, 1996) but others did not find statistically significant associations for these alleles (Glew *et al.*, 1992; Apple *et al.*, 1994; Allen *et al.*, 1996; Lin *et al.*, 2001).

Other allele groups that have been reported to confer risk include *DRB1*11* (Duggan-Keen *et al.*, 1996), *DRB1*15* (Cuzick *et al.*, 2000; Maciag *et al.*, 2000; Beskow *et al.*, 2001) and *DQB1*06* (Gregoire *et al.*, 1994; Beskow *et al.*, 2001) and the related haplotypes *DRB1*0401–DQB1*0301* (Cuzick *et al.*, 2000), *DRB1*1101–DQB1*0301* (Lin *et al.*, 2001) and *DRB1*1501–DQB1*0602* (Apple *et al.*, 1994). In addition, *DRB1*13* and the *DRB1*13–DQB1*06* haplotype have been reported to confer protection against the development of cervical cancer (Apple *et al.*, 1995; Sastre-Garau *et al.*, 1996).

Class II *DRB1* and *DQB1* genes were examined in 315 women who had invasive squamous-cell cervical cancer and 381 population-based controls who were residents of the metropolitan area of Seattle, WA (USA) (Madeleine *et al.*, 2002). An increased risk for squamous-cell cancer was associated with two *DRB1* alleles (*DRB1*1001* and *DRB1*1101*) and one *DQ* allele (*DQB1*0301*). Decreased risks for squamous-cell cancer were associated with *DRB1*0301* and *DRB1*13*. The relative risks for squamous-cell cancer that contains HPV 16 were different from those for squamous-cell cancers that contain other high-risk HPV types for three alleles: *DRB1*0401*, *DRB1*07* and *DQB1*06*. The increased risks associated with the DQB1*0301 allele were specific to the *DRB1*0401–DQB1*0301* (odds ratio, 1.9; 95% CI, 1.2–3.1) and *DRB1*1101–DQB1*0301* (odds ratio, 2.9; 95% CI, 1.6–5.2) haplotypes. Similarly, the associations with disease for the *DRB1*0301* and *DRB1*13* alleles were specific to the *DRB1*0301–DQB1*02* (odds ratio, 0.7; 95% CI, 0.5–1.0) and *DRB1*13–DQB1*06* (odds ratio, 0.6; 95% CI, 0.4–0.9) haplotypes.

Fewer studies have examined the association between class I alleles and cervical cancer. In some cases, the risk was associated with the genotypes *A2* or *A*3303* (Wang *et al.*, 2002a), although the association with *A*3303* was seen only in a population of women from Portland (OR, USA) and not in two other populations that were examined. In contrast, the same study found a protective effect for *CW*0202* in all three populations, and a protective effect for *CW*0401* for both populations in the USA. The *B*07* allele has been examined frequently and some (Hildesheim *et al.*, 1998; Wang *et al.*, 2002b), but not all (Gostout *et al.*, 2003) studies found that it conferred risk. In the progression of HPV-related dysplasia, the *B*44* allele was associated with a strong risk (odds ratio, 9.0) in one

Table 60. Association of human leukocyte antigen (HLA) class II alleles with the risk for cervical cancer

Reference, study location	HLA groups/allele(s)	Total no. of cases	Type of cases	*p* Value or odds ratio (95% CI)
	DRB1*			
Madeleine *et al.* (2002), USA	DRB1*07	315	HPV 16 SCC	1.5 (1.0–2.3)
Allen *et al.* (1996), Sweden	DRB1*08			
	DRB1*0802	150	Cervical cancer	$p = 0.005$
Madeleine *et al.* (2002), USA	DRB1*10	315	SCC	5.6 (1.2–26.1)
			HPV SCC	7.3 (1.5–36.7)
	DRB1*1001		HPV SCC	7.3 (1.5–36.7)
Wank & Thomssen (1991), USA	DR5 (DRB1*11, DRB1*12)	66	Invasive cancer	$p = 0.009$
Duggan-Keen *et al.* (1996), United Kingdom	DRB1*11	150	Cervical cancer	NS
Lin *et al.* (2001), Senegal	DRB1*11	55	Invasive carcinoma	1.0 (0.4–2.2)
Madeleine *et al.* (2002), USA	DRB1*11	315	SCC	1.8 (1.2–2.9)
	DRB1*1101		SCC	2.4 (1.4–4.2)
			HPV 16 SCC	2.4 (1.3–4.6)
Wank & Thomssen (1991), USA	DR6 (DRB1*13, DRB1*14)	66		$p = 0.004$
Apple *et al.* (1994, 1995), USA	DRB1*13	98	SCC and ADC	0.3 (0.1–0.7)
Sastre-Garau *et al.* (1996), France	DRB1*1301/1302	126	Invasive cancer	$p = 0.0004$
Cuzick *et al.* (2000), United Kingdom	DRB1*1301	116	SCC	NS
Lin *et al.* (2001), Senegal	DRB1*13	55	Invasive cancer	0.5 (0.2–1.1)
Krul *et al.* (1999), Netherlands	DRB1*13	172	SCC	NS
Madeleine *et al.* (2002), USA	DRB1*13	315	SCC	0.6 (0.4–0.9)
			HPV 16 SCC	0.6 (0.3–0.9)
	DRB1*1301		SCC	0.6 (0.4–1.0)
			HPV 16 SCC	0.6 (0.3–1.1)
	DRB1*1302		SCC	0.6 (0.3–1.1)
			HPV 16 SCC	0.4 (0.2–1.0)

Table 60 (contd)

Reference, study location	HLA groups/allele(s)	Total no. of cases	Type of cases	*p* Value or odds ratio (95% CI)
Maciag *et al.* (2000), Brazil	DRB1*15	161	SCC	2.2 (1.3–3.9)
Krul *et al.* (1999), Netherlands	DRB1*15	172	SCC	NS
Madeleine *et al.* (2002), USA	DRB1*15	315	SCC	1.0 (0.7–1.4)
Beskow *et al.* (2001), Sweden	DRB1*1501	440	CIS	$p = 0.027$
Madeleine *et al.* (2002), USA	DRB1*1501	315	SCC	1.0 (0.7–1.4)
Cuzick *et al.* (2000), United Kingdom	DRB1*1501	116	HPV 16 SCC	$p = 0.05$
Gostout *et al.* (2003), USA	DR2 (1501)	127	Cervical cancer	$p = 0.023$
	DQB1*			
	DQ1 (DQB1*05, DQB1*06)			
Maciag *et al.* (2000), Brazil	DQB1*05	161	SCC	0.6 (0.4–0.9)
Madeleine *et al.* (2002), USA	DQB1*05	315	SCC	1.2 (0.9–1.7)
Beskow *et al.* (2001), Sweden	DQB1*0602		CIS	$p = 0.028$
Madeleine *et al.* (2002), USA	DQB1*0602	315	HPV 16 SCC	0.9 (0.6–1.4)
Lin *et al.* (2001), Senegal	DQB1*0602	55	Invasive carcinoma	0.6 (0.1–2.7)
Sastre-Garau *et al.* (1996), France	DQB1*0603	126	Invasive cancers	
Madeleine *et al.* (2002), USA	DQB1*0603	315	SCC	0.6 (0.4–1.0)
Gregoire *et al.* (1994), USA	DQB1*0604	66	SCC	$p = 0.02$
Helland *et al.* (1992), Norway	DQB1*0604	158	SCC	0.4 (0.1–1.1)
Madeleine *et al.* (2002), USA	DQB1*0604	315	SCC	0.6 (0.3–1.3)

Table 60 (contd)

Reference, study location	HLA groups/allele(s)	Total no. of cases	Type of cases	*p* Value or odds ratio (95% CI)
Wank & Thomssen (1991), USA	DQ3 (DQB1*07, DQB1*08, DQB1*09)	66	Invasive cancer	$p = 0.0009$
Helland *et al.* (1992), Norway	DQ3	158		2.0 (1.3–3.2)
Nawa *et al.* (1995), Japan	DQ3	23	SCC	$p = 0.0003$
Sastre-Garau *et al.* (1996), France	DQ3	126	Invasive cancer	$p = 0.03$
Allen *et al.* (1996), Sweden	DQ3	150	Cervical cancer	$p = 0.11$
Apple *et al.* (1994), USA	DQ3	28	SCC and ADC	NS
Krul *et al.* (1999), The Netherlands	DQ3	172	SCC	NS
Glew *et al.* (1992), United Kingdom	DQ3	53	SCC	NS
Madeleine *et al.* (2002), USA	DQB1*07	315	SCC HPV 16 SCC	1.6 (1.2–2.2) 1.5 (1.0–2.2)
Duggan-Keen *et al.* (1996), United Kingdom	DQB1*0301	150	Cervical cancer	$p = 0.04$
Cuzick *et al.* (2000), United Kingdom	DQB1*0301	116	SCC	$p = 0.02$
Helland *et al.* (1992), Norway	DQB1*0301	158	SCC	1.8 (1.1–3.0)
Madeleine *et al.* (2002), USA	DQB1*0301	315	SCC HPV 16 SCC	1.6 (1.2–2.2) 1.5 (1.0–2.2)
Gregoire *et al.* (1994), USA	DQB1*0301	66	SCC	NS
Wank *et al.* (1993), USA	DQB1*0301			8.71 $p = 0.0001$
Vandenvelde *et al.* (1993), Belgium	DQB1*0301/0302			NS
Lin *et al.* (2001), Senegal	DQB1*0301/0302	55	Invasive cancer	0.8 (0.4–1.9)
Madeleine *et al.* (2002), USA	DQB1*09 DQB1*0303	315	SCC SCC	1.3 (0.8–2.1) 0.9 (0.6–1.3)

Table 60 (contd)

Reference, study location	HLA groups/allele(s)	Total no. of cases	Type of cases	*p* Value or odds ratio (95% CI)
Gregoire *et al.* (1994), USA	DQB1*0303	66	SCC	2.7
Wank *et al.* (1993), USA	DQB1*0303			4.50 *p* = 0.0012
	DRB1*:DQB1*			
Cuzick *et al.* (2000), United Kingdom	DRB1*0401–DQB1*0301	116		*p* = 0.02
Madeleine *et al.* (2002), USA	DRB1*0401–DQB1*0301	315	HPV 16 SCC	1.9 (1.2–3.1)
Allen *et al.* (1996), Sweden	DRB1*0401–DQB1*0301	150		*p* = 0.01
Lin *et al.* (2001), Senegal	DRB1*1101–DQB1*0301	55	Invasive carcinoma	2.6 (1.0–7.1)
Madeleine *et al.* (2002), USA	DRB1*1101–DQB1*0301	315	HPV 16 SCC	2.9 (1.6–5.2)
Allen *et al.* (1996), Sweden	DRB1*1101–DQB1*0301	150	Cervical cancer	NS
Duggan-Keen *et al.* (1996), United Kingdom	DRB1*07–DQB1*0201	150		NS
Allen *et al.* (1996), Sweden	DRB1*0802–DQB1*0402	150	Cervical cancer	*p* = 0.001
Maciag *et al.* (2000), Brazil	DRB1*15–DQB1*0602	161	SCC	2.0 (1.2–3.6)
Cuzick *et al.* (2000), United Kingdom	DRB1*1501–QB1*0602	116	 HPV 16	NS 1.8 (1.0–3.3)
Apple *et al.* (1994, 1995), USA	DRB1*1501–DQB1*0602	98	SCC and ADC HPV 16	2.9 (1.3–6.7) 4.8 (1.9–11.8)
Madeleine *et al.* (2002), USA	DRB1*1501–DQB1*0602	315	HPV 16 SCC HPV 16	1.0 (0.7–1.4) 0.9 (0.6–1.4)
Allen *et al.* (1996), Sweden	DRB1*1501–DQB1*0602	150	Cervical cancer	NS
Madeleine *et al.*, USA	DRB1*0301–DQB1*02 DRB1*13–DQB1*06	315	HPV 16 SCC	0.7 (0.5–1.0) 0.6 (0.4–0.9)
Apple *et al.* (1994, 1995), USA	DRB1*13–DQB1*0603	98		0.3 (0.1–0.8)

ADC, adenocarcinoma; CIS, carcinoma *in* situ; NS, not significant; SCC, squamous-cell carcinoma

study (Bontkes *et al.*, 1998) and the *B*07–DQB1*0302* haplotype was associated with a strong risk (odds ratio, 8.2) in another (Wang *et al.*, 2002b).

Epidemiological studies in different populations have found various relationships between risk for cervical cancer and HLA polymorphisms. Comparisons are hampered by issues such as small sample sizes, inappropriate controls and chance findings. The probability of chance findings is increased by multiple comparisons that are often made between the extensive number of polymorphisms and the disease. Ethnic admixture within study groups of one race also may contribute to differences in the distribution of HLA polymorphisms between seemingly homogeneous populations. This heterogeneity may influence risk estimates by masking true effects.

2.7.2 *Infectious co-factors*

The central etiological role of HPV (a sexually transmitted infection) in cervical tumorigenesis has led to hypotheses that other microbial agents that also infect the cervicovaginal epithelium could act as co-factors and increase or decrease the risk for cervical cancer in the presence of a high-risk HPV infection (Lacey, 1992). The proposed mechanisms of co-factors (described in Section 4.1.5(*a*)) include direct biological interactions, such as viral co-activation of HPV replication, and indirect effects, such as damage to the epithelial barrier that protects against HPV infection. In this section, special emphasis has been placed on studies of co-factors that controlled for the effects HPV infection in the analyses.

Two sources of bias were common concerns in the studies reviewed: (*a*) the inability to assess temporality; many studies assessed exposure using serological assays that do not distinguish between current and past infections. Moreover, most studies that tested directly for infections in the cervix were cross-sectional. Therefore, it was generally not known whether the presumptive infectious co-factor was present concurrently with a high-risk HPV infection, or whether it preceded or followed the development of cervical neoplasia. This most probably caused bias towards the null. (*b*) residual confounding by HPV; detection of a sexually transmitted infection, even among women who are all currently positive for HPV DNA, may be a surrogate marker for high-risk behaviours or high-risk sexual partners and a consequently greater cumulative lifetime exposure to high-risk HPV. Because of the very strong association of HPV with cervical cancer, a small degree of residual confounding by HPV could account for moderate effects that are putatively associated with a sexually transmitted infection.

(*a*) *Herpes simplex virus (HSV)*

Genital HSV infection is one of the microbial agents that is most frequently studied as a potential co-factor for cervical cancer. Before the causal role of HPV in the development of cervical cancer was firmly established, HSV was itself regarded as a candidate etiological agent — one that, similarly to HPV, could help explain the association of cervical cancer with sexual behaviour (Brinton, 1992). Although in-vitro studies conducted during

the 1970s demonstrated the carcinogenic potential of HSV (Duff & Rapp, 1971a,b; Duff, 1975), HSV DNA was not consistently detected in cervical cancer specimens (Brinton, 1992). An increased understanding of the causal role of HPV and the publication of a large prospective study that found no association of HSV-2 seroantibodies with incident cervical cancer (Vonka *et al*., 1984) shifted the focus away from HSV-2 as an etiological agent (Brinton, 1992; Ferrera *et al*., 1997a; Lehtinen *et al*., 2002). In retrospect, the study by Vonka *et al.* (1984) may have lacked statistical power because of the small number of cases observed during follow-up, and concerns were expressed regarding over-matching (Brinton, 1992). Moreover, laboratory studies demonstrated that HSV-2 DNA does not need to persist for HSV-2 to play a role in the transformation of cervical epithelial cells (i.e. a possible 'hit and run' mechanism) (Galloway & McDougall, 1983; Jones, 1995).

HSV-2 has a much greater tropism for genital tissue and recurs with greater frequency in the genital tract than HSV-1 (Engelberg *et al*., 2003; Sacks *et al*., 2004). Therefore, seroepidemiological studies conducted during or after the 1990s, when assays that could distinguish between HSV-2 and HSV-1 infection came into use, are of most interest (Ashley & Wald, 1999).

The majority of seroepidemiological studies found a moderate or no association between HSV-2 antibodies and cervical neoplasia (Table 61). Lehtinen *et al.* (2002) pooled data and specimens from three population-based Nordic cohorts to form a collective population of more than 500 000 women. No difference was found between the baseline seroprevalence of HSV-2 among 178 incident cervical cancer cases and 525 controls after adjustment for antibodies to HPV 16/18/33 VLPs and cigarette smoking (odds ratio, 1.0; 95% CI, 0.6–1.7).

A study by the IARC (Smith *et al.*, 2002a) that pooled data from seven separate case–control investigations conducted in Brazil, Colombia, Morocco, Peru, the Philippines, Spain and Thailand found a higher seroprevalence of HSV-2 in 1158 cases of squamous-cell carcinoma and 105 cases of adeno-/adenosquamous carcinoma than in 1117 controls; after limiting the analysis to HPV DNA-positive subjects and adjusting for seropositivity to *C. trachomatis*, the associations of HSV-2 seroantibodies with squamous-cell carcinoma (odds ratio, 2.2; 95% CI, 1.4–3.4) and adeno-/adenosquamous carcinoma (odds ratio, 3.4; 95% CI, 1.5–7.7) were still significant.

Several additional investigations reported significant associations of HSV-2 seroantibodies with cervical cancer (Hildesheim *et al*., 1991; Jha *et al*., 1993; Becker *et al*., 1994; Koffa *et al*., 1995; Daling *et al*., 1996; Thomas *et al*., 2001b, c) or cervical neoplasia (Olsen *et al*., 1998b), but most of these studies either did not control statistically for HPV infection despite having tested for HPV (Becker *et al*., 1994; Thomas *et al*., 2001b,c) or observed an association only among HPV-negative women (Jha *et al*., 1993; Koffa *et al*., 1995; Daling *et al*., 1996).

Studies that tested for HSV DNA in the cervix also gave conflicting results. A high prevalence of HSV DNA was detected in neoplastic cervical specimens using sensitive PCR methods in some studies (Koffa *et al*., 1995; Han *et al*., 1997) but these data were not confirmed by others (Vecchione *et al*., 1994; Tran-Thanh *et al*., 2003). After laboratory data

Table 61. Selected epidemiological studies (that assessed exposure to HPV) of seroantibodies to herpes simplex virus-2 (HSV-2) and risk for cervical neoplasia

Reference, study location	Study design	No. and type of cases	No. and type of controls	HSV-2 sero-prevalence	%	Association or odds ratio (95% CI)	Best epidemio-logical control for HPV and method of HPV detection	Comments
Hildesheim *et al.* (1991), Costa Rica, Panama, Colombia, Mexico	Case–control	766 ICC	1532 normal cytology	*HPV 16/18-positive* Cases Controls	 57 39	*HPV 16/18* 1.6 (1.3–1.9) *HPV 16/18 and HSV-2* 8.8 (5.9–13.0)	Filter in-situ hybridization for HPV 16/18/6/11	An insensitive and non-specific assay for HPV, complicating the interpretation of these data.; results suggest interaction between HPV 16/18 and HSV-2.
Peng *et al.* (1991), China	Case–control	101 ICC	146 normal cytology	ICC Controls	42 29	*ICC* 1.3 (0.7–2.3)	Adjusted for HPV 16 and 33 DNA by PCR	HPV DNA assay detected few high-risk types.
Koutsky *et al.* (1992), USA	Prospective cohort of cytologically normal women enrolled through an STD clinic	28 CIN2/3	213 who did not develop CIN2/3	Cases Controls	45 43	*CIN2/3* 1.0 (0.5–2.3)	Adjustment for HPV by Virapap or dot-filter hybridization	HPV assays were insensitive but few HPV-negative women developed CIN2/3; no details of HSV assay given
Jha *et al.* (1993), United Kingdom	Case–control	219 ICC	387 normal cytology	Invasive cancer Controls	11 5	*HPV-adjusted* 2.2 (1.1–4.5) *HPV-positive* 1.8 (0.4–8.6) *HPV-negative* 3.0 (1.5–6.0)	Adjusted by antibodies to HPV 16/18 E7	Insensitivity of HPV 16/18 E7 assay increases change of residual confounding by HPV; HSV-2 ELISA may not have been type-specific; blood collected years after diagnosis
Becker *et al.* (1994), USA	Case–control	128 Hispanic and 73 non-Hispanic CIN2/3	216 Hispanic and 121 non-Hispanic with normal cytology	*All subjects* CIN2/3 Controls	 36 29	*All subjects* 1.3 (0.8–1.9) *Hispanic* 3.1 (1.6–6.2) *Non-Hispanic* 0.7 (0.4–1.2)	Tested for HPV by PCR	No adjustment for HPV

Table 61 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	HSV-2 sero-prevalence	%	Association or odds ratio (95% CI)	Best epidemio-logical control for HPV and method of HPV detection	Comments
Dillner *et al.* (1994), Finland	Case–control	94 ICC	188 normal cytology	IgA in cases IgG in cases Data for controls not reported	28 92	*IgA* 1.0 (0.6–1.8) *IgG* 1.1 (0.4–2.6)	Tested for multiple HPV 16 peptide antibodies	No adjustment for HPV
de Sanjosé *et al.* (1994), Spain and Columbia	Case–control	*Spain* 249 CIN3 223 ICC *Columbia* 276 CIN 3 150 ICC	*Spain* 242 matched to CIN3 238 matched to cancer *Columbia* 270 matched to CIN 3 149 matched to cancers	*Spain* CIN3 Controls ICC Controls *Columbia* CIN3 Controls ICC Controls	 14 11 26 12 61 50 73 60	*Spain* CIN3 1.2 (0.7–2.0) ICC 1.1 (0.6–1.7) *Columbia* CIN3 1.1 (0.7–1.7) ICC 1.1 (0.6–2.1)	Adjusted for HPV DNA by PCR	HSV assay may have had some cross-reactivity between HSV-1 and HSV-2 (Lacey, 1992).
Bosch *et al.* (1996), Spain	Case–control of patients' male partners	306 husbands of women with CIN3 or SCC	327 husbands of women with normal cytology	Data not shown		No association	Adjusted for penile HPV DNA by PCR; HPV in husbands strongly associated with case status	Husbands' HSV-2 seroprevalence data are indirect evidence for or against HSV-2 as a co-factor.
Daling *et al.* (1996), USA	Case–control	264 SCC	541 normal cytology	Squamous carcinoma Controls	37 26	*All subjects* 1.2 (0.8–1.8) *HPV sero- and DNA-negative* 3.6 (1.6–8.0)	Stratified and/or adjusted for HPV 16 serology (all subjects), HPV DNA (cases) by PCR	Only 77% of cases were HPV DNA-positive and given HPV serology insensitivity, residual HPV confounding was a concern.

Table 61 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	HSV-2 sero-prevalence	%	Association or odds ratio (95% CI)	Best epidemio-logical control for HPV and method of HPV detection	Comments
Lehtinen *et al.* (1996), Finland	Nested case–control	27 ICC 72 in-situ cancer	143 cancer-free women	Cancer Controls	15 26	0.6 (0.2–1.4)	Statistical adjustment for HPV 16 VLP antibodies	Analysis controlled for detection of HPV 16 antibodies
Muñoz *et al.* (1996a), Colombia	Case–control of patients' male partners	210 husbands of women with CIN3 92 husbands of women with SCC	262 husbands of women with normal cytology	Data not shown		No association	Tested for penile HPV DNA by PCR	Husbands' HSV-2 seroprevalence data are indirect evidence for or against HSV-2 as a co-factor.
Ferrera *et al.* (1997a), Honduras	Case–control	25 CIN3 48 ICC	50 normal cytology matched to CIN3, 93 matched to ICC	All subjects tested	92	No significant association	Statistical adjustment for HPV DNA by PCR	Few details of the HSV-2 assay were provided, and its specificity could not be confirmed.
Olsen *et al.* (1998), Norway	Case–control	94 CIN2/3	228 normal cytology	CIN2/3 Controls	41 25	2.6 (1.0–6.3)	Adjusted for HPV DNA by PCR and antibodies to HPV 16 VLPs	Stronger association among HPV 16 DNA-positive subgroup of cases and controls. HSV-1 antibodies also associated with CIN2/3
Yoshikawa *et al.* (1999), Japan	Cross-sectional	94 CIN1 40 CIN2 33 CIN3	130 normal cytology	Any CIN Controls	79 72	2.0 (0.7–5.9)	Adjusted for HPV DNA by PCR	Details of HSV assay not reported
Thomas *et al.* (2001c), Thailand	Case–control	190 SCC	291 normal cytology	Squamous carcinoma Controls	58 50	1.4 (1.0–2.0)	Tested for HPV DNA by PCR, but no control for HPV in analyses	

Table 61 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	HSV-2 sero-prevalence	%	Association or odds ratio (95% CI)	Best epidemio-logical control for HPV and method of HPV detection	Comments
Lehtinen *et al.* (2002), Finland, Norway and Sweden	Nested case–control set in three cohorts	178 cancers identified through cancer registries	527 cancer-free women	Controls Cases	12 15	1.0 (0.6–1.7)	Statistical adjust-ment for HPV 16/18/31 by VLP serology	A meta-analysis of prior longitudinal studies also reported in this paper found no HSV-2 effect; total cohort size, 550 000
Smith *et al.* (2002a), Brazil, Columbia, Morocco, Peru, Philippines and Thailand	Pooled analysis of multiple case–control studies	1158 SCC 105 adeno-carcinomas	1117 normal cytology	Controls Squamous carcinoma Adenocarcinoma	26 44 44	*Squamous carcinoma* 2.2 (1.4–3.4) *Adenocarcinoma* 3.4 (1.5–7.7)	Analysis limited to HPV DNA-positive by PCR	Based on comparison with the 164 HPV DNA-positive normal controls
Castle *et al.* (2003b), Jamaica	Cross-sectional	92 CIN3/ICC 117 CIN2	201 CIN1	CIN1 CIN2 CIN3/cancer	61 62 74	*CIN3/cancer* 1.2 (0.6–2.3) *CIN2* 0.8 (0.5–1.5)	Analysis limited to HPV DNA-positive by PCR	
Yokoyama *et al.* (2003), Japan	Cohort	41 CIN1 and 43 CIN2 that persisted or progressed	73 CIN1 and 28 CIN2 that regressed	Regressed Persisted/progressed	79 77	Regressed 1.1 (0.6–1.9)	Adjusted for HPV DNA by PCR	Details of HSV assay not reported

CI, confidence interval; CIN, cervical intraepithelial neoplasia; ELISA, enzyme-linked immunosorbent assay; ICC, invasive cervical cancer; Ig, immunoglobulin; SCC, squamous-cell carcinoma; STD, sexually transmitted disease; VLP, virus-like particles

had shown that selected HSV-2 oncogenes (e.g. *Xho-2*) may be integrated into and persist in cervical cancer cells, Tran-Thanh *et al*. (2003) tested 200 cervical cancer specimens and 244 normal specimens and failed to detect these or other HSV-2 sequences in any cervical specimens.

Molecular epidemiological data have provided only inconsistent support for the hypothesis that HSV-2 is a co-factor in cervical tumorigenesis. Null results were often observed despite the potentially positive bias related to residual confounding by HPV, especially since the chronic and recurrent nature of genital HSV-2 infection would suggest that studies of HSV-2 might be less affected than studies of transient (e.g. bacterial) infections by an inability to address temporality.

(*b*) *Other herpes viruses*

All three β-herpesviruses, cytomegalovirus (CMV), human herpes virus (HHV)-6 and HHV-7 have been detected in cervical specimens (Han *et al*., 1997; Chan *et al*., 2001). While several studies that used sensitive assays to test for viral DNA have suggested that these viruses may be by-standers rather than co-factors in cervical tumorigenesis (Thompson *et al*., 1994; Boyle & Smith, 1999; Chan *et al*., 2001; Tran-Thanh *et al*., 2002), a few small studies reported associations between CMV and cervical neoplasia (Koffa *et al*., 1995), between HHV-6 and cervical cancer (Chen *et al*., 1994; Yadav *et al*., 1996) and between HHV-7 and high-grade cervical neoplasia (Lanham *et al*., 2001). Serological studies have also given conflicting results. Yokoyama *et al*. (2003) found that higher CMV IgG seroantibody titres were significantly associated with a greater risk of persistent CIN and Koutsky *et al*. (1992) reported a higher incidence of CIN2/3 in young patients with sexually transmitted diseases who were CMV-seropositive. Other studies, however, observed no association of CMV seroantibodies with cervical neoplasia (Jha *et al*., 1993; Dillner *et al*., 1994; de Sanjosé *et al*., 1994; Ferrera *et al*., 1997a; Yoshikawa *et al*., 1999).

Epstein-Barr virus (EBV), a γ-herpesvirus, can infect epithelial cells and may play a role in nasopharyngeal carcinoma. However, the role of EBV in cervical tumorigenesis is controversial and consideration must be given to the fact that positive PCR results can occur due to the ability of EBV to infect infiltrating immune cells (Shoji *et al*., 1997; Boyle & Smith, 1999). Several studies using standard PCR (Koffa *et al*., 1995; Voog *et al*., 1997; Ammatuna *et al*., 2000; Lanham *et al*., 2001) and studies that used in-situ PCR and in-situ hybridization to identify the specific infected cells (Payne *et al*., 1995; O'Leary *et al*., 1997; Shoji *et al*., 1997; Elgui de Oliveira *et al*., 1999) found no association of EBV with the presence of cervical neoplasia, whereas positive findings were reported by others using similar methods (Landers *et al*., 1993; Se Thoe *et al*., 1993; Sasagawa *et al*., 2000). In two seroepidemiological studies, serum EBV IgG antibodies were not associated with cervical cancer (Jha *et al*., 1993; Dillner *et al*., 1994).

(*c*) Chlamydia trachomatis

C. trachomatis is the most common sexually transmitted bacterial infection; it is an obligate intracellular bacterium that has prevalence rates of 3–10% among young sexually

active women in the general community, which can rise to 24% in high-risk populations (Burstein *et al*., 1998; Stamm, 1999; Burstein *et al*., 2001; Turner *et al*., 2002). Because 85–90% of *C. trachomatis* infections are asymptomatic, many remain undiagnosed (Turner *et al*., 2002) and untreated, and can persist for several months or even years (Stamm, 1999; Peipert, 2003; Stephens, 2003). *C. trachomatis* infection may also recur, or even possibly be reactivated, similarly to viral infections (Stephens, 2003; Hogan *et al*., 2004). Infection with *C. trachomatis* is associated with squamous metaplasia and hypertrophic ectopy and, when infection is chronic, may lead to sequelae, including pelvic inflammatory disease (Paavonen *et al*., 1988; Stamm, 1999). Therefore, although *C. trachomatis* is a treatable bacterial infection, it is an important cause of chronic intracellular cervical infection and, as a result, long-term co-infection with high-risk HPV may not be uncommon.

C. trachomatis has also been studied extensively as a potential co-factor in cervical tumorigenesis. In comparison with HSV-2, many more epidemiological studies have reported a positive association between this bacterium and high-grade cervical neoplasia and/or invasive cervical cancer (Table 62).

Two large epidemiological investigations observed highly significant associations between *C. trachomatis* seroantibodies and cervical squamous-cell carcinoma (Koskela *et al.*, 2000; Smith, J.S. *et al.*, 2004). The Nordic nested case–control study found that 30% of 149 squamous-cell carcinoma cases compared with 13% of 442 controls were seropositive for *C. trachomatis* at baseline, a difference that persisted after adjustment for detection of antibodies to HPV 16, 18 and/or 31 VLPs (odds ratio, 2.2; 95% CI, 1.3–3.5) (Koskela *et al*., 2000). In the IARC study, 53% of 1139 squamous-cell carcinoma cases but only 31% of 1100 controls were seropositive for *C. trachomatis* and the strength of this association increased with increasing *C. trachomatis* antibody titre (p for trend < 0.001) (Smith, J.S. *et al*., 2004). No association with adeno-/adenosquamous carcinoma was observed in either study. The Nordic nested case–control study also reported a possible association of *C. trachomatis* serovar with risk for cervical cancer (Anttila *et al*., 2001); this association was not found in the IARC study (Smith, J.S. *et al*., 2004).

Similar associations between antibodies to *C. trachomatis* and cervical neoplasia were observed in several other seroepidemiological studies that controlled for HPV (Koutsky *et al*., 1992; de Sanjosé *et al*., 1994; Smith *et al*., 2002b; Matsumoto *et al*., 2003). Two cross-sectional studies observed a significant association between *C. trachomatis* antibodies in men and CIN3 or cancer in their wives (Bosch *et al*,. 1996; Muñoz *et al*., 1996a). Only a minority of studies did not detect a significant association between *C. trachomatis* antibodies and cervical neoplasia (Lehtinen *et al*., 1996; Ferrera *et al*., 1997a).

Furthermore, detection of *C. trachomatis* DNA in the cervix was also associated with neoplasia in several studies. A nested case–control investigation based on the Swedish contingent of the Nordic cohort found that *C. trachomatis* DNA in prediagnostic (still normal) Pap smears was strongly related to the risk for subsequent cervical cancer (odds ratio, 17; 95% CI, 2.6–∞) (Wallin *et al*., 2002). A cross-sectional study showed an association between HSIL (odds ratio, 5.8; 95% CI, 1.5–22) but not squamous cancer (odds ratio, 2.1; 95% CI, 0.36–12) and cervical *C. trachomatis* DNA in HPV DNA-positive subjects

Table 62. Selected epidemiological studies (that assessed exposure to HPV) on the association between Chlamydia trachomatis (CT) infection, cervical neoplasia and cervical cancer

Reference, study location	Study design	No. and type of cases	No. and type of controls	Prevalence of *Chlamydia*	%	Association(s) and/or odds ratio (95% CI)	Best epidemiological control for HPV and method of HPV detection	Comments
Koutsky *et al.* (1992), USA	Prospective cohort of cytologically normal women enrolled through an STD clinic	28 incident CIN2/3	213 who did not develop CIN2/3	*CT culture* Cases Controls *CT seroprevalence* Cases Controls	 21 13 75 55	*CT culture* 1.1 (0.5–2.8) *CT seroprevalence* 2.4 (1.0–5.7)	Adjustment for HPV by Virapap or dot-filter hybridization	HPV assays used were insensitive, but few HPV-negative women developed CIN2/3; no details of the CT seroassay provided
Jha *et al.* (1993), United Kingdom	Case–control	219 ICC	387 normal cytology	ICC Controls	17 7	*HPV-adjusted* 2.2 (1.2–3.9) *HPV-positive* 1.5 (0.4–5.6) *HPV-negative* 3.1 (1.7–5.5)	Adjusted or stratified by antibodies to HPV 16/18 E7	Insensitivity of HPV 16/18 E7 assay increases chance that CT could be surrogate for HPV; blood collected years after diagnosis
Becker *et al.* (1994), USA	Case–control	128 Hispanic and 73 non-Hispanic CIN2/3	216 Hispanic, and 121 non-Hispanic women with normal cytology	*CT seroprevalence* CIN2/3 Controls	 85 82	 1.1 (0.7–1.9)	Tested for HPV DNA by PCR, but no control for HPV in analyses	No adjustment for HPV
de Sanjosé *et al.* (1994), Spain and Columbia	Case–control	*Spain* 249 CIN3 223 ICC *Columbia* 276 CIN3 150 ICC	*Spain* 242 normal cytology matched to CIN3 cases 238 normal cytology matched to cancer cases *Columbia* 270 normal cytology matched to CIN3 cases 149 normal cytology matched to cancer cases	*CT seroprevalence* *Spain* CIN3 Controls ICC Controls *Columbia* CIN3 Controls ICC Controls	 29 11 28 16 48 25 53 41	*Spain* CIN3 2.2 (1.1–4.6) ICC 1.7 (0.9–3.1) *Columbia* CIN3 1.8 (1.1–2.9) ICC 0.9 (0.5–1.8)	Adjusted for HPV DNA status determined using consensus PCR	Increasing CT titre had stronger association with case status, but assay specificity was not optimal (Smith, J.S. *et al.*, 2004).
Bosch *et al.* (1996), Spain	Case–control of patients' male partners	306 husbands of women with CIN3 or SCC	327 husbands of normal cytology women	*CT seroprevalence* Case husbands Control husbands	 21 8	 2.6 (1.4–4.6)	Adjusted for penile HPV DNA by PCR	Husbands' CT seroprevalence data are indirect evidence of CT as a co-factor; HPV in husbands was strongly associated with cancer in wives.

Table 62 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	Prevalence of *Chlamydia*	%	Association(s) and/or odds ratio (95% CI)	Best epidemiological control for HPV and method of HPV detection	Comments
Lehtinen *et al.* (1996), Finland	Nested case–control	72 in-situ cancer or ICC	143 matched controls from same cohort	*CT seroprevalence* Cancer Controls	 10 4	3.0 (0.7–13.4)	Statistical adjustment for HPV 16 VLP antibodies	
Muñoz *et al.* (1996a), Colombia	Case–control of patients' male partners	210 husbands of women with CIN3 92 husbands of women with SCC	262 husbands of normal cytology women	*CT seroprevalence* Case husbands Control husbands	 29 15	2.4 (1.4–4.1)	Tested for penile HPV DNA by PCR, but this was not associated in wives.	Husbands' CT seroprevalence data are indirect evidence of CT as a co-factor, but null association of HPV DNA with cancer may imply residual HPV confounding.
Muñoz *et al.* (1996b), Spain, Colombia	Case–control	85 HPV DNA-negative and normal cytology	725 HPV DNA-negative and normal cytology	*CT seroprevalence* HPV-negative HPV-positive	 20 40	2.3 (1.2–4.2)	HPV DNA by PCR	Some data incorporated in Smith, J.S. *et al.* (2004)
Ferrera *et al.* (1997a), Honduras	Case–control	25 CIN3 50 ICC	50 normal cytology matched to CIN3, 95 matched to ICC	*CT seroprevalence* ICC Controls CIN 3 Controls	 70 62 80 68	*ICC* 0.95 (0.36–2.5) *CIN3* 2.0 (0.5–7.9)	Statistical adjustment for HPV DNA by PCR	
Lehmann *et al.* (1999), Germany	Cross-sectional	29 HPV DNA-positive	115 HPV DNA-negative	*CT DNA* Any HPV HPV-negative	 10 2	$p < 0.05$	HPV DNA by PCR	No control for shared sexual risk factors between CT and HPV
Koskela *et al.* (2000), Finland, Norway and Sweden	Nested case–control	149 SCC 32 ADC	442 women from same cohorts who remained cancer-free, matched to SCC and 94 to ADC	*CT seroprevalence* SCC Controls ADC Controls *CT DNA prevalence* SCC	 30 13 9 7 10	*SCC* 2.2 (1.3–3.5) *ADC* 0.4 (0.1–1.7)	Adjustment for HPV 1618/33 by VLP serology	Some data reported earlier (Dillner *et al.*, 1994, 1997); current data later re-analysed to assess effect of CT serovar (Anttila *et al.*, 2001) and possible interactions between HPV 16 and CT (Hakama *et al.*, 2000; Luostarinen *et al.*, 2004)

Table 62 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	Prevalence of *Chlamydia*	%	Association(s) and/or odds ratio (95% CI)	Best epidemiological control for HPV and method of HPV detection	Comments
Giuliano *et al.* (2001, 2002b), USA–Mexico border	Cross-sectional	259 high-risk HPV 65 low-risk HPV	2153 HPV-negative	*CT DNA* HPV-negative Any HPV High-risk HPV Low-risk HPV	 8 16 18 9	*Any HPV* 1.8 (1.2–2.7) *High-risk HPV* 2.1 (1.4–3.2) *Low-risk HPV* 1.2 (0.5–3.2)	HPV DNA by PCR	Adjusted for sexual behaviour, age, other risk factors
Smith *et al.* (2002b), Africa, Asia, South America, Spain	Case–control	455 SCC 44 ADC	539 normal cytology	*CT seroprevalence* Controls SCC ADC	 22 48 30	*SCC* 2.1 (1.1–4.0) *ADC* Not significant	Cancer analysis limited to HPV DNA-positive by PCR	Some data incorporated in Smith, J.S. *et al.* (2004)
Tamim *et al.* (2002), Lebanon	Cross-sectional	61 HPV DNA-positive 49 HPV DNA-positive	478 HPV DNA-negative 80 HPV DNA-negative	HPV-negative HPV-positive *CT DNA* HPV-negative HPV-positive	22 26 13 59	 1.4 (0.7–2.7) 10.2 (4.2–24.3)	HPV DNA by PCR	No control for shared sexual risk factors between CT and HPV-positive. CT associated with 'abnormal cytology' in HPV-negative strata. Updates Finan *et al.* (2002).
Wallin *et al.* (2002), Sweden	Nested case–control	118 ICC	118 cancer-free women from same cohorts	*CT DNA prevalence* Controls Invasive cancers	 0 8	 17.1 (2.6–∞)	Adjusted analysis for detection of HPV by PCR	CT DNA and HPV DNA detected in baseline specimens, obtained years before cancer
Castle *et al.* (2003b), Jamaica	Cross-sectional	117 CIN2 92 CIN3	201 CIN1	*CT DNA* CIN1 CIN2 CIN3 *CT seroprevalence* CIN1 CIN2 CIN3	 9 10 9 20 17 22	By laboratory method *CT DNA* *p* trend = 0.96 *CT serology* *p* trend = 0.61	Analysis limited to HPV DNA-positive by PCR	Assessed CT gradient by grade of neoplasia; association of CT with HPV DNA not assessed, no comparison of CIN with normal controls
Matsumoto *et al.* (2003), Japan	Cross-sectional	80 CIN1 34 CIN2 27 CIN3	109 normal cytology	*CT seroprevalence* Any CIN Control	 23 11	**Any CIN adjusted for:** *HPV DNA and age* 1.7 (0.7–4.3) *HPV serology and age* 2.7 (1.3–6.0)	Adjusted for HPV DNA by PCR, and/or HPV 16/52/58 by VLP serology	Disparity in results raises concern that false-negative HPV serology resulted in residual HPV confounding; some data reported in Yoshikawa *et al.* (1999)

Table 62 (contd)

Reference, study location	Study design	No. and type of cases	No. and type of controls	Prevalence of *Chlamydia*	%	Association(s) and/or odds ratio (95% CI)	Best epidemiological control for HPV and method of HPV detection	Comments
Molano *et al.* (2003b), Colombia	Cross-sectional	216 high-risk HPV-positive 52 low-risk HPV-positive 9 other HPV	1536 HPV-negative	*CT DNA* HPV-negative > 1 HPV Any HPV High-risk HPV	 5 10 7 7	*> 1 HPV* 2.5 (1.1–5.9) *Any HPV* 1.3 (0.8–2.4) *High-risk HPV* 1.3 (0.7–2.4)	HPV DNA by PCR	Adjusted for no. of regular and casual sex partners, age at first intercourse, condom use; no association of CT with abnormal Pap test, but analysis combined ASCUS and SIL.
Yokoyama *et al.* (2003), Japan	Cohort	41 CIN1 and 43 CIN2	73 CIN1 and 28 CIN2 that regressed	*CT IgA Seroprevalence* Regressed Persisted/progressed	24 29	0.8 (0.5–1.3)	Adjusted for HPV DNA by PCR	Tested for CT IgA (rather than IgG — as in most studies); no report of assay sensitivity or specificity
Giuliano *et al.* (2004), USA–Mexico border	Cross-sectional	30 HSIL	1876 normal cytology	*CT DNA* Control HSIL	 7 13	1.1 (0.3–4.1)	Analysis limited to HPV DNA-positive by PCR	Subjects same as those in study of Giuliano *et al.* (2001)
da Silva *et al.* (2004), Brazil	Case–control	26 HPV DNA-positive	26 HPV DNA-negative	*CT DNA* HPV-negative HPV-positive	 8 35	6.4 (1.1–55.4)	HPV DNA by PCR	No control for shared sexual risk factors
Smith, J.S. *et al.* (2004), Brazil, Colombia, Morocco, Peru, Philippines, Spain, Thailand	Pooled analysis of multiple case–control studies	1139 SCC 99 ADC	1100 normal cytology	*CT seroprevalence* Controls SCC ADC	 31 53 39	*SCC* 1.8 (1.2–2.7) *ADC* 1.0 (0.5–2.0)	Analysis limited to HPV DNA-positive by PCR	Based on comparison with 164 HPV DNA-positive normal controls; some data from Smith *et al.* (2002b) and de Sanjosé *et al.* (1994) included; association of SCC increased with CT titre.
Golijow *et al.* (2005), Argentina	Cross-sectional	75 HSIL 35 SCC	79 normal cytology	*CT DNA* Control HSIL SCC	 11 47 20	*HSIL* 5.8 (1.5–22) *SCC* 2.1 (0.36–12)	Analysis limited to HPV DNA-positive by PCR	Based on comparison with 24 HPV DNA-positive normal controls

ADC, adenocarcinoma; ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; Ig, immunoglobulin; PCR, polymerase chain reaction; SCC, squamous-cell carcinoma; SIL, squamous intraepithelial lesion

(Golijow *et al*. 2005). Another cross-sectional study that did not find an association between *C. trachomatis* DNA and HSIL (Giuliano *et al*., 2004) reported an association between the detection of HPV and *C. trachomatis* DNA after adjustment for sexual behaviour (Giuliano *et al*., 2001, 2002b). Several other studies (Muñoz *et al*., 1996b; Lehmann *et al*., 1999; Tamim *et al*., 2002; Molano *et al*., 2003b; da Silva *et al*. 2004), but not all (Smith, J.S. *et al*., 2002b, 2004; Golijow *et al*., 2005), also observed an association between *C. trachomatis* (DNA or antibodies) and detection of HPV.

Only limited data, however, address the more specific question of the stage at which *C. trachomatis* might have its effects in the multistage process of HPV-associated cervical tumorigenesis and whether it has an effect on (*a*) the risk for HPV infection, (*b*) the persistence of HPV, (*c*) the development of neoplastic cervical lesions and/or (*d*) the persistence and progression of cervical lesions after their development. Most of the data that are available do not support a role of *C. trachomatis* infection in the progression of cervical neoplasia. *C. trachomatis* was not associated with the persistence or progression of lesions in a study of 114 prevalent CIN1 and 71 CIN2 that used *C. trachomatis* IgA seroantibodies as markers of active infection (Yokoyama *et al*., 2003) or in follow-up studies conducted during the 1980s that detected *C. trachomatis* by culture (Syrjanen, K. *et al*., 1986, 1987; Yliskoski *et al*., 1992). Similarly, a cross-sectional investigation found no differences in the detection of *C. trachomatis* DNA or *C. trachomatis* IgG seroantibodies by grade of neoplasia in colposcopy patients with CIN1 ($n = 201$), CIN2 ($n = 117$) or CIN3 ($n = 92$) (Castle *et al*., 2003), and at least one study that had detected a greater prevalence of *C. trachomatis* DNA in SIL relative to normal specimens did not find differences in prevalence between LSIL, HSIL and cancer (Golijow *et al*., 2005).

Overall, the data reported to date provide initial evidence of a possible epidemiological association between *C. trachomatis* and cervical neoplasia. Although the possibility of residual confounding by HPV can not be excluded, the frequent null results reported for HSV-2 and other sexually transmitted infections make it more difficult to attribute the association of *C. trachomatis* and cervical neoplasia entirely to their shared sexual risk factors. The exact stage(s), however, of the multistage process of HPV-associated tumorigenesis that might be affected by *C. trachomatis* has not been examined carefully and remains uncertain.

(*d*) *Other non-viral infections*

Several other non-viral infectious agents have been postulated as co-factors for cervical cancer. An association of *Trichomonas vaginalis* with cervical neoplasia was observed in several studies (Zhang & Begg, 1994), including a few large prospective cohort investigations (Gram *et al*., 1992; Zhang *et al*., 1995; Viikki *et al*. 2000). However, these studies did not control appropriately for HPV and were noted to have other limitations (Boyle & Smith, 1999; Watts *et al*., 2005). In contrast, two recent cross-sectional investigations (Becker *et al*., 1994; Schiff *et al*., 2000) and one prospective cohort study (Watts *et al*., 2005) found no relation between *T. vaginalis* and CIN2/3 or incident SIL, respectively, and neither was bacterial vaginosis found to be associated with cervical neoplasia in several

recent investigations (Peters *et al*., 1995; Frega *et al*., 1997; Castle *et al*., 2001; Boyle *et al*., 2003; Watts *et al*., 2005). Two studies that did report a significant association between bacterial vaginosis and cervical neoplasia did not control for possible confounding factors (Platz-Chrisensen *et al*., 1994; Schiff *et al*., 2000). Studies of the relationship between bacterial vaginosis and HPV infection gave conflicting results (Peters *et al*., 1995; Sikstrom *et al*., 1997; Castle *et al*., 2001; Jamieson *et al*., 2002; Mao *et al*., 2003; Watts *et al*., 2005).

A few epidemiological investigations reviewed by Boyle and Smith (1999) have assessed the possible association of *Neisseria ghonnorrhoeae* with cervical neoplasia and most reported no association (Takac, 1998; Boyle & Smith, 1999). Among the studies that reported a positive association, a large case–control study found some increase in risk for CIN3 or cancer with seroantibodies to *N. ghonnorrhoeae* in the women (de Sanjosé *et al*., 1994) but no association with seroantibodies in their husbands (Bosch *et al*., 1996; Muñoz *et al*., 1996a). A prospective study of young patients at clinics for sexually transmitted disease reported an increased risk for incident CIN2/3 among culture-positive women, but no information on other possibly correlated risk factors was available (Koutsky *et al*., 1992).

There is also little evidence to suggest that *Candida albicans* (Becker *et al*., 1994; Takac, 1998; Schiff *et al*., 2000) or *Treponema pallidum* (de Sanjosé *et al*., 1994; Bosch *et al*., 1996; Muñoz *et al*., 1996a; Ferrera *et al*., 1997a; Schiff *et al*., 2000; Thomas *et al*., 2001c) are co-factors for cervical tumorigenesis.

(*e*) *Inflammation caused by various infections*

Although only infection with *C. trachomatis* has been consistently associated with cervical neoplasia in epidemiological studies, it has been suggested that cervical inflammation in general, regardless of the specific microbial agent involved, may be a risk factor for progression of HPV infection (Castle & Giuliano, 2003). If this assumption is correct, it might help to explain some of the variable findings reviewed above. Consistent with this hypothesis, a cross-sectional study observed that the specific level of inflammation (graded by the number of invading neutrophils) was directly associated with a risk for CIN2/3 (Castle *et al*., 2001). In other studies, CIN2/3 was found to be more strongly associated with 'any' cervical co-infection than with co-infection assessed on an agent-specific basis (Schiff *et al*., 2000), and data from two independent studies suggested that the risk for cervical cancer increased with increasing numbers of possible co-infections detected (Schmauz *et al*., 1989; Dillner *et al*., 1994). Moreover, a small cohort study observed that variations in the detection of HPV DNA over time were related to the presence of any one of a number of cervicovaginal infections rather than to one specific infectious agent (McNicol *et al*., 1994).

(*f*) *Possible protective effects of adeno-associated virus*

Adeno-associated virus (AAV) is the one infectious agent that may reduce the risk for cervical neoplasia. AAV can suppress papillomavirus replication and cellular transformation *in vitro* (Hermonat, 1992, 1994a). However, although a few epidemiological studies

found that detection of AAV DNA was associated with a decreased risk for the presence and/or grade of neoplasia (Walz *et al*., 1997; Coker *et al*., 2001), most studies did not (Strickler *et al*., 1999b; Odunsi *et al*., 2000; Lanham *et al*., 2001; Ahn *et al*., 2003; Grce *et al*., 2004). Serological studies have also given conflicting results. Although several earlier seroepidemiological studies found possible inverse associations of AAV antibodies with cervical neoplasia (Sprecher-Goldberger *et al*., 1971; Mayor *et al*., 1976; Georg-Fries *et al*., 1984; Tobiasch *et al*., 1994), two subsequent serological studies gave negative results. The first found no association of AAV antibodies with cervical neoplasia or invasive cervical cancer in two separate, independent populations of patients (Strickler *et al*., 1999). The second study found a non-significant inverse association with cervical cancer (odds ratio, 0.4; 95% CI, 0.1–1.6) after controlling for HPV, and no association with CIN3 (odds ratio, 1.4; 95% CI, 0.3–6.8) (Smith *et al*., 2001).

2.8 Special populations

2.8.1 *Skin cancer in patients with epidermodysplasia verruciformis (EV) and HPV infection*

EV is a very rare, inherited condition that was first described by Lewandowsky and Lutz (1922). During the following 60 years, approximately 250 cases were reported worldwide (Lutzner & Blanchet-Bardon, 1985). The condition is usually recognized before puberty and is characterized by widespread HPV infection and the later development of multiple cutaneous squamous-cell carcinomas, predominantly at sites that are exposed to the sun. Although basal-cell carcinomas have been described in EV patients, they appear to be rare, and there are no reports of increased risk for malignant melanoma in EV patients (Orth *et al.*, 1980; Orth, 1986, 1987; Majewski *et al*., 1997; Pfister, 2003). Two cases of eccrine carcinoma and a single case of a malignant proliferating trichilemmal tumour have been described in EV patients (Motegi *et al*., 2003). No published standardized mortality ratios (SMRs) are available for skin cancer in EV patients, but a squamous-cell carcinoma:basal-cell carcinoma ratio of 16:1 was reported in one study of 66 EV patients (Tanigaki *et al.*, 1986). About half of these patients had developed warts by the age of 10 years, whilst squamous-cell carcinoma developed between the ages of 30 and 50 years. The average time lag between onset of EV-type skin warts and squamous-cell carcinoma was 24.5 years (Tanigaki *et al*., 1986). Other virus-associated cancers, including liver cancer associated with chronic hepatitis B virus infection (van Voorst Vader *et al*., 1986; see also IARC, 1994), genital carcinoma, tonsillar carcinoma and Burkitt lymphoma, have rarely been described in EV patients (Lutzner & Blanchet-Bardon, 1985; Ishiji *et al*., 2000).

The limited epidemiological data on EV have pointed to a considerable time lag between primary HPV infection and the development of HPV-associated changes. HPV infection is probably acquired during the first days after birth, particularly in EV families; even in the general population, 45% of skin swab samples were positive for HPV DNA shortly after birth (Antonsson *et al*., 2003). There is one report of possible vertical

transmission in an EV patient (Favre *et al*., 1998b). The same HPV types as those found in the skin lesions of the mother were detectable in her amniotic fluid, placenta and genital scrapes. In contrast, EV-type skin warts never start to appear before 4–5 years of age and carcinomas develop much later. Majewski and Jablonska (1997) observed EV patients with skin autografts from the uninvolved internal aspect of the arm that covered areas of the forehead that had been excised for carcinomas. Within the grafted skin, benign lesions started to develop only several years after transplantation. No carcinoma developed for up to 20 years of graft life, whereas premalignant and malignant changes appeared around the grafts. This suggests that HPV-associated skin carcinogenesis is a very slow process.

The high level of consanguinity in EV families suggests an autosomal recessive mode of inheritance (Lutzner, 1978; Tanigaki *et al*., 1986) but, in one family, the inheritance appeared to be X-linked (Androphy *et al*., 1985). Genetic linkage analyses of consanguineous EV families identified susceptibility loci on chromosomes 17q25 and 2p21-p24 (Ramoz *et al*., 1999, 2000). A more detailed analysis of three Algerian and two Colombian EV1 (17q25)-linked families revealed homozygous nonsense mutations in one of two adjacent novel genes, *EVER1* and *EVER2*, in affected family members (Ramoz *et al*., 2002). No such mutations were observed in 90 unrelated individuals. The predicted full-length EVER1 and EVER2 proteins share 28.4% of their amino acids. They have features of integral membrane proteins and are localized in the endoplasmic reticulum.

The immunogenetic background responsible for defects in immunosurveillance of EV-HPV infections and the inability to eliminate EV-HPV-infected cells remains poorly defined (Majewski *et al*., 1997). A comparison of the prevalence of interleukin (IL) 10 promoter polymorphisms in 22 Brazilian EV patients and 27 healthy individuals indicated that genotypes that determine a low IL10 production are significantly increased in EV patients (de Oliveira *et al*., 2003). This is not easy to interpret because IL10 is generally regarded as immunosuppressive, although it has been speculated that low levels of IL10 would allow higher production of tumour necrosis factor-α (TNFα), which could impede antigen-presenting Langerhans cells.

Mutations in exons 5–8 of the *TP53* gene were detected in one of three benign lesions, two of five actinic keratoses, three of nine Bowen carcinomas *in situ* and five of eight squamous-cell carcinomas of two EV patients (Padlewska *et al*., 2001). Five of nine mutations characterized by sequencing were C→T transitions at dicytidine sites that are considered to be ultraviolet (UV) signature mutations. The other four mutations could be caused by reactive oxygen species that result from exposure to the UVA component of sunlight or from oxidative metabolism.

The importance of sunlight (see also IARC, 1992) in the development of EV-associated squamous-cell carcinomas is suggested by the fact that, although skin warts are found on all body sites, the carcinomas occur almost exclusively on sites exposed to the sun (Tanigaki *et al*., 1986). Furthermore, squamous-cell carcinomas appear to develop more frequently in Caucasian EV patients who live in subtropical and tropical climates than in those who live in temperate climates, and are rare in black EV patients. Only two of 33 (6%) black South African EV patients developed squamous-cell carcinomas (van Voorst Vader *et al.*, 1987)

compared with 40–50% of Caucasian patients who lived in Europe (Orth *et al.*, 1979), 58% of Japanese patients who lived in Japan (Tanigaki *et al*., 1986) and 100% of patients who lived in South America (Rueda & Rodriguez, 1976).

HPV types in warts and skin cancers in EV patients

(*a*) *Skin warts*

EV patients develop a variety of skin warts: common warts (verruca vulgaris), consistently plane warts (verruca planar, that are usually somewhat flatter than plane warts in the general population) and frequently the so-called EV-specific lesions, namely, red plaque-like lesions and scaly, pityriasis versicolor-like lesions (Orth *et al*., 1979; Orth, 1987; Majewski *et al*., 1997).

Table 63 summarizes studies of HPV typing of EV-associated skin warts, all of which used restriction enzyme cleavage and hybridization methods without amplification. Reports are based on a limited number of specimens, often from single patients, and only one study included information on control material (Jacyk *et al*., 1993a). Some studies did not specify the type of skin wart examined and many did not specify the number of samples examined.

A large number of HPV types has been found in plane warts and EV-specific lesions. The phylogenetically related types HPV 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 38, 47 and 50 all belong to the papillomavirus genus beta according to the new nomenclature (de Villiers *et al*., 2004a). They are widely referred to as EV-HPV because they were originally found only in EV patients. In addition to EV-HPV, HPV 3 and 10, which are found in plane warts in the general population, were frequently detected.

A very similar spectrum of HPV types has been identified in five EV patients by PCR with degenerate HPV primers (HPV 8, 19, 20, 24, 38, 5-related and 9-related from genus beta, and HPV 2 and 57 that are found in common warts in the general population) (Harris *et al*., 1997; Surentheran *et al*., 1998).

Cutaneous lesions induced by EV-HPV are highly polymorphic, and include verruca planar, red plaque-like lesions, pityriasis versicolor-like lesions and lesions similar to seborrheic keratoses. However, they share a specific cytopathic effect that is identical for various EV-HPVs, the intensity of which depends only on the viral load and the activity of the disease (Majewski *et al*., 1997). The cytopathic effect is characterized by large clear cells with clear nucleoplasma and cytoplasmic keratohyaline granules. A keratinocyte differentiation-dependent viral transcription pattern has been revealed by in-situ hybridization of benign HPV 5-induced lesions, which is characteristic of productive warts with strong E4-specific signals almost throughout the epithelium and L1/2-specific signals in the superficial layers of the stratum granulosum (Haller *et al*., 1995). HPV 3 and 10 are predominantly associated with plane warts, which may be large, pigmented and confluent. The cytopathic effect is again specific with a ‘bird’s eye-like’ appearance of cells due to vacuolization around pyknotic dark nuclei (Majewski *et al*., 1997).

Table 63. HPV types detected by southern blot in skin warts in epidermoplasia verruciformis (EV) patients with multiple lesions

Reference, study location	Types included	No. of cases	Clinical description	HPV type-specific positivity						Comments
				1–3, 10	5	8	17	20	Others[a]	
Orth *et al.* (1979)[b], Europe	1, 2, 3, 4, 5, 8, 9, 12	14	VP RP PV	+ +	 + +	 +			2, 9 9, 12 12	Frozen and paraffin-embedded tissue
Ostrow *et al.* (1982), USA	5	2	NA		+					Frozen tissue
Pfister *et al.* (1983a) [Turkish patient]	1, 2, 3, 4, 5, 6, 8, 10, 11, 20, 25, 29	1	NA		+	+		+[c]	19[c], 25[c], one type not specified	Frozen tissue
Kremsdorf *et al.* (1984), France	14a, 14b, 15, 17a, 17b, 19, 20, 21, 22, 23, 24	8	VV/VP PV				 +	+	14a, 14b, 15, 21 19, 21, 23, 24	Frozen tissue
Lutzner *et al.* (1984), France	2, 3, 5, 8, 9, 14, 15, 17, 20, 21, 22, 23, 24	11	NA	+	+	+		+	2, 14, 22, 9, 9-related	Frozen tissue
van Voorst Vader *et al.* (1986), Netherlands	5, 8, 17, 19, 20, 24	1	NA		+	+	+		19, 24	Frozen tissue
Kanda *et al.* (1989), Japan	1, 2, 3, 5, 8, 12, 14, 17, 20, 21, 38	12	VP, VV PV RP RP	+	 +		 +	 + +	14, 38 12, 14, 38 (multiple HPV types in some lesions) 14, 21	Frozen tissue

Table 63 (contd)

Reference, study location	Types included	No. of cases	Clinical description	HPV type-specific positivity						Comments
				1–3, 10	5	8	17	20	Others[a]	
Jacyk & de Villiers (1993), South Africa	20 not specified	20	VP PV	+	+ +		 +			Frozen tissue
Jacyk *et al.* (1993a), South Africa	20 not specified	5	Seborrheic keratoses PV VP	 +	+ + +		 +		No HPV found in 10 keratoses from non-EV patients	Frozen tissue; includes information on controls
Jacyk *et al.* (1993b), South Africa	20 not specified	1	PV VV						9 4, 9	Frozen tissue
Yutsudo *et al.* (1994), Japan	3, 17, 20, 38 and others not specified	1 1	PV VV, VP	 +			+	+	38	Fresh tissue
Adachi *et al.* (1996), Japan	3, 5, 8, 9, 12, 14, 17, 20, 25, 47; type-specific primers for HPV 5, 14, 21, 47	1	NA		+				14, 21, 47	Paraffin-embedded tissue

NA, not available; PV, pityriasis versicolor-like lesions; RP, red plaque-like lesions; VP, verruca planar; VV, verruca vulgaris

[a] Of those types tested

[b] EV-HPV were named HPV 4 in the original paper and further differentiated and later renamed as HPV 5, 8, 9 and 12 (Orth *et al.*, 1980; Kremsdorf *et al.*, 1984).

[c] Specified later (Gassenmaier *et al.*, 1984)

(*b*) *HPV types in squamous-cell carcinoma of EV patients*

Table 64 summarizes data on HPV types in EV-associated skin cancer, which are derived mostly from single case reports. All studies were performed using hybridization methods without amplification. In the limited number of invasive squamous-cell carcinomas analysed, HPV 5, 8, 14, 17, 20 and 47 have been identified. HPV-21 DNA was disclosed by PCR amplification and sequence analysis in a malignant proliferating trichilemmal tumour (Motegi *et al*., 2003). The dominance of HPV-5 and -8 in malignant tumours from Europe and the USA is in contrast to the presence of multiple EV-HPV types in benign lesions of the same patients (Pfister *et al*., 1983b; Van Voorst Vader *et al*., 1986; reviewed in Orth, 1987), which may point to an increased carcinogenic potential. However, due to the overall small number of patients, data on prevalence should still be regarded as preliminary. In contrast, HPV 3 or 10 were never detected in EV-associated squamous-cell carcinomas and no malignant conversion was observed in EV patients only infected by HPV 3 (Majewski *et al*., 1997).

HPV 5, 8, 17, 20 and 47 DNAs have been found in squamous-cell carcinomas as episomal oligomers and monomers, some in concatemeric form (approximately 100 copies/cell) (Pfister *et al*., 1983b; Yutsudo *et al*., 1985; Deau *et al.*, 1991; Yutsudo *et al*., 1994; Adachi *et al*., 1996). This is in contrast to the frequently observed integration of HPV DNA in cervical cancers. HPV 14 was identified only rarely in a skin carcinoma and appeared to be integrated into the cellular DNA (Orth, 1987). In another case, HPV 5 DNA was integrated in a metastasis whereas viral genomes persisted extrachromosomally in the corresponding primary tumour (Yabe *et al*., 1989, 1991, 1999). Transcripts of HPV 5, 17 and 20 have been demonstrated in squamous-cell carcinomas (Orth, 1987; Yutsudo & Hakura, 1987; Yutsudo *et al*., 1994).

Both wild-type HPV genomes and those with deletions have been found in primary and metastatic tumours (Ostrow *et al*., 1982; Yabe *et al*., 1989; Deau *et al*., 1991). Some cancers contain mostly or only viral DNA with deletions, which primarily affect the late genes but may extend into the non-coding genome region. In addition, sequence variants of the HPV 5 and 8 *E6* gene have been demonstrated in some EV-associated cancers (Deau *et al*., 1991). The significance of these findings and their role in transformation remains unclear.

2.8.2 *Studies of the incidence of HPV-associated neoplasia in transplant patients*

A consistent increase in the incidence of malignancies in organ transplant recipients has been attributed to the effect of chronic immunosuppression that is required to prevent rejection of the transplanted tissue. Immunocompromised individuals are at increased risk for HPV-associated anogenital and cutaneous cancers compared with age-matched healthy individuals. Organ transplant recipients have an almost 100-fold increased risk for squamous-cell skin carcinoma and a 10-fold increased risk for basal-cell carcinoma (Leigh *et al.*, 1999). In Australia, where exposure to UV light is high, the cumulative incidence of skin cancer increased progressively from 7% after 1 year of immunosuppression to 40%

Table 64. HPV types detected by southern blot in skin cancer in epidermoplasia verruciformis (EV) patients

Reference, study location	Types included	No. of cases	HPV type-specific positivity				Comments
			5	8	17	20	
Ostrow *et al*. (1982), USA	5	2	+				Frozen tissue; HPV 5 found in PV lesions, primary SCC and metastatic SCC in same patient; wild-type and sub-genomic HPV 5 found in primary and metastatic tumour
Pfister *et al*. (1983b) [Turkish patient]	1, 2, 3, 4, 5, 6, 8, 10, 11, 20, 25, 29	1	+				Frozen tissue; 100 copies/cell; oligomeric DNA, some persisting in concatemeric form
Lutzner *et al*. (1984), France	1, 2, 3, 5, 8, 9, 14, 15, 17, 20, 21, 22, 23, 24	5	+	+			Frozen tissue; HPV 14 found in one SCC; the seven skin cancers include three Bowen disease and four SCC.
Yutsudo *et al*., (1985), Japan	17	1			+		Fresh tissue; 100 copies/cell; episomal DNA as oligomers and monomers; HPV transcripts in SCC suggests infection.
Orth (1986, 1987), International	1, 2, 3, 4, 5, 8, 9, 12, 14a/b	14	+	+			Frozen tissue; HPV-14b (1 tumour) (approximately 100–300 copies/cell)
van Voorst Vader *et al.* (1986), Netherlands	1, 2, 3, 4, 5, 8, 9, 10, 12, 14, 15, 17, 19–24	1	+				Frozen tissue; HPV 17 and 24 found in peri-lesional tissue
Yabe *et al*. (1989, 1991, 1999), Japan	5	1	+				Tissue storage not specified; HPV 5 found in benign lesions; deleted forms of HPV 5 found in both primary and metastatic tumours from same patient
Yutsudo *et al*. (1994), Japan	3, 17, 20, 38 and others not specified	1				+	Fresh tissue; 100 copies/cell; episomal DNA as oligomers and monomers; HPV transcripts in SCC suggests infection.
Adachi *et al*. (1996), Japan	3, 5, 8, 9, 12, 14, 17, 20, 25, 47; type-specific primers for 5, 14, 21, 47	1					Paraffin-embedded tissue; HPV 47 episomal DNA as oligomers and monomers
Ishiji *et al*. (2000), Japan	3, 5, 16, 20, 57, 58, 60	1				+	Paraffin-embedded tissue; HPV 20 DNA also disclosed by in-situ hybridization in the nuclei of some cancer cells

PV, pityriasis versicolor-like lesion; SCC, squamous-cell carcinoma

after 9 years and 70% after 20 years (Bouwes Bavinck *et al.*, 1996). These data suggest the interplay of UV light and immunosuppression as risk factors in the development of skin cancer (IARC, 1992). The exact role of immunosuppression in conferring increased risk is not known. Current data suggest that it is most strongly associated with the early stages of dysplasia, and that progression to cancer *per se* is not associated with immunosuppression. Similarly, the biology of HPV infection among immunocompromised individuals is not yet known in detail. Also, questions remain about the biology of HPV infection among transplant patients compared with immunocompromised individuals who are HIV-positive (Palefsky & Holly, 2003).

The first reports of cutaneous and anogenital lesions as a result of HPV infection in immunosuppressed transplant recipients appeared in the 1980s. Lutzner *et al*. (1980) described two immunosuppressed renal allograft recipients who developed skin lesions. In both patients, structural antigens of HPV 5 were identified in these lesions by immunofluorescence. The histological and ultrastructural features observed were similar to those previously seen in patients with HPV 5-associated EV, the only condition in which this HPV type had been detected until that time. The data suggested a role of this potentially high-risk virus in skin cancers that are known to occur with increased frequency in immunosuppressed allograft recipients.

Lower genital cytopathology was evaluated in 105 immunosuppressed renal transplant recipients (Halpert *et al*., 1986). Evidence of HPV infection was found in 17.5% and lower genital neoplasia was observed in 9.5% of the patients. The rate of viral infection in the immunosuppressed patients was ninefold greater than that in a general population and 17-fold greater than that in a matched immunocompetent population. The rate of cervical neoplasia was 16-fold greater than that in the general population and nine-fold greater than that in a matched immunocompetent population. In one-third of patients with HPV lesions and one-half of patients with neoplastic lesions, multiple lower genital sites were also involved. Of the risk factors evaluated, only the number of sexual partners was associated with the development of HPV-related lower genital neoplasia.

Studies on the incidence of HPV-associated cancer in transplant patients have been reviewed previously (IARC, 1995). A number of more recent studies are reviewed below.

(*a*) *HPV infection, CIN and invasive cervical and anogenital carcinomas in transplanted patients*

Studies up to 1994 on the prevalence of anogenital and cervical lesions and/or HPV infection in immunosuppressed women following transplantation were reviewed previously (IARC, 1995) and are summarized in Table 65. At least seven studies of transplanted patients have been published subsequently.

To test the hypothesis that renal allograft recipients are at high risk for anal HPV infection and AIN, 133 renal allograft recipients and 145 control patients underwent anoscopy and biopsy (Ogunbiyi *et al.*, 1994). PCR was used to detect HPV 16 DNA in biopsy samples. A histological diagnosis of anal HPV infection or AIN was made in 32 allograft recipients: HPV infection was detected in five patients, 20 had AIN1, three had AIN2,

Table 65. Prevalence or risk of cervical HPV infection, cervical intraepithelial neoplasia (CIN) and invasive carcinoma of the cervix and anogenital carcinomas in transplanted patients

Reference, study location	Method of detection	No. and % with HPV or lesion				Relative risk (95% CI) or *p* value	Comments
		Transplanted patients		Controls			
		No.	%	No.	%		
HPV infection							
Schneider *et al.* (1983), USA	Cytology (koilocytotic atypia)	11/132	8.5	–		9[a] (3.4–20.2)	Frozen tissue
Halpert *et al.* (1986), USA	Cytology	18/81	22.8	2/81	2.5	17[b] (5.0–50.6)	Frozen tissue
MacLean *et al.* (1986), New Zealand	Cytology	5/24	21	–			Frozen tissue
Alloub *et al.* (1989), United Kingdom	DNA hybridization	22/49	45	26/69	38		Paraffin-embedded tissue
	HPV 6/11	9/49	18.4	22/69	32	$p = 0.36$	
	HPV 16/18	10/49	20.4	4/69	6	$p < 0.005$	
	Mixed 6/11 and 6/18	3/49	6.1	–			
Gentile *et al.* (1991), Italy	Cytology/histology	12/39	31	–			Frozen tissue
Gitsch *et al.* (1992), Germany	Histology (condyloma)	7/23	30	–			Frozen tissue
Fairley *et al.* (1994a), Australia	PCR (*L1* consensus primers)	15/69	22	1/22	4.5	$p = 0.05$	Frozen tissue; the *p* value was obtained using a Fisher's exact test.
Ogunbiyi *et al.* (1994), United Kingdom	Anoscopy/biopsy	5/133	3.8	0	0	–	Frozen tissue

Table 65 (contd)

Reference, study location	Method of detection	No. and % with HPV or lesion				Relative risk (95% CI) or *p* value	Comments
		Transplanted patients		Controls			
		No.	%	No.	%		
Ozsaran *et al.* (1999), Turkey	Histology	2/48	4.2	–	–	–	Frozen tissue
Roka *et al.* (2004), Austria	Hybrid Capture 2 with high-acid low-risk probes	14/60	23.3	–	–	–	Frozen tissue; kidney and liver; transplanted patients
Invasive carcinoma							
Schneider *et al.* (1983), USA	Cytology	6/132	4.5	–			Frozen tissue
MacLean *et al.* (1986), New Zealand	Cytology	0/24	0	–			Frozen tissue; mean time since transplant, 61 months
Fairley *et al.* (1994b), Australia and New Zealand	Cytology	12 cases	NA	–		3.3 (1.7–5.8)	Frozen tissue; mean follow-up, 5.8 years; SIR comparing patients on dialysis with transplanted patients
Birkeland *et al.* (1995), Denmark, Finland, Norway and Sweden	Histology	28 cases	NA	–		8.6 (5.7–13)	Frozen tissue; mean follow-up, 4.8 years; SIR; population-based cancer registry data
Ozsaran *et al.* (1999), Turkey	Histology	20/48	4.7	–	–	–	–
Brown *et al.* (2000), USA	PCR MY11/09 and specific primers for 6, 11, 16, 18	13/16	81	8/13	62	$p = 0.02$	Paraffin-embedded tissue

Table 65 (contd)

Reference, study location	Method of detection	No. and % with HPV or lesion				Relative risk (95% CI) or *p* value	Comments
		Transplanted patients		Controls			
		No.	%	No.	%		
CIN							
Porreco *et al.* (1975), USA	Cytology	3/131	2.3	–		[14 (2.8–40)]	Frozen tissue; mean follow-up, 3.6 years
Cordiner *et al.* (1980), United Kingdom	Cytology/histology	5/26	19	–			Frozen tissue; after a mean of 3.8 years of immunosuppression
Ingoldby *et al.* (1980), United Kingdom	Cytology	0/50	0	–			Paraffin-embedded tissue; 3 years of follow-up
Schneider *et al.* (1983), USA	Cytology	6/132	4.5	–			Frozen tissue; mean time to CIN since transplant, 38 months
Halpert *et al.* (1986), USA	Cytology	10/81	12	2/81	2.5	[5.6 (1.1–38)]	Frozen tissue; mean time since transplant, 47 months
Alloub *et al.* (1989), United Kingdom	Histology	24/49	49	7/69	10	[8.5 (3.0–25)]	Paraffin-embedded tissue
Gentile *et al.* (1991), Italy	Cytology/histology	1/39	2.6	–			Frozen tissue; mean time since transplant, 77 months
Gitsch *et al.* (1992), Austria	Histology	2/23	8.7	–			Frozen tissue
David *et al.* (1993), Germany	Cytology/histology	5/58	8.6	–			Frozen tissue

Table 65 (contd)

Reference, study location	Method of detection	No. and % with HPV or lesion				Relative risk (95% CI) or *p* value	Comments
		Transplanted patients		Controls			
		No.	%	No.	%		
Fairley *et al.* (1994a), Australia	Cytology	5/69	7.2	0/22	0		Frozen tissue
Ogunbiyi *et al.* (1994), United Kingdom	Anoscopy/biopsy	27/133	20.3	1/145	0.68	$p < 0.05$	Frozen tissue
Longuet *et al.* (1996), France	Southern blot hybrid	4/81	4.9	0/3000	0	–	Frozen tissue
Sasadeusz *et al.* (2001), Australia	Cytology	Before transplant 7/77	9.1	–		2.2 (1.1–4.2)	Includes four high-grade smears; the risks are for transplanted women compared with the general population.
		After transplant 11/87	12.6	–		7.0 (4.8–10.2)	
Malouf *et al.* (2004), Australia	Cytology	17/166	10.2	–			Includes CIN3; incidence of CIN3 lesions in lung transplanted patients versus women screened from the New South Wales cytology registry

[ ] Calculated by the Working Group
CI, confidence interval; CIN, cervical intraepithelial neoplasia; PCR, polymerase chain reaction; SIR, standardized incidence ratio
[a] Compared with the general population
[b] Compared with the matched immunocompetent population

three had AIN3 and one patient had anal cancer. One subject with AIN was detected in the control group. HPV 16 DNA was detected in 47% and 12.4% of anal biopsies in the transplant recipients and the controls, respectively. Renal allograft recipients were found to be at high risk for anal HPV infection and neoplasia ($p < 0.05$).

The presence of HPV and the ensuing risk for CIN were studied in 48 renal transplant patients who received immunosuppressive therapy (Özsaran *et al*., 1999). Cervical smears were analysed and colposcopy was conducted. Genital neoplasia was found in 20 of the patients. Koilocytosis developed in six of eight (75%) patients who received high-dose immunosuppressive therapy that was necessitated by rejection of the transplant. HPV was found in two of 48 patients, both of whom had koilocytosis in their cervical biopsies. The data show that renal transplant patients who receive immunosuppressive therapy are at increased risk for CIN.

The prevalence of anal HPV infection was studied in organ transplant patients before immunosuppressive therapy (Roka *et al*., 2004). Patients (40 men, 20 women) who underwent solid-organ transplantation (kidney, liver) for the first time were routinely screened for anal HPV infection. Anal swabs were obtained within 24 h after transplantation and analysed for the presence of mucosal-type HPV DNA by liquid DNA/RNA hybridization. Some type of HPV DNA was detected in 14 patients (23.3%), nine patients (15%) were positive for high-risk HPV, eight (13.4%) were positive for low-risk HPV and three (5%) were positive for both types. The prevalence of HPV infection tended to be higher in liver transplant than in kidney transplant recipients (29.4% versus 20.9%), but the difference was not significant. The prevalence of previous HPV infection (23.3%) before immunosuppressive therapy was started was higher than that found in previous studies or in a control group. In particular, the rate of infection with high-risk HPV types was 15%.

HPV types were analysed in lower genital tract neoplasms of renal transplant recipients and compared with virus types found in immunocompetent patients who had similar neoplasms and in normal immunocompetent controls (Brown *et al.*, 2000). Twenty specimens from lower genital tract neoplasms of 16 renal transplant patients, 13 specimens from 13 immunocompetent patients with similar histology and samples from 13 patients with normal lower genital-tract histology were analysed by PCR for the presence of HPV infection. Primers included the L1 region consensus primers and primers specific for the HPV E6 region for subtypes 6, 11, 16 and 18. HPV infection was detected in 21 of 46 specimens tested. Thirteen of the HPV-positive specimens were from transplant patients and eight were from immunocompetent patients (five with and three without disease). This difference was statistically significant between the transplant and immunocompetent group ($p = 0.02$). Although no difference in HPV 6 and/or 11 was detected between the two groups, the difference in HPV types 16 and/or 18 approached statistical significance ($p = 0.06$). High-risk HPV 16 and/or 18 were found at a higher rate in transplant patients than in their immunocompetent counterparts. The combination of reduced immune function and increased HPV 16 and/or 18 infection rate places these patients at increased risk for aggressive lower genital tract neoplastic progression.

The genome of a novel HPV type was cloned from an iatrogenically immunosuppressed woman with persistent low-grade vaginal AIN (Longuet *et al.*, 1996). HPV 74 was found to be phylogenetically related to the low-risk HPV types 6, 11, 44 and 55. HPV 74 or a variant of this type was found in specimens from three additional immunosuppressed women but not in about 3000 anogenital specimens from immunocompetent patients.

Immunocompromised patients, such as renal allograft recipients, have a higher rate of cytological abnormalities following infection with HPV. This is thought to be due to prolonged persistence of the virus because of impaired clearance by the immune system. A retrospective review was conducted of the cervical cytology of women who underwent bone-marrow transplantation and who had had cervical smears performed in the period 1990–98 (Sasadeusz *et al.*, 2001). The number of cytological abnormalities was significantly higher than that in the general population, both before (age-adjusted odds ratio, 2.2; $p = 0.02$) and after bone-marrow transplantation (odds ratio, 7.0; $p < 0.0001$). After transplantation, allogeneic recipients had more abnormalities than autologous patients (odds ratio, 2.6; $p = 0.02$) although only allogeneic recipients had a higher level of abnormalities after transplantation than before (allogeneic odds ratio, 6.8; $p = 0.004$). These observations suggested that pre-transplant disease and treatment factors increase the risk for cytological abnormalities and that transplant-related factors such as conditioning therapy and immunosuppression further increase this risk.

The incidence and outcomes of HPV infection and cervical abnormalities after lung transplantation were investigated in a retrospective cross-sectional study of 166 female recipients who underwent transplantation between February 1989 and June 2001 (Malouf *et al.*, 2004). The incidences of low-grade epithelial abnormality of the cervix, CIN1 and the earliest pre-cancerous changes of the cervical epithelial cells (CIN3) in the post-transplant cohort were 42.2 and 30, respectively, per 1000 women screened. In a large reference population of 20–69-year-old women, these figures were 8.3 and 6.2, respectively, per 1000 women screened. It was concluded that the incidence of cervical abnormalities in lung transplant recipients is about five times higher than that in the general population.

(*b*) *HPV DNA in transplant-associated skin lesions*

(i) *Skin warts*

Individual case reports documented the presence of multiple HPV types, including EV-associated types (Soler *et al.*, 1992; Purdie *et al.*, 1993), in skin warts of transplant recipients. HPV 27 (Ostrow *et al.*, 1989b) and HPV 49 (Favre *et al.*, 1989) were first identified in warts of transplant recipients and HPV 26 in warts of a patient with an unusual immune deficiency syndrome (Ostrow *et al.*, 1984).

Table 66 summarizes the results of larger studies (i.e. that include more than five lesions) of transplant-associated viral warts. In most studies that used in-situ hybridization or southern blot, the overall detection rate of HPV DNA ranged from 60 to 90%. Three studies that used PCR amplification with several sets of genus- and species-specific, degene-

Table 66. Prevalence of HPV DNA in skin warts of transplant recipients

Reference, study location	Method of detection (types included)	No. of cases (warts)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Gassenmaier *et al*. (1986), Germany	Southern blot (1, 2, 3, 4, 5/8, 16/18)	16	8/16 (50)	6/16	1/16	1/16	–	Paraffin-embedded tissue
Rüdlinger *et al*. (1986), United Kingdom	Southern blot (1–4, 10, 5, 6/11, 16)	54	39/54 (72)	39/54	0/54	0/54	–	Frozen tissue; multiple HPV types found in single lesions; no control warts examined
van der Leest *et al.* (1987), USA	Southern blot (1–6)	32 (44)	39/44 (89)	50/44*	4/44*	0/44	–	Frozen tissue; *double infections in 15 cases
Barr *et al*. (1989), United Kingdom	Dot blot and southern blot (1, 2, 4, 5/8)	77	NA	NA	12/77	–	–	Frozen tissue; no control warts examined
Wilson *et al*. (1989), United Kingdom	Southern blot (1, 2, 3, 4, 5, 8)	18	13/18 (72)	9/18	0/18	–	4/18 not further characterized	Frozen tissue; viral genome in HPV 2 warts showed poly-morphism at PvuII and PstI sites.
Blessing *et al*. (1990), United Kingdom	ISH (4, 5, 8)	20	4/20 (20)	0/20	4/20	–	–	Frozen tissue; simple warts, dysplastic warts and EV-like lesions (3) studied; no specimen contained > 1 HPV type; no control wart samples
Obalek *et al*. (1992), France and Poland	Southern blot (1–7, 10, 16, 18, 28, 41, 50)	56 (82)	72/82 (88)	72/82	10/82	0/82	2/82 detected under non-stringent hybridization conditions	Frozen tissue; EV HPV always co-detected with HPV 3 or related types.

Table 66 (contd)

Reference, study location	Method of detection (types included)	No. of cases (warts)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Euvrard *et al.* (1993), France	ISH (1a, 2a, 5, 16/18)	17	14/17 (82)	9/17	0/17	10/17	–	Frozen and paraffin-embedded tissue; multiple HPV types found in single lesions; no control warts examined
Soler *et al.* (1993), France	Southern blot, ISH and PCR (5, 6/11, 16/18, 1a, 2a)	18 transplant 3 non-transplant	11/18 (61) 0/3 (0)	1/18 0/3	1/18 0/3	4/18 0/3	–	Frozen tissue
Trenfield *et al.* (1993), Australia	Southern blot (1, 2, 3, 4, 5/8, 10, 11, 16/18, 41)	18	5/18 (28)	5/18	0/18	0/18	–	Frozen tissue
Hepburn *et al.* (1994), New Zealand	Dot blot (1–5, 6/11, 8, 41, 48, 49)	36 (44)	19 (43)	26/44	4/44	5/44	41 (1)	Multiple types found in some lesions
Pélisson *et al.* (1994), France	ISH (1a, 2a, 5, 6a, 11a, 16 and 18)	8 transplant 7 non-transplant 7 non-transplant normal skin	5/8 (63) 4/7 (57) 0/7 (0)	4/8 4/7 0/7	1/8 0/7 0/7	4/8 0/7 0/7	– – –	Frozen tissue; simple warts examined; multiple HPV types found in single lesions
Shamanin *et al.* (1994b), United Kingdom	PCR and direct sequencing (1–4, 10, 5/8, 6/11, 16/18 and others)	50	28 (60)	15/50	6/50	1/50	Uncharacterized (14)	Frozen tissue; benign warts and EV-like lesions (3) studied; no control warts examined
Stark *et al.* (1994), United Kingdom	Southern blot and PCR (1, 2, 5/8, 6/11, 16/18 and others)	18 transplant 6 non-transplant	10/18 (55) 2/6 (33)	4/18 2/6	3/18 0/6	3/18 0/6	0/18 0/6	Frozen tissue

Table 66 (contd)

Reference, study location	Method of detection (types included)	No. of cases (warts)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
de Villiers *et al*. (1997), United Kingdom	Nested PCR (2 sets of degenerate primers)	8 (15)	1/15 (93)	10/15	2/15	0/15	–	Frozen tissue.
Harwood *et al*. (1999), United Kingdom	Several nested PCR with degenerate, cutaneous, mucosal, EV-HPV-specific primers and direct sequencing	23 (51)	51/51 (100)	47/51	41/51	14/51	7 (4), 41 (5)	Co-detection of two or more distinct HPV types in 94% of lesions
Berkhout *et al*. (2000), Netherlands	Nested PCR with degenerate primers, EV, alpha 2/4, direct sequencing	12 VV 7 VP 16 VS	9/12 (75) 6/7 (86) 10/16 (63)	6/12 6/7 4/16	2, 6, 3*/12 0, 1, 2*/7 7, 7, 5*/16			Frozen tissue; *subgroups of beta-HPV, multiple HPV types in some lesions
O'Connor *et al*. (2001b), Ireland	Nested PCR with mucosal and EV-HPV-specific degenerate primers	11	11/11 (100)	6/11	10/11	0/11	–	Co-detection of two or more distinct HPV types in 6 warts

EV, epidermodysplasia verruciformis; ISH, in-situ hybridization; NA, not available; PCR, polymerase chain reaction; VP, verruca plana; VS, verruca seborrheica; VV, verruca vulgaris

[a] Of those types tested

[b] Alpha 2-, alpha 4-, gamma- and mu-HPV

rate primers detected HPV DNA in more than 90% of skin warts (de Villiers *et al.*, 1997; Harwood *et al.*, 1999; O'Connor *et al.*, 2001b).

Common skin-associated HPV types from the genera alpha2, alpha4, gamma and mu, including HPV 1, 2, 3, 4, 10, 27, 28, 57 and 77, were the most common types to be identified in studies in which appropriate probes were used (83–92%). Depending on the genus-specific sensitivity of the detection system, EV-HPV types (genus beta) were found in 10–90% of the warts. In earlier studies, mucosal HPV types 6/11 and 16/18 were found in 23 of 199 (11%) transplant samples and in none of 16 (0%) controls when probes that detect these HPV types were employed [$p = 0.32$]. A large PCR-based study of 51 warts identified mucosal types in 14 of these (27%). This study employed the most comprehensive set of PCR primers, which allowed co-detection of two or more distinct HPV types in 94% of the lesions (Harwood *et al.*, 1999). Mixed infections were seen predominantly with cutaneous and EV-HPV types. In contrast, in immunocompetent individuals, single HPV types only were detected with the same complex PCR system in all but one of 20 warts from 15 immunocompetent individuals (Harwood *et al.*, 1999). It should be emphasized that no EV phenotype was usually expressed in mixed infections of skin warts of transplant patients (van der Leest *et al.*, 1987; Obalek *et al.*, 1992; Harwood *et al.*, 1999). However, Morrison *et al.* (2002) described verrucae planae with the histological diagnosis of EV in eight of 17 patients who had had organ transplants (HPV 11) or acquired immunodeficiency syndrome (AIDS) (HPV 6) and identified HPV 8 and HPV 5 by in-situ hybridization.

(ii) *Verrucous keratoses (precancerous lesions)*

Table 67 summarizes the prevalence of HPV DNA in case series (of more than five lesions) of verrucous keratoses. HPV DNA detection rates in transplant-associated verrucous keratoses were approximately 20–30% in most studies that used southern blot or in-situ hybridization without amplification. In early PCR-based studies, detection rates were between 24 and 48% (Shamanin *et al.*, 1994b; Stark *et al*., 1994; Tieben *et al*., 1994), which increased to 49–88% by the use of more sophisticated and comprehensive primer systems (de Jong-Tieben *et al*., 1995; de Villiers *et al*., 1997; Berkhout *et al*., 2000; Harwood *et al*., 2000). HPV DNA was also found in 80% of precancerous lesions by a nested PCR with degenerate primers (de Jong-Tieben *et al*., 1995) but in no more than 30% by single-step PCRs that had been developed earlier (Tieben *et al*., 1994).

This underlines the importance of assay sensitivity and indicates that most HPV DNA persists at low copy levels in precancerous skin lesions and in skin cancers (see Sections (iii) and (iv)) of transplant recipients. This notion corresponds to observations of skin cancers in the general population. In contrast, the prevalence of HPV DNA in skin warts was similar when determined by southern blot (60–90%) and highly sensitive PCRs (100%).

The combination of data from studies that used probes designed to detect type-specific HPV, albeit with different methodologies, showed that overall common skin-associated HPV types were found in 27 of 219 (12%) transplant samples compared with four of 23 (17%) control samples [$p = 0.68$]. These types were found in 11 of 17 lesions (65%) by broad-spectrum PCR assays. When comparing studies with similar methodologies, common

Table 67. Prevalence of HPV DNA in verrucous keratoses of transplant recipients

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV-type specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Rüdlinger *et al.* (1986), United Kingdom	ISH (1a, 2, 3, 4, 5/8, 6/11, 16)	11	1/11 (9)	1/11	0/11	0/11	–	Frozen tissue; no control samples examined
Barr *et al.* (1989), United Kingdom	Dot blot (1, 2, 4, 5/8)	NA	NA	NA	7/44	NA	NA	–
Blessing *et al.* (1990), United Kingdom	ISH (4, 5/8)	19	5/19 (26)	2/19	3/19	–	–	Frozen tissue; no control samples examined
Euvrard *et al.* (1991), France	ISH (1, 2, 5, 16/18)	7	0/7 (0)	0/7	0/7	0/7	–	Frozen tissue
Viac *et al.* (1992), France	ISH (multiple probes)	11	4/11 (36)	2/11	0/11	0/11	Uncharacterized (2/11)	Frozen tissue
Euvrard *et al.* (1993), France	ISH (1, 2, 5, 16/18)	21	5/21 (24)	5/21	1/19	3/21	–	Multiple HPV types identified in single lesions; no control tissue examined
Soler *et al.* (1993), France	Southern blot, ISH and PCR (1, 2, 3, 4, 5/8, 6/11, 16/18)	18	11/18 (61)	4/18	1/18	15/18	–	Frozen tissue; multiple HPV types found in single lesions
Trenfield *et al.* (1993), Australia	Southern blot (1, 2, 3, 4, 5/8, 11, 16/18)	26	4/26 (15)	3/26	1/26	0/26	–	Frozen tissue

Table 67 (contd)

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV-type specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
McGregor *et al*. (1994), United Kingdom	PCR (5/8, 6/11, 16/18)	31 transplant 13 non-transplant	0/31 (0) 0/13 (0)	– –	0/31 0/13	0/31 0/13	–	Paraffin-embedded tissue
Pélisson *et al*. (1994), France	ISH (1, 2a, 3, 4, 5, 6a/11a, 16/18)	10 transplant 2 non-transplant	4/10 (40) 0/2 (0)	2/10 0/2	1/10 0/2	4/10 0/2	–	Frozen tissue; multiple HPV types found in single lesions
Shamanin *et al*. (1994b), United Kingdom	Southern blot and PCR (1–4, 10, 5/8, 6/11, 16/18 and others)	40	19/40 (48)	6/40	6/40	0/40	Uncharacterized (7/40)	Frozen tissue; no control samples studied
Stark *et al*. (1994), United Kingdom	Southern blot and PCR (1, 2, 3, 4, 5/8, 6/11, 16/18)	46 transplant 21 non-transplant	11/46 (24) 4/21 (19)	5/46 3/21	2/46 2/21	1/46 0/21	Unknown (3/46)	Frozen tissue; no control samples examined
Tieben *et al*. (1994), Netherlands	PCR and direct sequencing (multiple probes)	10	3/10 (30)	1/10	1/10	0/10	Uncharacterized (1/10)	Frozen tissue; no control samples
de Jong-Tieben *et al*. (1995), Netherlands	PCR (degenerate nested primers, direct sequencing)	15 AK 5 BD	14/15 (93) 2/5 (40)		14/15 2/5			Frozen tissue; frequently more than one HPV type detected
de Villiers *et al*. (1997), United Kingdom	Nested PCR (2 sets of degenerate primers, sequencing)	12 (17)	11/17 (65)	1/17	6/17		7 (1)	Frozen tissue

Table 67 (contd)

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV-type specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Harwood *et al.* (2000), United Kingdom	Degenerate PCR for EV, cutaneous, and mucosal HPV	9 (17)	15/17 (88)	11/17	12/17	2/1		Frozen tissue; 55% of 11 lesions of immunocompetent patients HPV-positive (see Table 24?)
Berkhout *et al.* (2000), Netherlands	Nested PCR with degenerate primers, EV, alpha 2/4, direct sequencing	56	38/56 (68)	NA/56	21/56			Frozen tissue
de Jong-Tieben *et al*. (2000), Netherlands	PCR (degenerate nested primers, direct sequencing)	37 AK 11 BD	18/37 (49) 8/11 (73)		18/37 8/11			Three of 28 (11%) clinically normal skin samples EV-HPV-positive
Forslund *et al.* (2003a), Australia	PCR with FAP- and HPV 38-specific primers, cloning and sequencing	6	2/6 (33)	1/6	2/6			Frozen tissue; 67% of perilesional and buttock swabs were HPV-positive; 70% of 10 lesions of immunocompetent patients HPV-positive (see Table 24?)

AK, actinic keratoses; BD, Bowen's disease; EV, epidermodysplasia verruciformis; ISH, in-situ hybridization; NA, not available; PCR, polymerase chain reaction

[a] Of those tested

[b] Alpha 2-, alpha 4-, gamma- and mu-HPV

cutaneous HPV types are clearly less prevalent in precancerous lesions than in skin warts. Mucosal HPV types were found in about 10% of transplant samples. The high prevalence of HPV now detected in precancerous lesions is due to the frequent detection of EV-HPV types in an average of 65% of transplant samples compared with 6% in earlier studies.

(iii) *Squamous-cell carcinoma*

Table 68 summarizes the prevalence of HPV DNA in case series of transplant-associated squamous-cell carcinoma (see also Table 21 in IARC, 1995 for comparison).

Rates of detection of HPV DNA in these tumours in studies that used southern blot and in-situ hybridization and in early studies that used PCR varied extremely from 0 to 100%. In one case–control study that used PCR and multiple probes, HPV DNA was found in two of nine (22%) control squamous-cell carcinomas compared with 10 of 30 (33%) transplant-associated squamous-cell carcinomas (Stark *et al*., 1994). Consistently high rates of detection of HPV DNA ranging from 54 to 91% have been found since 1995 by the use of sophisticated and comprehensive primer systems. Direct comparisons within individual studies consistently showed higher HPV DNA prevalences in squamous-cell carcinomas of immunosuppressed patients than in those of immunocompetent patients (Harwood *et al*., 2000; Meyer *et al*., 2000; O'Connor *et al*., 2001b; Forslund *et al*., 2003b).

The combination of the data from studies before 1995 showed that common skin-associated HPV types were found in 34 of 452 (7%) transplant and one of nine (11%) control samples. These types were found in 24 of 44 carcinomas (55%) by the broadest PCR spectrum employed (Harwood *et al*., 2000). Mucosal HPV types were found in about 10% of transplant-associated squamous-cell carcinomas. As in the case of precancerous lesions, the high prevalence of HPV found in squamous-cell carcinoma during the past 10 years is due to the frequent detection of a broad spectrum of beta-HPV, including EV-HPV and related types, in 70–80% of the cases.

HPV 8 was detected in a primary squamous-cell carcinoma from the arm and its lymph node metastasis (Morrison *et al.*, 2002).

(iv) *Basal-cell carcinoma*

Table 69 summarizes the prevalence of HPV DNA in case series of basal-cell carcinoma. In studies from 2000 and later, the overall rates of detection were between 33 and 80%. Combining the data from these four studies, the overall detection rate was 30 of 52 (57%); common skin-associated HPV types were found in 23%, beta-HPV in 44% and mucosal HPV in 4%.

(v) *Multiple lesions*

In patients from whom multiple lesions were analysed, certain HPV types were found to prevail in both benign, precancerous and malignant lesions located on different anatomical sites, and partially removed on different occasions up to 7 years apart (Höpfl *et al*., 1997; de Villiers *et al*., 1997; Harwood *et al*., 2000). However, overall no single HPV type seemed to prevail in cutaneous precancerous lesions or skin cancers of transplant recipients.

Table 68. Prevalence of HPV DNA in squamous-cell carcinoma (SCC) of transplant recipients

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Barr *et al.* (1989), United Kingdom	Dot blot (1, 2, 4, 5/8)	25	16/25 (64)	1/25	15/25	–	–	Frozen tissue
Magee *et al.* (1989), USA	ISH (1–4, 16/18, 6/11)	8	8/8 (100)	0/8	–	8/8	–	–
Blessing *et al.* (1990), United Kingdom	ISH (4, 5/8)	11	2/11 (18)	2/11	0/11	–	–	Frozen tissue
Dyall-Smith *et al.* (1991), United Kingdom	PCR amplification (1–4, 5, 7, 9, 11, 16/18, 19, 25)	188	0/188 (0)	0/188	0/188	0/188	–	Frozen tissue; no control SCC studied
Viac *et al.* (1992), France	ISH (multiple probes)	8	2/8 (25)	1/8	0/8	1/8	–	–
Euvrard *et al.* (1993), France	ISH (1, 2, 5, 16/18)	46	25/46 (54)	20/46	2/46	15/46	–	Frozen tissue; multiple HPV types found in single lesions; no control samples studied
Purdie *et al.* (1993), United Kingdom	Dot blot and Southern blot (1–4, 10, 5/8, 6/11, 16/18)	10	6/10 (60)	2/10	2/10	0/10	Unknown (4/10)	–
Smith *et al.* (1993), Australia	PCR amplification (probes not specified)	20	0/20 (0)	–	–	–	–	–
Soler *et al.* (1993), France	Southern blot, PCR and ISH (1–4, 5/8, 6/11, 16/18)	26	21/26 (81)	0/26	6/26	20/26	–	Frozen tissue; multiple HPV types found in single lesions
Trenfield *et al.* (1993), Australia	Southern blot (multiple probes)	40	2/40 (5)	1/40	1/40	0/40	–	Frozen tissue

Table 68 (contd)

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
McGregor *et al.* (1994), United Kingdom	PCR amplification (5/8, 6/11, 16/18)	14 transplant 22 non-transplant	0/14 (0) 0/22 (0)	– –	0/14 0/22	0/14 0/22	–	Paraffin-embedded material
Pélisson *et al.* (1994), France	ISH (1a, 2a, 5, 6a/11a, 16/18)	13	8/13 (62)	3/13	1/13	7/13	–	Frozen tissue; no control SCC studied
Shamanin *et al.* (1994b), United Kingdom	Southern blot and PCR (1, 2, 3, 5, 7, 10, 37, 40)	23	13/23 (57)	4/23	0/23	0/23	41 (1); unknown (8)	Frozen tissue; no control samples examined
Stark *et al.* (1994), United Kingdom	Southern blot and PCR (1–4, 5/8, 6/11, 16/18)	30 transplant patients 9 controls	10/30 (33) 2/9 (22)	3/30 1/9	0/30 1/9	2/30 0/9	Unknown (6)	Frozen samples
Tieben *et al.* (1994), Netherlands	PCR and direct sequencing (multiple probes)	24	5/24 (21)	1/24	3/24	0/24	Unknown (2)	Frozen tissue
Berkhout *et al.* (1995), Netherlands	PCR (degenerate nested primer, direct sequencing)	53	43/53 (81)	0/53	43/53	0/53		Multiple HPV types found in some lesions
Shamanin *et al.* (1996), United Kingdom	Broad-range PCR with degenerate primers	20	13/20 (65)	7/20	2/20	1/20	41, 54, 61 (1 each), 69 (4)	Frozen tissue
de Villiers *et al.* (1997), United Kingdom	Nested PCR (2 sets of degenerate primers)	22 11 intra-epidermal carcinomas	20/22 (91) 10/11 (91)	9/28	> 70%	1*		Frozen tissue; *HPV 11 in a SCC on the thumb

Table 68 (contd)

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types (no. of lesions)	
Harwood *et al.* (2000), United Kingdom	Degenerate PCR for EV, cutaneous, and mucosal HPV	18 (44)	37/44 (84)	24/44	33/44	4//44	66 (1)	Frozen tissue; multiple HPV types found in single lesions
Berkhout *et al.* (2000), Netherlands	Nested PCR with degenerate primers, EV, alpha 2/4, direct sequencing	81	63/81 (78)	18/81	32,41, 23*/81			Frozen tissue; *subgroups of beta-HPV, multiple HPV types in some lesions. 10/31 clinically normal skin samples HPV-positive
de Jong-Tieben *et al*. (2000), Netherlands	PCR (degenerate nested primers, direct sequencing)	50	34/50 (68)		34/50			Three of 24 (13%) clinically normal skin samples EV-HPV-positive
Meyer *et al*. (2000), Germany	Nested PCR with mucosa, cutaneous, and EV-HPV-specific degenerate and type-specific (5, 8) primers	9	6/9 (67)		5/9		70 (1)	Frozen tissue
O'Connor *et al*. (2001b), Ireland	Nested PCR with mucosa and EV-HPV-specific degenerate primers	9	8/9 (89)	0/9	8/9	0/9	–	
Forslund *et al.* (2003a), Norway	PCR with FAP-specific primers	60	33/60 (55)	NA	NA	NA	NA	One HPV 10 and mostly beta-HPV in 8 patients
Forslund *et al.* (2003c), Australia	PCR with FAP- and HPV38-specific primers, cloning and sequencing	11	6/11 (54)	2/11	5/11	–	–	Frozen tissue; 91% of perilesional and 73% of buttock swabs were HPV-positive.

EV, epidermoplasia verruciformis; ISH, in-situ hybridization; KA, keratoacanthoma; NA, not available; PCR, polymerase chain reaction

[a] Of those types tested

[b] Alpha 2-, alpha 4-, gamma- and mu-HPV

Table 69. Prevalence of HPV DNA in basal-cell carcinoma (BCC) of transplant recipients

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types	
Rüdlinger *et al*. (1986), United Kingdom	Southern blot (1–4, 5/8, 6/11, 16)	1	0/1 (0)	0/1	0/1	0/1	–	–
Obalek *et al*. (1988), Poland	Southern blot (1, 4, 5, 10, 11, 16/38)	2	2/2 (100)	2/2	0/2	0/2	–	–
Euvrard *et al*. (1993), France	ISH (mixed probe)	2	0/2 (0)	0/2	0/2	0/2	–	–
Trenfield *et al*. (1993), Australia	Southern blot (1–4, 5/8, 11, 16/18)	11	1/11 (9)	1/11	0/11	0/11	–	–
McGregor *et al*. (1994), United Kingdom	PCR amplification (5/8, 6/11, 16/18)	11 (transplant) 15 (non-transplant)	0/11 (0) transplant 0/15 (0) non-transplant	–	0/11 0/15	0/11 0/15	–	Paraffin embedded tissue
Pélisson *et al*. (1994), France	ISH (1, 2, 5, 6/11, 16/18)	4	3/4 (75)	1/4	0/4	3/4	–	Frozen tissue; no control BCC samples
Tieben *et al*. (1994), Netherlands	PCR (four consensus primers designed to detect cutaneous HPV types)	4	0/4 (0)	0/4	0/4	0/4	0/4	Frozen tissue
Shamanin *et al*. (1996), United Kingdom	Broad-range PCR with degenerate primers	5	3/5 (60)	3/5	0/5	0/5	51, 56	Frozen tissue; multiple HPV types in two cases
Harwood *et al*. (2000), United Kingdom	Degenerate PCR for EV-, cutaneous and mucosal HPV	15 (24)	18/24 (75)	9/24	13/24	2/24		Frozen tissue

Table 69 (contd)

Reference, study location	Method of detection (types included)	No. of cases (lesions)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
				1–4, related types[b]	EV-, beta-HPV	6/11, 16/18	Other types	
Berkhout *et al*. (2000), Netherlands	Nested PCR with degenerate primers, EV, alpha 2/4, direct sequencing	14	5/14 (36)	2/14	0,3, 4*/14			*Subgroups of beta-HPV; multiple HPV types in some lesions
de Jong-Tieben *et al*. (2000), Netherlands	PCR (degenerate nested primers, direct sequencing)	9	3/9 (33)		3/9			Three of 24 (13%) clinically normal skin samples EV-HPV-positive
Forslund *et al.* (2003c), Australia	PCR with FAP- and HPV 38- specific primers, cloning and sequencing	5	4/5 (80)	1/5	3/5			Frozen tissue; 83% of perilesional and 60% of buttock swabs were HPV-positive.

See Table 7 for a description of the primers used.
EV, epidermodysplasia verruciformis; ISH, in-situ hybridization; PCR, polymerase chain reaction
[a] Of those types tested
[b] Alpha 2-, alpha 4-, gamma- and mu-HPV

(*c*) *HPV infection and cancer at other sites in transplant patients*

(i) *Head and neck region*

Three cases of head and neck squamous-cell carcinoma were reported in patients who were 18, 29 and 53 years of age at the time of tumour diagnosis after renal, cardiac or bone-marrow transplantation (Bradford *et al.*, 1990). Time from transplant to diagnosis of tumour ranged from 7 months to 12 years. Only the youngest patient had no history of exposure to the traditional pre-disposing factors, tobacco and alcohol use. Histopathology of all three tumours showed features of koilocytosis with hyperkeratosis and parakeratosis suggestive of HPV infection (Bradford *et al*., 1990) [the Working Group noted that this paper does not provide specific data on the presence of HPV].

A 36-year-old renal transplant recipient who took cyclosporin A presented with bilateral nasal polypoid lesions that involved the nasal septum and lateral nasal walls (Harris *et al.*, 1998b). Pathological findings from surgical excision demonstrated an inverted papilloma with focal atypia and mild dysplasia. DNA from the tissue was tested by PCR and revealed the presence of HPV type 6. Analysis of RNA showed transcription in the tissue of the HPV early proteins E6 and E7. Histologically normal nasal tissue from the same patient contained HPV DNA and transcripts similar to those described in the inverted papilloma specimen (Harris *et al*., 1998b).

HPV infection in oral cyclosporin-induced gingival overgrowth was investigated in renal transplant recipients by assessing morphological changes and by the use of in-situ hybridization with HPV-specific probes (Bustos *et al.*, 2001). Biopsies of gingival overgrowth lesions from 13 renal transplant recipients and four samples of healthy mucosa from these patients were analysed. The pathologist was not aware of the HPV result. Twelve of the 13 samples studied (92.3%) contained HPV, of which four tested positive for HPV 6/11 and one for HPV 16. In 11 of the HPV-positive cases, koilocytotic atypia was found. The four biopsies of normal mucosa from gingival overgrowth patients also contained HPV DNA. These data show that suppression of T-cell function by cyclosporin therapy can result in an increase in HPV infection and add to the growth-stimulating activity of cyclosporin in the oral mucosa.

A total of 10 paraffin-embedded biopsy specimens of epithelial tumours from six heart transplant recipients were studied for the presence of HPV (Auvinen *et al*., 2002). These cases included all epithelial cancer cases among the malignancies seen in 249 heart transplant patients at Helsinki University. HPV DNA was amplified by PCR. A specimen from one patient revealed the presence of HPV 16. In this patient, who had received a heart transplant in 1991 and subsequent chemotherapy including cyclosporin, a tonsillar tumour (epidermoid carcinoma) was discovered in 1997.

(ii) *Urinary tract*

Three cases of de-novo lower urinary tract carcinoma in renal transplant recipients were reported, which showed the potential for unusually rapid urothelial extension and invasion in chronically immunosuppressed individuals (Lemmers & Barry, 1990). Two patients had a history of perianal condylomata acuminata. Tumours from one of these

harboured the genetic sequences of HPV type 6. One patient had multiple manifestations of cyclophosphamide-related urothelial injury, including bladder carcinoma.

One report described two cases of rapidly progressive, multifocal transitional-cell carcinomas of the bladder that developed in two patients after renal and cardiac transplantation, respectively (Noel *et al*., 1994). In both cases, HPV 16 DNA was detected using the PCR amplification method. This HPV type has not been previously described in this type of tumour in transplant recipients. HPV infection may play a role in the development of rapidly progressive multifocal transitional-cell carcinoma in the bladder of immunosuppressed patients.

2.8.3 *Studies in human immunodeficiency virus (HIV)-infected persons*

(*a*) *Studies of the uterine cervix*

(i) *Prevalence of cervical HPV infection and SIL* (Table 70)

In a study from Italy that included 221 women at high risk for HIV, among the 121 HIV-positive women, 58 (47%) had HPV lesions, 23 (40%) of whom had CIN1–3. In the 100 HIV-negative women, 23 (23%) had HPV lesions; among these 23 women, six (26%) had CIN1–3. These findings suggest that HIV infection is associated with HPV lesions and that cervical cytological abnormalities develop in this situation (Branca *et al.*, 1995).

Murphy *et al*. (1995) performed a retrospective study of 136 HIV-positive women who attended an inner city ambulatory HIV clinic over a 6-year period between 1987 and 1992 in Dublin, Ireland. During this time, a total of 165 HIV-infected women attended for management of their HIV disease. The results of cervical cytological specimens (smears) were available for 136 (82.4%) women. Forty-one (30.1%) women had mild dysplasia/CIN1, 21 (15%) had CIN2 and 17 (12.5%) had CIN3. The overall prevalence of dysplasia was 58.1%. Twenty-seven (34.2%) of the women with CIN had cytological evidence of HPV infection. No association between the clinical stage of HIV disease and the presence or degree of SIL was observed (Murphy *et al.*, 1995).

Sun *et al*. (1995) compared the prevalence of HPV infection and CIN in more than 650 HIV-positive and HIV-negative women from the New York area: 60% of HIV-positive and 36% of HIV-negative women had detectable cervical HPV DNA ($p < 0.001$); 27% of HIV-positive women had HPV 16, 24% had HPV 18 and 51% had more than one type. Of the HIV-negative women, 17% had HPV 16, 9% had HPV 18 and 26% had multiple types. Latent HPV infection was defined as the presence of HPV in the absence of disease. Among 208 HIV-positive women with HPV infection, 126 (61.6%) had no evidence of CIN compared with 97.3% of HIV-negative women with HPV infection. Thus, HPV infection in HIV-positive women appears to be more probably associated with CIN than that in HIV-negative women.

Bongain *et al*. (1996) studied 111 HIV-positive women in Nice, France, 39 of whom were pregnant. Each participant underwent four cervical biopsies: 9.9% had CIN2 and 8.2% had CIN3. No significant differences in the prevalence of CIN were noted between

transmission group, Centers for Disease Control stage of disease, $CD4^+$ cell count and pregnancy.

Langley *et al*. (1996) studied 68 HIV-1-positive, 58 HIV-2-positive, 14 HIV-1-positive/HIV-2-positive and 619 HIV-negative women who attended clinics for sexually transmitted diseases in Senegal. HPV was detected in 43% of women by PCR and in 7% by southern transfer hybridization; 7.4% of all women had SIL. Both HIV-1 and HIV-2 were associated with HPV infection. HIV-2 was also associated with SIL but the association between HIV-1 and SIL did not reach statistical significance. Most lesions were LSIL. HIV-positive women who had SIL had a lower ratio of CD4:CD8 cells than HIV-positive women without SIL ($p = 0.003$).

Petry *et al*. (1996) compared the prevalence of cervical HPV infection and CIN among 62 HIV-positive women and 77 HIV-negative women who were immunosuppressed due to other causes. HIV-positive women had a higher prevalence of cervical HPV infection and CIN than HIV-negative immunosuppressed women.

Sopracordevole *et al*. (1996) assessed the relationship between HIV status, level of $CD4^+$ cells and SIL in 51 HIV-positive women in Aviano, Italy. Thirty of 51 patients (58.8%) had confirmed SIL. There was no significant difference in the $CD4^+$ cell count between women with or without SIL, which suggests that the expression of HPV-related dysplasia is a complex process.

Cappiello *et al.* (1997) assessed the association between different HPV genotypes, HIV infection and CIN in a multisite study carried out in Italy. The women were intravenous drug users or sexual partners of intravenous drug users. CIN was detected in 36% of HIV-positive women and in 9% of HIV-negative women. The prevalence of HPV did not differ significantly between HIV-positive and HIV-negative women. The most frequently detected genotypes in both groups were HPV 16 and HPV 18 and were similar between HIV-positive and HIV-negative women. HIV-positive women showed a wider spectrum of HPV genotypes, including low-risk and rare types.

Chiasson *et al*. (1997) studied the prevalence of CIN and vulvovaginal lesions in a group of HIV-positive and HIV-negative women from the New York area, USA. Vulvovaginal condylomata acuminata were found in 5.6% of HIV-positive and 0.8% of HIV-negative women. Multicentric disease was more common among HIV-positive than HIV-negative women and HIV-positive women with vulvovaginal disease were more likely to have CIN than those without (odds ratio, 2.9; 95% CI, 1.1–74). In a multivariate analysis, HIV positivity (adjusted odds ratio, 5.3; 95% CI, 1.3–35.3) and HPV infection (adjusted odds ratio, 6.1; 95% CI, 1.7–39.4) were associated with the detection of vulvovaginal condylomata.

Drapkin *et al*. (1997) performed a retrospective chart review of 89 HIV-positive and 100 HIV-negative women who attended Duke University clinics in southeastern USA. SIL was found in 49.4% of HIV-positive and in 23.0% of HIV-negative women (odds ratio, 3.3; 95% CI, 1.7–6.1).

Table 70. Prevalence of cervical HPV infection and squamous intraepithelial lesions (SIL) in HIV-positive and HIV-negative women

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Branca *et al*. (1995), Italy	No HPV DNA detection	121	100			23.2 HIV+, 11.0 HIV–		Cytology, histology	HIV– women were at high risk for HPV infection.
Murphy *et al*. (1995), Ireland	No HPV DNA detection	136				58.1 SIL HIV+, 27.9 HSIL		Cytology	Retrospective chart review; 80.9% of women acquired HIV through IDU.
Sun *et al*. (1995), USA	MY09/MY11 primers with RFLP typing and E6 primers (16, 18)	344	325	60.4 HIV+, 35.7 HIV–	$p < 0.001$	*LSIL* 14.2 HIV+, 2.8 HIV– *HSIL* 4.9 HIV+, 0.6 HIV–		Cytology, histology	HPV testing performed on cervicovaginal lavage specimen
Bongain *et al*. (1996), France		111				6.1 CIN1 9.9 CIN2 8.2 CIN3		Histology	Of 111 participants 39 were pregnant at time of study; each patient had 4 cervical biopsies.
Langley *et al*. (1996), Senegal	PCR with SBH	68 HIV-1, 58 HIV-2, 14 HIV-1 and HIV-2	619	57.1 HIV-1, 50.0 HIV-2, 75.0 HIV-1 and HIV-2, 40.1 HIV–	2.9 (1.7–4.9) 1.7 (1.0–2.9) 4.9 (0.8–10.3)	*SIL* 7.5 HIV-1, 11.1 HIV-2, 16.7 HIV-1 and HIV-2, 6.8 HIV–	1.8 (0.7–4.7) 2.9 (1.2–7.2) 5.2 (1.4–19.6)	Cytology, histology	Adjusted odds ratio; women enrolled from STD clinics in Senegal; HPV testing performed on endocervical swabs
Petry *et al*. (1996), Germany	Viratype	62	77 allograft recipients, 19 immuno-suppressed	50.0 HIV+, 19.5 HIV– allograft recipients, 31.6 HIV– immuno-suppressed		46.8 HIV+, 16.9 HIV– allograft recipients, 31.6 HIV– immuno-suppressed		Cytology, histology	Cervical swab material used for HPV testing

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Sopracordevole *et al*. (1996), Italy	No HPV DNA detection	51				58.8 SIL		Cytology, histology	
Cappiello, *et al*. (1997), Italy	MY09/MY11 PCR and RFLP (specific types not specified)	135	101	40.3 HIV+, 29.6 HIV–	1.61 (0.89–1.21)	35.8 HIV+, 9.2 HIV–	5.52 (2.43–12.91)	Cytology, histology	HPV typing performed on cells scraped from glass slides; participants or their sexual partners were IDUs.
Chiasson *et al*. (1997), USA	PCR on cervicovaginal lavage specimen using L1 consensus primers	396	375	61.7 HIV+, 35.7 HIV–	$p < 0.001$	5.6 HIV+, 0.8 HIV–	$p < 0.001$	Cytology, histology	Study examined prevalent condyloma of vulva, vagina and perianal region and multicentric condyloma
Drapkin *et al*. (1997), USA	No HPV DNA detection	89	100			*SIL* 49.4 HIV+, 23.0 HIV–	3.3 (1.7–6.1)	Cytology	Retrospective chart review of women in southeastern USA
Ferrera *et al*. (1997b), Honduras	GP5/GP6 PCR with SB analysis (6, 11, 16, 18, 31, 33)	23	28	56.5 HIV+, 18.0 HIV–	6.0 (1.5–26.7)				Study of prostitutes in Tegucigalpa, Honduras; HPV testing on wooden spatula specimen
Frankel *et al*. (1997), USA		55 with adequate cytology results				23 genital condyloma, 47.3 cervical SIL on cytology, 2 vaginal SIL		Cytology	Study of women hospitalized with HIV infection at Yale-New Haven Hospital from October 1994 to April 1995
Rezza *et al*. (1997), Italy	MY09/MY11 primers with RFLP (16, 18, 31, 33, 35, 53, 58)	135	101	40.0 HIV+, 31.7 HIV–	$p = 0.19$	*LSIL* 35.6 HIV+, 8.9 HIV–	5.64 (2.5–13.2)	Cytology	HPV testing performed on cervical cytobrush sample
Calore *et al*. (1998), Brazil	No HPV DNA detection	82				LSIL, 19.5 HIV+ HSIL, 6.1 HIV+		Cytology	Women aged 13–21 years

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
La Ruche *et al*. (1998), Cote d'Ivoire	MY09/ MY11 primers with RFLP analysis for typing	151 HIV+ and HIV– women with LSIL 60 HIV+ and HIV– women with HSIL	151 HIV+ and HIV– 240 HIV+ and HIV–	68.2 LSIL 30.5 LSIL controls 81.7 HSIL 20.4 HSIL controls	4.4 (2.6–7.4) 14.3 (6.6–30.9)			Cytology, histology	Cervical Viva-brush specimen used for HPV testing; controls were chosen at random among women shown not to have cervical lesions; adjusted odds ratio
Maiman *et al.* (1998), USA	MY09/MY11 primers with probing (6, 11, 32, 40, 42, 43, 44, 53, 54, 55, 61, 70, Pap 155, Pap 291, AE2, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73)	253	220	75.1 HIV+, 46.8 HIV–	$p < 0.0001$	*LSIL* 15.4 HIV+, 3.6 HIV–, *HSIL* 7.9 HIV+, 1.6 HIV– *SIL* 50.8 HIV+, CD4 < 200; 28.3 HIV+, CD4 < 200–499; 24.6 HIV+, CD4 > 500	$p < 0.0001$	Cytology, histology	Cervicovaginal lavage specimen used for HPV testing
Rezza *et al*. (1998), Italy	MY09/MY11 primers with RFLP typing (16, 18, 31, 33, 35, 53, 58, 11, 44, 46, 54, 59, 66, CP 6108, CP 8304)	135	101	40.0 HIV+, 31.7 HIV–					HPV testing performed on cytobrush specimen

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Rugpao *et al.* (1998), Thailand	Viratype Plus with typing (16, 18, 31, 35, 45, 52, 56 and 6, 11, 42, 43, 44)	224	257	*HR type* 15.8 HIV+, 3.6 HIV– *LR type* 5.4 HIV+, 8.3 HIV–	5.0 (1.8–14.6) 1.8 (0.6–4.9)	*SIL* 10.6 HIV+, 2.5 (HIV–)	5.3 (2.0–15.2)	Cytology	Women were sexual partners of men with HIV-1 infection.
Six *et al*. (1998), France	SBH (6/11/42, 16/18/33, 31/35/39) and MY09/MY11 PCR with probing (16, 18, 33) and GP1/GP2 primers with probing (all other HPV types)	253	160			*SIL* 265 HIV+, 7.5 HIV– *Among HIV+* 14.9, CD4 > 500; 26.0, CD4 200–500; 38.7, CD4 < 200	 1.5 (0.5–4.2) 3.4 (1.4–8.3) 4.4 (1.7–11.4)	Cytology (with biopsy for HSIL)	HPV testing on cervical cytobrush sample; 278 women were followed prospectively at 6-month intervals for 1 year; reference category was HIV– women.
Uberti-Foppa *et al*. (1998), Italy	HC2 and MY09/MY11 primers with typing by RFLP	168	100	*HC* 66.1 HIV+, 15.0 HIV– *PCR* 91 HIV+, 48 HIV–	$p < 0.001$ $p < 0.001$			Cytology, histology	Collection method for HPV testing not specified; 91 HIV+ women acquired HIV through heterosexual contact and 74 through IDU.
Cu-Uvin *et al*. (1999), USA	MY09/MY11/ HMBO1 L1 PCR	851	434	64.3 HIV+, 27.6 HIV–	4.7 (3.7–6.1)	–			HERS population; HPV testing in cervicovaginal lavage specimen

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Goncalves *et al.* (1999), Brazil	MY09/MY11 PCR with RFLP typing (more than 44 types)	141		65.7 vaginal samples, 64.4 cervical samples, 47.4 perianal samples				Cytology, histology	HPV testing on cytobrush specimens from cervical, vaginal and perianal areas
Hankins *et al*. (1999), Canada	MY09/MY11 primers with probing (14 different types)	375		67.2 HIV+		10.9 confirmed SIL HIV+		Cytology	Women were participants in the Canadian Women's HIV Study.
Kapiga *et al*. (1999), Tanzania	No HPV DNA detection	691				*SIL* 2.9 HIV+; CD4 > 500, 2.1; CD4 200–500, 2.3; CD4 > 500, 8.8	*p* for trend = 0.02	Cytology	Participants were HIV+ pregnant women; cytology collected 3–6 months after delivery
Leroy *et al*. (1999), Rwanda	No HPV DNA detection	103	107			24.3 HIV+, 6.5 HIV–	4.6 (1.8–12.3)	Cytology	All women were pregnant.
Luque *et al*. (1999), USA	HC2	93		47.3 overall, 21.5 HR, 12.9 LR	2.57 (1.29–13.56)	51.3 HIV VL > 10 000 copies/mL, 24.3 HIV VL < 10 000 copies/mL	2.11* (1.12–10.19)	Cytology	Sampling from Cervex brush; *relative risk

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Massad *et al*. (1999), USA	MY09/MY11/HMB01 with probing (6, 11, 16,18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	1713	482			*LSIL* 14.9 HIV+, 2.3 HIV– *HSIL* 2.5 HIV+, 1.2 HIV–	8.9 (4.81–16.4) 2.68 (1.13–6.34)	Cytology	Women were participants in the WIHS; HPV testing was performed on a cervicovaginal lavage specimen.
Palefsky *et al*. (1999), USA	MY09 MY11/HMB01 with probing (6, 11, 16,18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	1778	500	63.4 HIV+, 29.8 HIV–	4.08 (3.29–5.05)			Cytology	Women were participants in the WIHS; HPV testing was performed on a cervicovaginal lavage specimen.
Stratton *et al*. (1999), USA	No HPV DNA detection	452 pregnant 126 non-pregnant				*LSIL* 17.0 pregnant, 23.8 non-pregnant *HSIL* 2.0 pregnant, 2.4 non-pregnant		Cytology	Women were participants in the WITS; 240 women had a Pap smear *post partum*.
Temmerman *et al*. (1999), Kenya	GP5+/GP6+ primers with probing (6, 11, 16, 18, 31, 33)	51	469	41.2 HIV+, 14.3 HIV–	3.91 (2.00–7.65)	17.6 HIV+, 5.1 HIV–	4.77 (1.84–12.36)	Cytology	HPV testing on endocervical brush specimens; women recruited from a family planning clinic in Nairobi

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Ahdieh *et al*. (2000), USA	MY09/MY11/HMB01 PCR primers	184	84	69.6 HIV+, 75.7 $CD4^+$ < 200, 68.5 $CD4^+$ ≥ 200, 26.2 HIV–					Women were participants in the ALIVE cohort and followed semi-annually; HPV was tested on cervicovaginal lavage specimen.
Ammatuna *et al*. (2000), Italy	HC2 and 2-step PCR combining MY09/ MY11 primers with GP5+/GP6+ PCR	110		60.9 HIV+		53.6 HIV+	3.55 (1.96–6.48)	Cytology, histology	HPV typing performed on Ayre's spatula specimen
Branca *et al*. (2000), Italy	MY09/MY11 PCR with RFLP analysis (16, 18, 31, 33, 35, 53, 58)	266	193			*LSIL* 21.8 HIV+, 6.6 HIV– *HSIL* 7.6 HIV+, 3.4 HIV–	3.9 (2.2–7.0) for SIL	Cytology	Women were participants in the DIANAIDS cohort; HIV– women were at high risk for HPV infection; an Ayre's spatula specimen was used for HPV testing.
Cubie *et al*. (2000), Scotland, United Kingdom	HC2	63		12.5 HIV VL < 500, 15.4 HIV VL 500–5000, 32.4 HIV VL 5000–50 000, 52.9 HIV VL > 50 000		25.0 HIV VL < 500, 23.1 HIV VL 500–5000, 37.8 HIV VL 5000–50 000, 52.9 HIV VL > 50 000		Cytology, histology	Sampling from Cervex brush; women followed at 6-month intervals

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
French *et al*. (2000), USA	MY09/MY11/HMB01 (6, 11, 16,18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	1314		66.2 overall, 96.2 SIL, 61.3 normal cytology	11.45 (5.51–23.82)			Cytology	Women were participants in the WIHS; HPV testing was performed on a cervicovaginal lavage specimen; women in this analysis had measurement of serum retinol; multivariate odds ratio
Heard *et al*. (2000), France	MY09/MY11 primers (16, 18, 33) plus SBH with probing (6/11/42, 16/18/33, 31/35/39, other types) and sequencing of unidentifiable types	307		49.5 by PCR 55.5 by SBH		13.7 CIN1 13.3 CIN2/3		Cytology, histology	HPV testing on cotton swab and cervical spatula specimens
Marais *et al*. (2000), South Africa	MY09/MY11 primers and an HPV 16-specific primer set	47	52	85.1 HIV+, 42.3 HIV–	$p = 0.00001$				HPV testing performed on cervicovaginal lavage specimens; participants were commercial sex workers.
Moscicki *et al*. (2000), USA	MY09/MY11 primers (6, 11, 42, 44, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58)	133	55	77.4 HIV+, 54.5 HIV– *HR* 54.9 HIV+, 29.1 HIV–	1.4 (1.1–1.8) 1.8 (1.2–2.7)	*SIL* 33.1 HIV+, 10.9 HIV–		Cytology	Young women aged 13–18 years were participants in the REACH cohort; HPV testing was performed on a cervicovaginal lavage specimen; most of the women had high $CD4^+$ levels, with 50% having $CD4^+ > 500/mm^3$.

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Torrisi *et al.* (2000), Italy	MY09/MY11 PCR with restriction endonuclease typing	104	106 previously normal (Group 2), 112 previously abnormal cytology (Group 3)	53.8 HIV+, 6.6 Group 2, 41.9 Group 3		*SIL* 50 HIV+, 5.7 Group 2, 56.3 Group 3		Cytology, histology	HPV performed on cervicovaginal lavage specimen
Womack *et al.* (2000), Zimbabwe	HC2	249	217	64.3 HIV+, 27.6 HIV–		17.3 HIV+, 5.9 HIV–		Cytology, histology	HPV typing performed on Cyto-soft cervical brush specimen; primary care setting in Harare, Zimbabwe
Duerr *et al.* (2001), USA	SBH/HC2 and MY09/MY11 primers (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 70, Pap 155, Pap 291, W13B)	709	341	64.5 HIV+, 29.2 HIV–	$p < 0.001$	18.8 HIV+, 5.3 HIV–	$p < 0.001$		Cervicovaginal lavage specimen used for HPV testing; women were participants in the HERS.
Hameed *et al.* (2001), USA	HC	209		48 HIV+		11 LSIL 0.1 HSIL		Cytology	Study of HPV testing and cytology among women being followed for HIV infection; HPV testing performed on cervical swab material

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Mayaud *et al*. (2001), Tanzania	MY09/MY11 primers with reverse line blot assay and specific priming (6, 11, 16, 18, 31)	100	555	*Age* *< 20 years* 41 HIV+, 34 HIV– *20–29 years* 33 HIV+, 36 HIV– *≥ 30 years* 33 HIV+, 25 HIV–	1.02 (0.6–16)			Cytology	Pregnant women in Tanzania studied; endocervical cytobrush specimen used for HPV testing
Thomas *et al*. (2001a), Thailand	MY09/MY11 PCR (6/11, 16, 18, 31, 33, 35, 39, 45)	37	214	*6/11* 27.0 HIV+, 8.4 HIV– *16* 24.3 HIV+, 12.1 HIV– *31/33/35/39* 27.0 HIV+, 11.7 HIV–	1.1 (0.4–2.9) 1.2 (0.4–3.2) 1.2 (0.5–3.3)			Cytology	Study of commercial sex workers in Bangkok; HPV testing performed on cervical samples obtained with a Teflon-coated swab
Volkow *et al*. (2001), Mexico	MY09/MY11 primers and GP5/GP6 primers, specific priming of HPV 16 E6/E7 and HPV 18 LCR	85	44 with HIV+ male partner, 55 commercial sex workers	68.7 HIV+, 28.6 HIV–		*SIL* 17.8 HIV+, 12.5 HIV– *HSIL* 8.2 HIV+, 1.8 HIV–	$p < 0.05$	Cytology, histology	HPV testing performed on cervical cytobrush
Chirenje *et al*. (2002), Zimbabwe	No HPV DNA detection	207	355			25.6 HIV+, 6.7 HIV–	$p < 0.001$	Cytology, histology	Women aged 18–50 years recruited from family health centres and family planning clinics in Harare

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Jamieson *et al.* (2002), USA	SBH (11, 16, 18, 51, 52, 53) and MY09/MY11 primers (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 70, Pap 155, Pap 291, W13B)	767	390	63.7 HIV+, 27.4 HIV–	2.3 (2.0–2.8)			Cytology	Cervicovaginal lavage specimen used for HPV testing. Women were participants in the HER.
Levi *et al.* (2002), Brazil	SPF10 primers with reverse line blot (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74)	208		98.1 HIV+; most common types: 6, 39.2; 51, 31.9; 11, 26.0; 18, 24.0; 16, 22.5		13.4% Pap III on cytology		Cytology, histology	Cervical cytobrush specimens were used for HPV testing.
Tate & Anderson (2002), USA	No HPV DNA detection	43	103			73 HIV+, 27 HIV–	$p = 0.019$	Cytology, histology	
Hawes *et al.* (2003), Senegal	MY09/MY11 primers (16, 18, 31, 33, 35, 45, 51, 52, 56)	335 HIV-1 only, 69 HIV-2 only, 29 HIV-1 and HIV-2	3686	69.1 HIV-1 only, 61.8 HIV-2 only, 67.9 HIV-1 and 2, 25.3 HIV–		*SIL/ICC* 17.2 HIV-1 only, 19.5 HIV-2 only, 34.4 HIV-1 and 2, 4.0 HIV–	 2.2 (1.0–4.8) 6.0 (2.1–17.1) 8.0 (2.0–3.15)	Cytology, histology	Cervical cell samples obtained for HPV testing; instrument not specified

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Baay *et al*. (2004), Zimbabwe	GP5+/GP6+ primers with probing for HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and LR types (6, 11, 34, 40, 42, 43, 44, 54)	61	174	54.0 HIV+ 27.0 HIV–	3.18 (1.67–6.10)	15.3 HIV+, 6.0 HIV–	$p = 0.037$	Cytology	Cervicovaginal lavage samples used for HPV testing
Branca *et al*. (2004), Italy	MY09/MY11 PCR plus type-specific priming (E6/E7) (6/11, 16, 18, 31, 33, 35, 45, 52, 53, 58, 66)	17	227	35.7 HIV+, 29.2 HIV–	$p = 0.43$	*HSIL on Pap* 50 HIV+, 18.2 HIV–	4.5 (1.08–18.8)	Cytology, histology	Women were patients referred for assessment of abnormal Pap smears; exocervical and endocervical specimens used for HPV testing
Levi *et al*. (2004), Brazil	HC2 and PGMY primers with line blot probing	255	36	87 HIV+, 100 HIV–				Cytology, histology	Cervical brush samples used for HPV testing; HIV+ women were enrolled during routine gynaecological visit; HIV–controls were all referred to the gynaecologist due to suspicion of CIN or condyloma.

Table 70 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p*-value		
Strickler *et al.* (2005), USA	MY09/MY11/HMB01 (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	1848	514		$p < 0.001$ incident HPV detection versus persistence of HPV infection			Cytology	Women were participants in the WIHS; HPV testing was performed on a cervicovaginal lavage specimen; relationship between HIV viral load, $CD4^+$ level and prevalence, incidence and persistence of HPV infection or SIL examined; interaction was stronger for prevalent and incident infection or SIL than persistent infection or SIL.

See Table 7 for a description of the primers used.

ALIVE, AIDS Link to Intravenous Drug Experience; CI, confidence interval; CIN, cervical intraepithelial neoplasia; DIANAIDS, Italian collaborative study on HIV/HPV; HC2, Hybrid Capture 2; HERS, HIV Epidemiology Research Study; HIV, human immunodeficiency virus; HIV+, HIV-positive; HIV–, HIV-negative; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; IDU, intravenous drug user(s); LCR, long control region; LR, low-risk; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou test; PCR, polymerase chain reaction; REACH, Reaching for Excellence in Adolescent Care and Health; RFLP, restriction fragment length polymorphism; SBH, Southern blot hybridization; STD, sexually transmitted disease; VL, viral load; WIHS, Women's Interagency HIV Study; WITS, Women and Infant Transmission Study

Ferrera *et al*. (1997b) studied the prevalence of HPV infection in 23 HIV-positive and 28 HIV-negative prostitutes in Tegucigalpa, Honduras: 56.5% of HIV-positive and 18.0% HIV-negative women were positive for HPV DNA (odds ratio, 6.0; 95% CI, 1.5–26.7).

Frankel *et al*. (1997) characterized the prevalence of SIL among a group of HIV-positive women admitted to Yale-New Haven Hospital for an HIV-related illness. Of these, 55 women had cytology adequate for interpretation and 47.3% had evidence of cervical SIL.

Rezza *et al*. (1997) studied 135 HIV-positive and 101 HIV-negative women in Italy. SIL was diagnosed in 35.6% of HIV-positive and 8.9% of HIV-negative women (odds ratio, 5.64; 95% CI, 2.48–13.17). HPV DNA was detected in 72% of women with SIL and in 25% of women without SIL. HPV DNA was detected more often among HIV-positive (40%) than among HIV-negative (32%) women (not statistically significant). Multivariate analysis of risk factors for SIL included the use of oral contraceptives (adjusted odds ratio, 2.64; 95% CI, 1.16–6.00), being HIV-positive with < 200 $CD4^+$ cells (adjusted odds ratio, 7.29; 95% CI, 2.12–24.02) and having a high-risk HPV type (adjusted odds ratio, 17.53; 95% CI, 6.25–49.23).

In a young population (13–21 years) of 82 HIV-positive women in Brazil (Calore *et al.*, 1998), 21 (26%) showed characteristic features of HPV-infection and SIL.

La Ruche *et al*. (1998) reported on 2170 HIV-positive and HIV-negative women in Abidjan, Cote d'Ivoire. Women with LSIL, HSIL or cervical cancer were enrolled as were controls without cervical disease. Risk factors for cervical lesions that were studied included cervical HPV infection and HIV-1 and HIV-2 positivity. In a multivariate analyses, LSIL was associated with HPV positivity (adjusted odds ratio, 4.4; 95% CI, 2.6–7.4), HIV-1 positivity (adjusted odds ratio, 2.1; 95% CI, 1.2–3.7) and parity of more than three children (adjusted odds ratio, 2.1; 95% CI, 1.1–3.9). Risk factors for HSIL in the multivariate analysis included HPV positivity (adjusted odds ratio, 14.3; 95% CI, 6.6–30.9), HIV-1 positivity (adjusted odds ratio, 2.3; 95% CI, 1.1–4.7), tobacco chewing (adjusted odds ratio, 5.4; 95% CI, 1.3–22.9) and illiteracy (adjusted odds ratio, 2.1; 95% CI, 1.0–4.3). The only risk factor for cervical cancer that was significant was HPV positivity (adjusted odds ratio, 13.3; 95% CI, 3.2–55.5). A wide diversity of HPV types was found in LSIL and HSIL, but HPV 16 was the most common.

Maiman *et al*. (1998) examined risk factors for SIL in 253 HIV-positive and 220 HIV-negative women in the New York area, USA, and evaluated the sensitivity and specificity of cervical cytology in HIV-positive women. The sensitivity and specificity of cytology for all grades of CIN were 0.60 and 0.80 and those for high-grade CIN were 0.83 and 0.74, respectively. Abnormal cytology was found in 32.9% of HIV-positive women and 7.6% of HIV-negative women. In a multivariate analysis, risk factors for abnormal cytology were HIV infection with > 500 $CD4^+$ cells ($p = 0.002$), 200–499 $CD4^+$ cells ($p < 0.001$) and < 200 $CD4^+$ cells ($p < 0.001$) and HPV infection ($p < 0.001$).

Rezza *et al.* (1998) studied risk factors for cervical HPV infection in 135 HIV-positive and 101 HIV-negative Italian women at risk for HIV infection (intravenous drug users or sexual partners of men at risk for HIV infection). Women who were intravenous drug users

were at nearly threefold higher risk for HPV infection than heterosexual women (adjusted odds ratio, 2.7; 95% CI, 1.4–5.0), and this difference was not influenced by HIV serostatus.

Rugpao *et al*. (1998) studied 224 HIV-positive and 257 HIV-negative women who were partners of men with HIV-1 infection in Chiang Mai, Thailand. HIV-positive women were five times more likely to have an infection with a high-risk HPV type and cervical SIL. The prevalence of SIL increased with decreasing $CD4^+$ cell count, but the trend was not significant.

Six *et al.* (1998) investigated the impact of HIV infection on the prevalence, incidence and short-term prognosis of CIN in a prospective study among women from France and French Guyana with a 1-year follow-up. Prevalence of CIN was 7.5% among HIV-negative women and 26.5% among HIV-positive women. Factors associated independently with prevalence of CIN were lower level of $CD4^+$ cells, infection with HPV 16, 18 and 33 and related types, infection with HPV 31, 35 and 39 and related types, lifetime number of sexual partners, younger age, past history of CIN and lack of past cervical screening. Of 344 women, 278 were followed for 1 year at 6-month intervals. Incidence of CIN ranged from 4.9% in HIV-negative women to 27% in HIV-positive women with $< 500 \times 10^6$/L $CD4^+$ cells ($p < 0.001$). Progression from LSIL to HSIL during follow-up was detected in 38.1% of HIV-positive women with $\leq 500 \times 10^6$/L $CD4^+$ cells but not in HIV-negative women or HIV-positive women with $> 500 \times 10^6$/L $CD4^+$ cells. HPV 16, 18 and 33 and related types were also associated with higher incidence of CIN and progression from low-grade to high-grade CIN.

Uberti-Foppa *et al*. (1998) studied the prevalence of HPV and cytological abnormalities in HIV-positive women in Milan, Italy. Using Hybrid Capture 2, 66% of HIV-positive women and 15% of HIV-negative women were positive for HPV DNA ($p < 0.0001$). PCR gave positive results for HPV DNA in 91% and 48%, respectively ($p < 0.001$). No significant difference was observed with respect to prevalence of HPV between women who acquired it through intravenous drug use and women who acquired HIV through heterosexual contact ($p = 0.09$). LSIL and HSIL were both more common among intravenous drug users than among women who acquired HIV through sexual contact.

Cu-Uvin *et al*. (1999) examined the prevalence of cervical HPV infection in 851 HIV-positive women and 434 HIV-negative women at high risk in the HIV Epidemiology Research Study. HPV infection was more prevalent among HIV-positive women (64% versus 28%). This study showed no statistically significant difference in the prevalence of lower genital tract infections other than HPV between HIV-positive and HIV-negative women.

Goncalves *et al*. (1999) studied cervical cytology and HPV from cervical, vaginal and perianal scrapes from 141 HIV-positive women in Santos City, Brazil. One or more specimens that were positive for HPV DNA were found in 80.8% of patients. Two or more HPV types were detected in 45% of the samples. The most frequent HPV types detected were 16 and 18 (30.5%) and, overall, 34.8% had high-risk types; 65.7% of vaginal samples, 64.4% of cervical samples and 47.4% of perianal samples were positive for HPV DNA.

Hankins *et al*. (1999) examined risk factors for prevalent HPV infection in 375 women who participated in the Canadian Women's HIV Study: 67.2% of the women were positive for HPV DNA and 10.9% had SIL. Women with SIL were more likely to have HPV infection than those without SIL ($p = 0.002$). In a multivariate analysis, risk factors for HPV infection included $CD4^+$ cell count $< 0.20 \times 10^9$/L (adjusted odds ratio, 1.99; 95% CI, 1.17–3.37), non-white race (adjusted odds ratio, 2.00; 95% CI, 1.17–3.42), inconsistent use of condoms in the 6 months before study entry (adjusted odds ratio, 2.02; 95% CI, 1.16–3.50) and lower age; women aged 30–39 years (adjusted odds ratio, 0.51; 95% CI, 0.30–0.87) and 40 years or older (adjusted odds ratio, 0.52; 95% CI, 0.26–1.0) had lower risks than women aged < 30 years.

Kapiga *et al*. (1999) studied the prevalence of and risk factors for cervical SIL among 691 HIV-positive women who attended antenatal clinics in Dar es Salaam, Tanzania. Cytology was collected 3–6 months *post partum*. Mid-upper arm circumference was measured as an indicator of body wasting. The prevalence of SIL in the study population was 2.9%; 55% of the lesions were LSIL and 45% were HSIL. Risk factors for SIL in multivariate analysis included having a $CD4^+$ cell count < 200/mm^3 (odds ratio, 6.15; 95% CI, 1.19–41.37) and decreased by 68% for each 5-cm increase in mid-upper arm circumference (odds ratio, 0.32; 95% CI, 0.10–0.93), which indicated that more advanced HIV-related immunosuppression and body wasting were the primary risk factors for SIL in this population.

Leroy *et al*. (1999) studied the prevalence of SIL and its association with HIV-1 infection among 103 HIV-positive and 107 HIV-negative pregnant women in Kigali, Rwanda. The prevalence of SIL was higher in HIV-positive women than in HIV-negative women: 24.3% versus 6.5% (odds ratio, 4.6; 95% CI, 1.8–12.3); that of LSIL was 14.6% in HIV-negative and 4.6% in HIV-positive women; and that of HSIL was 9.7% and 1.9%, respectively.

Luque *et al*. (1999) included 93 HIV-positive women in upstate New York (USA) in a cross-sectional study to evaluate the relationship between plasma HIV-1 RNA levels and cervical HPV infection. HIV-1 RNA plasma levels of > 10 000 copies/mL were associated with the detection of high-risk HPV DNA types in cervical specimens (relative risk, 2.57; 95% CI, 1.29–13.56). In addition, similar HIV-1 RNA plasma levels were associated with abnormal Pap smears (relative risk, 2.11; 95% CI, 1.12–10.19).

Massad *et al*. (1999) studied the prevalence of and risk factors for abnormal cervical cytology among 1713 HIV-positive women and 482 risk-matched HIV-negative control women who participated in the Women's Interagency HIV Study. Cervical cytology was abnormal in 38.3% of HIV-positive women and 16.2% of HIV-negative women. HSIL was found in only 2.5% of the HIV-positive women. In a multivariate analysis, risk factors for abnormal cytology included HIV infection, lower $CD4^+$ cell count, higher level of HIV RNA, HPV positivity, previous history of abnormal cytology, being employed and the number of male sex partners within 6 months of enrolment. Having more than one abortion was associated with a decreased risk for cytological abnormality.

Palefsky *et al*. (1999) characterized the prevalence of 39 different HPV types in 1778 HIV-positive and 500 HIV-negative women participating in the Women's Interagency HIV Study. HIV-positive women had an increased risk for HPV infection (odds ratio, 4.08; 95% CI, 3.29–5.05). The distribution of HPV types was wide in both HIV-positive and HIV-negative women. HPV 16 was found in 5.2% of HIV-positive and 2.0% of HIV-negative women ($p < 0.001$). The prevalence of most HPV types increased with progressively lower $CD4^+$ cell strata. HPV 16 was not one of these types (see comments by Strickler *et al*., 2003; Section 2.8.3(*a*)(ii)). In a multivariate analysis, HIV-positive women with a $CD4^+$ cell count $< 200/mm^3$ were at highest risk for HPV infection compared with HIV-negative women, regardless of HIV RNA load (odds ratio, 10.13; 95% CI, 7.32–14.04), followed by women with a $CD4^+$ cell count $> 200/mm^3$ and an HIV RNA load > 20 000 copies/mL (odds ratio, 5.78; 95% CI, 4.17–8.08) and those with a $CD4^+$ cell count $> 200/mm^3$ and an HIV RNA load < 20 000 copies/mL (odds ratio, 3.12; 95% CI, 2.36–4.12), after adjustment for other factors. Other risk factors for HPV infection among HIV-positive women included racial/ethnic background (African-American versus Caucasian; odds ratio, 1.64; 95% CI, 1.19–2.28), current tobacco smoking (odds ratio, 1.55; 95% CI, 1.20–1.99) and younger age (age < 30 years versus ≥ 40 years; odds ratio, 1.75; 95% CI, 1.23–2.49).

Stratton *et al*. (1999) studied the prevalence of SIL in a cohort of 452 pregnant and 126 non-pregnant HIV-positive women who participated in the Women and Infant Transmission Study. The prevalence of SIL was similar for pregnant (17.0%) and non-pregnant women (23.8%) ($p = 0.09$). In a multivariate analysis, a lower percentage of $CD4^+$ cells ($p < 0.001$), HSV infection ($p = 0.03$) and inflammation on the Pap smear ($p < 0.001$) were all associated with SIL, but pregnancy status was not.

Temmerman *et al*. (1999) studied 51 HIV-positive and 469 HIV-negative women at a family planning clinic in Nairobi, Kenya. In a multivariate analysis, detection of HPV was associated with HIV-1 infection (odds ratio, 3.9; 95% CI, 2.0–7.7) and the number of pregnancies (for ≥ 3 pregnancies compared with 0 or 1; odds ratio, 0.4; 95% CI, 0.2–0.9). HPV infection was strongly associated with high-grade CIN (odds ratio, 14.9; 95% CI, 6.8–32.8); 17.6% of HIV-positive and 5.1% of HIV-negative women had CIN as detected by a Pap test (odds ratio, 4.77; 95% CI, 1.84–12.36). In a multivariate model, predictors of high-grade CIN included HIV-1 positivity (odds ratio, 4.8; 95% CI, 1.8–12.4), the number of lifetime sexual partners (for ≥ 4 partners compared with 0 or 1; odds ratio, 3.8; 95% CI, 1.1–13.5) and education (for secondary compared with primary; odds ratio, 0.38; 95% CI, 0.17–0.88).

Ahdieh *et al*. (2000) studied 184 HIV-positive and 84 HIV-negative women who participated in the ATDS Link to Intravenous Drug Experience cohort and were followed semi-annually over a 6-year period. Of the 187 participants who were positive for HPV at least once, the probability of subsequent HPV positivity was 47.5% for HIV-negative women, 78.7% for HIV-positive women with $CD4^+$ cell counts ≥ 200 and 92.9% for HIV-positive women with $CD4^+$ cell counts < 200 cells/μL ($p < 0.001$). Compared with HIV-infected participants, the relative incidence of HPV clearance was 0.29% and 0.10%

among HIV-positive women with $CD4^+$ cell counts ≥ 200 and < 200 cells/μL ($p < 0.001$), respectively.

Ammatuna *et al*. (2000) studied the presence of HPV DNA in cervical scrapings from 110 HIV-positive women. Using PCR, HPV DNA was found in 60.9% of the samples. Using Hybrid Capture 2, low-risk HPV types were found in 19.4% of the patients, high-risk HPV types in 41.8% and both low-risk and high-risk types in 38.8%. CIN was found in 53.6% of the women. HPV was associated with the detection of CIN (odds ratio, 3.55; 95% CI, 1.96–6.48).

Branca *et al*. (2000) studied 266 HIV-positive and 193 HIV-negative women at high risk in Italy. HIV-positive women were more likely to have SIL (odds ratio, 3.9; 95% CI, 2.2–7.0), most of which were low-grade, while a high prevalence of HPV DNA PCR genotypes was observed in both groups: 48.5% of HIV-positive women and 52% of HIV-negative women had one or more high-risk HPV types detected by PCR.

In a study from Scotland, United Kingdom (Cubie *et al.*, 2000) that included 63 HIV-infected women, high-risk HPV types were detected in 25% of those with normal cytology, while over 80% of women with abnormal cytology were high-risk HPV-positive.

French *et al.* (2000) explored the relationship between vitamin A (retinol) deficiency and SIL in 1314 HIV-positive women who participated in the Women's Interagency HIV Study. At the baseline visit, 15.5% had retinol concentrations consistent with deficiency (< 1.05 μmol/L). In a multivariate model, SIL was associated with retinol concentrations < 1.05 μmol/L (odds ratio, 1.62; 95% CI, 1.02–2.58) together with HPV infection (odds ratio, 11.45; 95% CI, 5.51–23.82), older age (per 10 years; odds ratio, 0.57; 95% CI, 0.44–0.76), being Hispanic/Latin American (odds ratio, 1.86; 95% CI, 1.03–3.37), higher $CD4^+$ levels (per 100 cells/mm^3; odds ratio, 0.80; 95% CI, 0.72–0.88), a body mass index < 18.5 (odds ratio, 2.16; 95% CI, 1.05–4.47) or a body mass index > 25.0 (odds ratio, 0.66; 95% CI, 0.46–0.93).

Heard *et al*. (2000) studied risk factors for CIN in 307 HIV-positive women. CIN was diagnosed in 27.0% and HPV infection in 52.8% of the women. Among all HPV-positive women, high HPV load was found in 55.6%. High HPV viral load was more common among women with $CD4^+$ cell counts < 200/μL compared with those with $CD4^+$ cell counts > 200/μL ($p = 0.002$). High HPV viral load was also associated with an increased risk for CIN in a multivariate analysis in comparison with HPV-negative women (adjusted odds ratio, 16.8; 95% CI, 7.0–40.3). Low HPV viral load was a risk factor for CIN only in women with $CD4^+$ cell counts < 200/μL (adjusted odds ratio, 7.4; 95% CI, 1.3– 43.0).

Marais *et al*. (2000) studied the prevalence of HPV infection in 47 HIV-positive and 52 HIV-negative sex workers in South Africa as well the prevalence of antibodies to HPV 16 VLP by ELISA in cervicovaginal lavage and serum specimens. HIV-positive women had a significantly higher prevalence of HPV DNA than HIV-negative women (85% versus 42%; $p = 0.00001$). They also had a lower rate of positivity than HIV-negative women for serum IgA antibodies ($p = 0.012$) but a higher rate of positivity for cervical anti-VLP 16 IgG antibodies ($p = 0.002$).

Moscicki *et al*. (2000) studied the prevalence of cervical HPV infection and SIL in 133 HIV-positive and 55 HIV-negative women aged 13–18 years who participated in the Reaching for Excellence in Adolescent Care and Health cohort. Few of the HIV-infected women (6.6%) had $CD4^+$ cell levels < 200/mm^3. HPV infection was found in 77.4% of HIV-positive and 54.5% of HIV-negative women (relative risk, 1.4; 95% CI, 1.1–1.8). Among those with HPV infection, 70.1% of the HIV-positive and 30% of the HIV-negative women had abnormal cytology ($p < 0.001$). In a multivariate analysis, HIV positivity was a significant risk factor for both HPV infection (odds ratio, 3.3; 95% CI, 1.6–6.7) and SIL (odds ratio, 4.7; 95% CI, 1.8–14.8). $CD4^+$ cell count and HIV viral load were not associated with HPV infection or SIL.

Torrisi *et al*. (2000) examined the prevalence of HPV in 104 HIV-positive women, 106 HIV-negative women with previously normal cytology (Group 2) and 112 HIV-negative women with previously abnormal cytology (Group 3). SIL was found in 50% of HIV-positive versus 5.66% of HIV-negative Group 2 ($p < 0.001$) and 56.3% of HIV-negative Group 3 women ($p = 0.433$). HPV DNA positivity was found in 53.8% of HIV-positive, 6.6% of HIV-negative Group 2 and 42% HIV-negative Group 3 women. Multiple HPV types were found in 21.4% of HIV-positive women.

Womack *et al*. (2000) characterized cervical HPV infection in 466 women at high risk for HIV infection during primary cervical cancer screening in Zimbabwe. Compared with HIV-negative women, HIV-positive women had a more than twofold prevalence of HPV (64.3% versus 27.6%), a nearly threefold higher prevalence of high-grade CIN (17.3% versus 5.9%) and more than sevenfold the amount of HPV DNA. The amount of HPV DNA increased with severity of disease in both HIV-negative and HIV-positive women.

Duerr *et al*. (2001) examined risk factors for SIL among 709 HIV-positive and 341 HIV-negative women who participated in the HIV Epidemiology Research Study. SIL was more common among HIV-positive than among HIV-negative women (18.8% versus 5.3%; $p < 0.001$) as was HPV infection (64.5% versus 29.2%; $p < 0.001$). In a multivariate analysis, the association with SIL was higher for high-risk HPV types (adjusted prevalence ratio, 27.0; 95% CI, 12.5–58.4) than for low-risk types (adjusted prevalence ratio, 10.5; 95% CI, 4.5–24.6). Intermediate-risk types showed little difference from high-risk types (adjusted prevalence ratio, 25.0; 95% CI, 11.6–54.2). Lower $CD4^+$ cell levels were also associated with SIL but more weakly than HPV infection ($CD4^+$ < 200; adjusted prevalence ratio, 1.9; 95% CI, 1.2–3.0; $CD4^+$ 200–500; adjusted prevalence ratio, 1.6; 95% CI, 1.0–2.5).

Hameed *et al.* (2001) studied 209 HIV-positive women for whom Hybrid Capture and cytology data were available. One hundred and one women (48%) were positive for HPV subtypes by DNA typing by this method; 19/9% had SIL according to cytology, most of which were low-grade.

Mayaud *et al*. (2001) studied the relationship between HPV infection, HIV infection and SIL in 100 HIV-positive and 555 HIV-negative pregnant women in Tanzania. There was no association between HPV and HIV (odds ratio, 1.02; 95% CI, 0.6–1.6). SIL was

associated with HPV (odds ratio, 3.66; 95% CI, 1.9–7.0), but not with HIV (odds ratio, 1.54; 95% CI, 0.7–3.4).

Thomas *et al.* (2001a) reported no significant association between HPV and HIV infection in a study of sex workers in Bangkok.

Volkow *et al*. (2001) studied the prevalence of HPV infection and SIL in 85 HIV-positive and 99 HIV-negative women at high risk in Mexico. Cases included women who were positive for HIV and accepted to participate. HPV DNA was detected by PCR in 69% of HIV-positive women and 29% of HIV-negative women ($p < 0.0001$).

Chirenje *et al*. (2002) performed a cross-sectional study of the prevalence of CIN among 207 HIV-positive women and 355 HIV-negative women who attended a family health centre and family planning clinics in Harare, Zimbabwe. Cervical cytology was abnormal in 25.6% of HIV-positive women compared with 6.7% of HIV-negative women ($p < 0.001$).

Jamieson *et al*. (2002) examined risk factors for HPV infection and its association with cytological abnormalities at baseline in 767 HIV-positive women and 390 HIV-negative women in the HIV Epidemiology Research Study. HIV-positive women were more likely to have HPV infection than HIV-negative women (prevalence ratio, 2.3; 95% CI, 2.0–2.8). The distribution of HPV types was similar between the HIV-positive and HIV-negative women. HPV viral loads as measured by PCR dot blot signal strength were higher among HIV-positive than among HIV-negative women as was the proportion of HPV-positive women with multiple HPV types. Among women with high HPV viral load, HIV infection was not associated with SIL.

Levi *et al*. (2002) examined the prevalence of HPV infection and multiplicity of HPV types in 208 HIV-positive women in Brazil. Almost all women (98%) were HPV-positive; 78.9% had multiple HPV types with an average of three per patient. HPV 6 was the most common genotype (39.2%) followed by types 51 (31.9%), 11 (26.0%), 18 (24.0%) and 16 (22.5%); 28 patients (13.4%) had a Pap III score. The prevalence of high-risk genotypes increased with the cytological classification. There were no significant associations between the number of HPV genotypes, abnormal cytology, HIV viral load and $CD4^+$ cell count.

Tate and Anderson (2002) compared recurrence rates of CIN after ablation and hysterectomy in 43 HIV-positive women with those in 103 HIV-negative women. All patients were followed up for at least 24 months. Recurrence was greater in the HIV-positive women for all treatment modalities (73% versus 27%; $p = 0.019$). Higher recurrence rates were seen in women with $CD4^+$ cell counts < 200 cells/mm^3 compared with women with $CD4^+$ cell counts > 200/mm^3 (55% versus 26%; $p = 0.002$). The mean HIV viral load was also higher among women who had recurring disease than among those who did not (18 384 versus 3892; $p = 0.002$).

Hawes *et al*. (2003) studied 4119 women who attended an outpatient clinic in Senegal, an area in which both HIV-1 and HIV-2 are highly prevalent in the population. Among women infected with high-risk HPV, those with HIV-1 (odds ratio, 2.2; 95% CI, 1.0–4.8), HIV-2 (odds ratio, 6.0; 95% CI, 2.1–17.1) or both HIV-1 and HIV-2 (odds ratio, 8.0;

95% CI, 2.0–31.5) were more likely to have HSIL or cervical cancer than HIV-negative women. This relationship was not detected among women without high-risk HPV infection. HIV-2-positive women were more likely to have HSIL (odds ratio, 3.3; 95% CI, 0.9–12.4) or cervical cancer (odds ratio, 7.9; 95% CI, 1.1–57) than HIV-1-positive women. The authors hypothesized that the increase in risk associated with HIV-2 infection may reflect the longer periods of mild immunosuppression than are typically seen with HIV-1, and this may be relevant to the effect of highly active antiretroviral therapy (HAART) on the natural history of CIN.

Baay *et al*. (2004) studied the prevalence of cervical HPV infection in a population of women from rural Zimbabwe. The prevalence of HPV was higher in HIV-positive (54%) than in HIV-negative women (27%) (odds ratio, 3.18; 95% CI, 1.67–6.10). The most common HPV types in HIV-positive women were 33 (5.2%), 35 (4.6%), 45 (4.6%) and 58 (4.6%); HPV 16 was found in only 3.4%. Among HIV-negative women, the most common types were HPV 35 (11.5%), 6 (9.8%) and 58 (8.2%); HPV 16 was found in only 3.3%.

Branca *et al.* (2004) assessed risk factors and HPV-related mechanisms of CIN in 17 HIV-positive and 227 HIV-negative women in Italy. HPV prevalence was 36% in HIV-positive and 29% in HIV-negative women. HIV-positive women had more frequent HSIL Pap tests ($p = 0.04$), CIN2 or higher in cervical biopsy ($p = 0.049$) and external genital warts ($p = 0.019$).

Levi *et al*. (2004) studied HIV-positive women from Sao Paulo, Brazil. HPV-DNA prevalence was 87% in HIV-positive women, and 45% were infected by more than two types, compared with 8.3% in HIV-negative women. HPV 16 was the most common type found in HIV-positive women (30.9%) followed by types 52 (22.8%) and 59 (20.6%). In HIV-negative women, the most common types were HPV 51 (19.4%), 16 (16.7%) and 73 (16.7%). The number of HPV types detected among HIV-positive women increased, but not significantly, with increasing grade of Pap smear, whereas HPV viral load as measured by Hybrid Capture 2 (Group B high-risk types) was significantly increased ($p < 0.001$).

Strickler *et al*. (2005) studied the effect of HIV RNA level and $CD4^+$ cell count on the natural history of type-specific HPV infection in 1848 HIV-positive and 514 HIV-negative women who participated in the Women's Interagency HIV Study cohort. A strong interaction between the $CD4^+$ cell count and plasma HIV viral load was found for both prevalent ($p = 0.002$) and incident ($p = 0.001$) detection of HPV. The hazard ratio for incident HPV detection was highest among women with a $CD4^+$ cell count < 200/mm^3 (hazard ratio range, 4.0–5.0) or an HIV RNA level > 100 000 copies/mL; the relationship was weaker for persistent HPV infection. Although incident HPV detection was associated with the number of recent sexual partners (p for trend < 0.001), 22% of sexually inactive HIV-positive women with a $CD4^+$ cell count < 200/mm^3 also had at least one incidentally detected HPV type. There was strong interaction between the effects of HIV RNA and $CD4^+$ cell count on incident SIL, but only a weak effect on its persistence. The weak effect of HIV RNA viral load and $CD4^+$ cell count on HPV and persistence of

SIL may explain the limited number of HIV-positive women who develop HSIL and cervical cancer. The data on sexual activity and incident detection of HPV are consistent with the possibility that at least some of the HPV detected in HIV-positive women reflects reactivation of previously acquired HPV infection rather than a newly acquired infection.

(ii) *Natural history of cervical HPV infection and SIL* (Table 71)

Heard *et al*. (1995) followed 43 HIV-positive women who had normal cytology or SIL at baseline every 6 months for up to 18 months; 18 of 19 (95%) women who had SIL at baseline and who were not treated and eight of 13 (61%) women who were treated with surgery had persistent lesions.

Spinillo *et al*. (1996) studied 48 HIV-positive and 38 HIV-negative women with a history of intravenous drug use who attended an antenatal clinic during their first trimester of pregnancy. Participants were re-examined during their second and third trimesters and 8–12 weeks *post partum*: 27.1% of HIV-positive women and 7.9% of HIV-negative women had CIN at their baseline visit ($p = 0.027$). None of the lesions progressed throughout pregnancy in either HIV-positive or HIV-negative women.

Sun *et al*. (1997) compared the persistence of cervical HPV infection among 220 HIV-positive and 231 HIV-negative women in the New York City area, USA. HPV DNA was detected at baseline in 56% of the HIV-positive and 31% of the HIV-negative women. After four examinations, the cumulative prevalence of HPV infection was 83% in the HIV-positive and 62% in the HIV-negative women ($p < 0.001$). Twenty per cent of the HIV-positive and 3% of the HIV-negative women had persistent infections with high-risk types (18 or 45) ($p < 0.001$). The detection of HPV DNA in women who had previously had negative tests was not associated with sexual activity during the interval since the preceding examination, which suggests the possibility of reactivation of a previously acquired HPV infection as an explanation for the detection of at least some of these infections. The risk for persistent HPV infection in HIV-seropositive compared with HIV-negative women was 7.5 (95% CI, 3.6–16).

Minkoff *et al*. (1998) characterized the relationship between HIV status and infection with high-risk HPV types in 268 HIV-positive and 265 HIV-negative women in the New York area, USA. The prevalence at baseline of any HPV type was 73% among HIV-positive and 43% among HIV-negative women ($p < 0.0001$). The respective prevalence of high-risk HPV types was 32.5 and 17.0% ($p < 0.001$). The rate of detection of new high-risk HPV types was almost three times higher among HIV-positive than among HIV-negative women ($p < 0.01$). However, there was no difference in the rate of loss of detection of high-risk HPV types.

Eckert *et al*. (1999) compared the prevalence and type of HPV infection in the genital tract of 23 HIV-positive and 23 HIV-negative women who were matched for cytology. After matching, the groups had a similar prevalence of HPV DNA and of high-risk HPV types at baseline. On follow up, HIV-positive women were more likely to develop SIL (38% versus 10%; $p = 0.03$), to have visits at which HPV DNA was detected (68% versus

Table 71. Natural history of cervical HPV infection and squamous intraepithelial lesions (SIL) in HIV-positive and HIV-negative women

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
Heard *et al*. (1995), France	No HPV DNA detection	43 SIL				Untreated patients, 95; treated patients, 61		Cytology, histology	Participants followed every 6 months with a Pap smear for at least 18 months
Spinillo *et al*. (1996), Italy	ISH (6/11, 16/18, 31/33/35)	48 CIN1–3	38 CIN1–3	NR		27.1 HIV+, 7.9 HIV–	$p = 0.027$	Biopsy, cytology, histology	Participants were HIV+ women with history of IDU being seen for antenatal care in their first trimester; re-examined in second and third trimester and 8–12 weeks *post partum*.
Sun *et al*. (1997), USA	MY09/MY11 PCR and type-specific primers (16 and 18)	220	231	*At baseline* 56 HIV+, 31 HIV– *Persistent HPV infection* 83 HIV+, 62 HIV–	7.5 (3.6–16)	NR			Persistent HPV infection defined as detection of the same type of HPV at 2 or more examinations during the follow-up period of 3–12 months; HPV testing performed on cervicovaginal lavage; almost all CIN were low-grade.
Minkoff *et al*. (1998), USA	MY09/MY11 PCR primers (2, 6, 11, 13, 26, 32,34, 40, 42, 53, 54, 55, 57, 59, 61, 62, 64, 66, 69, 70, 72, Pap 155, Pap 291, AE2, AE5-8, W13B, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73)	268	265	73 HIV+, 43 HIV– *HR HPV* 32.5 HIV+, 17.0 HIV–	$p < 0.0001$ $p < 0.001$			Cytology, histology	HPV testing performed on cervicovaginal lavage specimen; women followed every 6 months
Eckert *et al*. (1999), USA	MY09/MY11 PCR primers (6/11, 31/33/35/39, 16/16/45)	23	23	73.9 HIV+, 65.2 HIV–	$p = 0.4$	*Baseline* 39 HIV+, 39 HIV– *Follow-up* 38 HIV+, 10 HIV–	$p = 0.03$	Cytology, histology	Dacron swabs used to measure HPV in the cervix/ectocervix and vaginal wall; participants matched by cytology results at baseline and followed every 4 months for 56 (HIV+) and 53 visits (HIV–)

Table 71 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
La Ruche *et al*. (1999), Cote d'Ivoire	MY09/MY11 PCR	38	56	83.3 HIV+ 58.8 HIV–	$p = 0.015$	*Persistent CIN* 76 HIV+, 18 HIV– *Progression to high-grade CIN* 18 HIV+, 0 HIV–	4.3* (2.4–7.7)	Cytology, histology on women with high-grade cytology referred for colposcopy	HPV testing using cervical Viba-Brush specimen; women followed after a median of 5 months from the initial smear; *relative risk
Petry *et al*. (1999), Germany	HC1	138		NR		15.9 CIN1, 12.3 CIN2 or -3		Cytology, histology	
Cubie *et al.* (2000), Scotland, United Kingdom	HC for HR HPV, HC2 with probes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)	63		Normal cytology, 25; abnormal cytology, 8.0; persistent HPV infection, 42.9		NR			
Ellerbrock *et al*. (2000), USA	MY09/MY11 PCR primers with RFLP typing and E6 primers (16 and 18)	328	325			91% HIV+, 75% HIV–	3.2 (1.7–61)	Cytology, histology	Women had no SIL at enrollment; HPV testing performed on cervicovaginal lavage specimen; Women followed for approximately 30 months

Table 71 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
Ahdieh *et al*. (2001), USA	MY09/MY11/ HMB01 PCR primers (16/18/31/45, 33/35/39/51/52/ 56/58/59/68, 6/11/26/40/42/53/54/55/ 66/73/82/ 83/84)	871	439	Cumulative pre-valence of HPV infection: increase from 73.4 to 90.2 HIV+ women with $CD4^+$ < 200, increase from 28.1 to 54.0 HIV–				Cytology	Women were participants in the HERS and were followed at 6-month intervals for assessment of type-specific HPV infection; Increased HPV viral load using PCR as indicated by increased dot blot signal strength from 1 to 4
Calore *et al*. (2001), Brazil		1587 (baseline), 409 (follow-up)				12.6 SIL or cervical cancer		Cytology	
Cohn *et al*. (2001), USA	HC 2, HC RLU	103		66 HIV+, 56.3 with HR types HIV+	*p* value = 0.0006	20 CIN after 1 year of follow-up		Cytology, biopsy	Women were participants in the American Foundation for AIDS Research Community Based Clinical Trials Network in 6 US cities; Cervical Dacron swabs used for HPV testing; Women studied at baseline, 6 months and 12 months; Cases had $CD4^+ \leq 500/mm^3$.
Massad *et al*. (2001), USA	MY09/MY11/ HMB01 PCR (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	1639	452	NR		73 HIV+, 42 HIV–	4.0 (2.6–6.1)	Cytology	Women were participants in the WIHS; HPV testing performed on a cervicovaginal lavage specimen; Women had measurement of serum retinol AND were followed every 6 months; Median follow-up, 4.0 years

Table 71 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
Conley *et al*. (2002), USA	PCR using L1 consensus primers and RFLP typing analysis; some samples studied with E6-specific primers (16 and 18)	481 (baseline), 385 (incidence analysis)	437 (baseline), 341 (incidence analysis)	54 HIV+, 32 HIV–	$p < 0.001$	*Vulvovaginal and perianal condyloma and dysplasia* 6.2 HIV+, 0.9 HIV– *Incident condyloma (all sites)* 7 HIV+, 1 HIV–	$p < 0.0001$ 13.8 (10.9–17.3)	Cytology, histology	Study examined incident condyloma of vulva, vagina and perianal region and multicentric lesions; HPV testing performed on cervicovaginal lavage specimen
Silverberg *et al*. (2002), USA	MY09/MY11 PCR primers (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, 83, 84)	2032 (WIHS)	551 (WIHS)	*HPV 6/11* 5.0 HIV+, 0.9 HIV– *Other HPV* 58.7 HIV+, 29.0 HIV–	$p < 0.001$	Genital warts *WIHS* 9.8 HIV+, 3.1 HIV– *HERS* 13.6 HIV+, 5.0 HIV–	$p < 0.001$ $p < 0.001$	Cytology, physical examination	Women were participants in the WIHS and HERS and were followed every 6 months.
		863 (HERS)	420 (HERS)	*HPV 6/11* 4.3 HIV+, 1.2 HIV– *Other HPV* 59.9 HIV+, 26.0 HIV–	$p < 0.001$				

Table 71 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
Branca *et al*. (2003), Italy	MY09/MY11 PCR primers with RFLP genotyping and confirmation of typing by sequencing	89	48	*At baseline* 38.6 HIV+, 27.1 HIV– *New infection during follow-up* 27.1 HPV/HIV+, 3.1 (HPV/HIV+) *Cleared infection during follow-up* 22.8 HPV+/HIV+, 69.2 HPV+/HIV–	 8.8 (1.20–64.6) 0.33 (0.16–0.67)	NR			Women were participants in the DIANAIDS project and were followed for a mean of 14 months.
Ford *et al*. (2003), Indonesia	PCR (primers not specified) with hybridization (6, 11, 16, 18, 31, 33, 35, 45, 52)	631 (baseline), 618 (18 months)		38.3 at baseline, 29.7 at 18 months		NR			Study of female sex workers in Bali, Indonesia; HPV testing performed at baseline and 18 months later; HPV testing performed on cervical swab specimen
Strickler *et al*. (2003), USA	PCR MY09/MY11/ HMB01 PCR (6, 11, 16,18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	2929		HPV 6 HPV 11 HPV 16 HPV 18 HPV 31	4.9 (2.0–12.02) 2.05 (0.93–4.50) 1.69 (1.01–2.81) 2.24 (1.23–4.08) 3.07 (1.55–6.07)	NR		Cytology	Hazard ratio estimates for association between $CD4^+ < 200$ versus ≥ 500 with incident detection of specific HPV types; women were participants in the WIHS and HERS; HPV testing was performed on a cervicovaginal lavage specimen.

Table 71 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p*-value	%/incidence	Odds ratio (95% CI)/ *p*-value		
Massad *et al*. (2004a), USA	PCR MY09/ MY11/HMB01 PCR (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	202	21	*HR types* 49.5 HIV+, 19.1 HIV– *LR types* 19.8 HIV+, 9.5 HIV–	$p < 0.001$	*Progression of CIN 1* 3.8 HIV+, 0 HIV–	0.4 (0.25–0.66)	Cytology, histology	Women were participants in the WIHS. HPV testing performed on a cervicovaginal lavage specimen; women with a histological diagnosis of CIN1 were included in the analysis; women were followed for a mean of 3.3 years.
Moscicki *et al*. (2004), USA	PCR MY09/ MY11/HMB01 PCR primers (16-like, 16, 31/33/35, 52, 58, 67, 18-like, 18, 39, 45, 59/68/70, 56-like, 56, 53/66, 26/69, 51, 6/11/42/43/44, 54/40, 13/32, 67/72, 2/57, 55)	172	84	NR		*LSIL at baseline* 22.5 HIV+, 10.2 HIV– *HSIL at baseline* 5.3 HIV+, 0 HIV–	$p < 0.002$ $p = 0 .13$	Cytology	Cervicovaginal lavage samples used for HPV testing; participants were women aged 13–18 years in the REACH cohort.

See Table 7 for a description of the primers used.

CI, confidence interval; CIN, cervical intraepithelial neoplasia; DIANAIDS, Italian collaborative study on HIV/HPV; HC, Hybrid Capture; HERS, HIV Epidemiology Research Study; HIV, human immunodeficiency virus; HIV–, HIV-negative; HIV+, HIV-positive; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; IDU, intravenous drug user(s); ISH, in-situ hybridization; LR, low-risk; LSIL, low-grade squamous intraepithelial lesion; NR, not reported; Pap, Papanicolaou test; PCR, polymerase chain reaction; REACH, Reaching for Excellence in Adolescent Care and Health; RFLP, restriction fragment length polymorphism; RLU, relative light unit; WIHS, Women's Interagency HIV Study

40%; $p = 0.04$) and to have more visits at which multiple HPV DNA types were detected (18% versus 0%; $p = 0.02$) than HIV-negative women.

La Ruche *et al*. (1999) performed a short-term prospective study of CIN in Abidjan, Cote d'Ivoire. Of 94 women with a cytological diagnosis of SIL, 36 were infected with HIV-1 and two with HIV-2. The average follow-up period after the initial smear was 5 months. HIV-positive women had a higher percentage of persistent CIN (76%) than HIV-negative women (18%) (relative risk, 4.3; 95% CI, 2.4–7.7). Progression to high-grade CIN occurred more frequently among HIV-positive (18%) than HIV-negative women (0%). In a multivariate analysis, persistence of lesions was associated with HIV positivity and an undetermined grade of CIN at baseline; HPV infection was not a significant risk factor after adjustment for the other factors in the model.

Petry *et al*. (1999) studied the role of HPV testing as a screening tool for incident SIL among 138 HIV-positive women in Germany. The prevalence of high-grade neoplasia (≥ CIN2) was 12.3% (17/138), and the total prevalence of cervical neoplasia including CIN1 was 26.8% (37/138).

Cubie *et al*. (2000) performed a prospective observational cohort study of 63 HIV-positive women in Edinburgh, United Kingdom. Abnormal cervical cytology, particularly that of low grade, was common in these HIV-infected women. Using Hybrid Capture, high-risk HPV types were detected in 25% of the women with normal cytology. Over 80% of those with abnormal cytology of any grade were positive for HPV. Persistent high-risk HPV infection, as defined by two or more consecutive HPV-positive results, was common and found in 27 of 63 (42.9%) women from whom multiple samples were obtained. Progression of cervical disease, even among the more strongly immunosuppressed women, was a rare event in this cohort.

Ellerbrock *et al*. (2000) studied risk factors for incident SIL in 328 HIV-positive and 325 HIV-negative women in the New York area, USA. During follow-up, the incidence of SIL was 8.3 cases per 100 person–years in HIV-positive and 1.8 cases per 100 person–years in HIV-negative women ($p < 0.001$). Of the incident SILs, 91% were LSIL in HIV-positive women and 75% in HIV-negative women. In a multivariate analysis, risk factors for incident SIL included HIV infection (relative risk, 3.2; 95% CI, 1.7–6.1), transient HPV DNA detection (relative risk, 5.5; 95% CI, 1.4–21.9), persistent infection with HPV DNA types other than 16 or 18 (relative risk, 7.6; 95% CI, 1.9–30.3), persistent infection with HPV DNA types 16 and 18 (relative risk, 11.6; 95% CI, 2.7–50.7) and younger age (< 37.5 years versus ≥ 37.5 years of age; relative risk, 2.1; 95% CI, 1.3–3.4).

Ahdieh *et al*. (2001) performed a prospective study of HPV infection in women who participated in the HIV Epidemiology Research Study. In a multivariate analysis, increased signal strength on dot blot, but not viral risk category, was independently associated with persistence of HPV infection among HIV-positive women (odds ratio, 2.5; 95% CI, 2.1–2.9). Persistence was 1.9 (95% CI, 1.5–2.3) times more common among women with a $CD4^+$ cell count < 200 cells/μL compared with those with a count > 500 cells/μL. Among the HPV types examined, HPV 16 had the highest incidence rate among HIV-negative women (1.67 per 100 person–years). Among HIV-positive women, HPV 18 had the highest

incidence rate among the high-risk types (2.61 per 100 person–years) but low-risk HPV 53 had the highest incidence rate overall (6.23 per 100 person–years).

Calore *et al*. (2001) studied cytological specimens from 1587 HIV-positive women in Brazil: 12.6% had SIL or cervical cancer in at least one specimen; 24 women progressed from normal to LSIL within 3 years and 11 progressed from normal to HSIL within 3 years.

Cohn *et al*. (2001) studied the 1-year incidence of CIN in 103 women who participated in the AIDS Research Community Based Clinical Trials Network in six cities in the USA. Higher HPV viral loads as measured by higher Hybrid Capture RLU ratios were associated with high-grade CIN ($p = 0.0006$). Incident CIN occurred in 20% of women during 1 year of follow-up, and was associated with higher HPV RLU ratios at baseline ($p = 0.03$).

Massad *et al*. (2001) studied the incidence, progression and regression rates of abnormal cervical cytology among 1639 HIV-positive and 452 HIV-negative women who participated in the Women's Interagency HIV Study. At least one abnormal smear was found during the whole follow-up among 73.0% of HIV-positive and 42.3% of HIV-negative women ($p < 0.001$). The incidence of HSIL was low among HIV-positive women and only 5.9% ever developed HSIL during follow-up. The incidence of SIL was 8.9/100 person–years among HIV-positive and 2.2/100 person–years among HIV-negative women (relative risk, 4.0; 95% CI, 2.6–6.1). Progression 6 months after an abnormal smear was found in 14% of HIV-positive women. HIV positivity, HPV positivity, lower $CD4^+$ cell count and higher HIV RNA level predicted the incidence of abnormal cytology. HPV positivity and higher HIV RNA level predicted the progression of abnormalities found at baseline. HPV negativity, higher $CD4^+$ lymphocyte count and lower HIV RNA level predicted regression of disease.

Conley *et al*. (2002) studied the incidence of vulvovaginal and perianal condylomata acuminata and intraepithelial neoplasia in 925 HIV-positive and HIV-negative women in the New York area, USA. Vulvovaginal and perianal condylomata acuminata or intraepithelial neoplasia were found in 6% of HIV-positive and 1% of HIV-negative women at baseline. Among women without lesions at enrolment, 9% of HIV-positive and 1% of HIV-negative women developed vulvovaginal or perianal lesions over a median follow-up of 3.2 years. Risk factors for incident disease included HIV-1 infection ($p = 0.013$), HPV infection ($p = 0.0013$), lower $CD4^+$ cell counts ($p = 0.0395$) and history of frequent intravenous drug use ($p = 0.02$).

Silverberg *et al*. (2002) examined the relationship between HIV infection and incidence of genital warts and infection with HPV 6 or 11 in both the Women's Interagency HIV Study and HIV Epidemiology Research Study populations. The prevalence of HPV 6 or 11 was 5.6 times higher and that of genital warts was 3.2 times higher in HIV-positive than in HIV-negative women in the Women's Interagency HIV Study and 3.6 times and 2.7 times higher, respectively, in the HIV Epidemiology Research Study. In the former, the risk for HPV 6 or 11 infection increased from 5.1 (95% CI, 2.9–8.8) among HIV-negative women to 8.8 (95% CI, 6.1–12.8) among HIV-positive women with $CD4^+$ cell counts > 200/mm^3 and to 12.8 (95% CI, 8.8–18.8) among HIV-positive women with $CD4^+$ cell counts ≤ 200/mm^3. In this study, infection with HPV 6 or 11 was associated with an increased risk for genital warts

compared with HPV negativity in HIV-negative women (odds ratio, 2.7; 95% CI, 1.6–4.6), HIV-positive women with $CD4^+$ cell counts > 200/mm^3 (odds ratio, 4.9; 95% CI, 3.2–7.7) and HIV-positive women with $CD4^+$ cell counts ≤ 200/mm^3 (odds ratio, 5.3; 95% CI, 3.3–8.5). The incidence of infection with HPV 6 or 11 and the incidence of genital warts measured in cases per 100 person–years were higher among HIV-positive women than among HIV-negative women in both of these studies.

Branca *et al*. (2003) studied the natural history of cervical HPV infection in 89 HIV-positive and 48 HIV-negative women who participated in the DIANAIDS study. New HPV infections during follow-up were more common among HIV-positive than HIV-negative women (odds ratio, 8.8; 95% CI, 1.2–64.6), and clearance of HPV infection at baseline was less frequent among HIV-positive than HIV-negative women (odds ratio, 0.33; 95% CI, 0.16–0.67). In a multivariate analysis, risk factors for HPV positivity at the end of the study included HIV positivity ($p < 0.001$), PCR positivity at entry ($p = 0.009$), p53 polymorphism at aa-72 ($p = 0.01$), high-risk HPV type ($p = 0.02$) and significant Pap smear at entry ($p = 0.04$).

Ford *et al*. (2003) studied female sex workers in Bali, Indonesia, for cervical HPV infection at baseline and again 18 months later. The prevalence of HPV infection was 38.3% at baseline, which declined to 29.7% after 18 months. The prevalence of HPV infection declined with age ($p < 0.01$). Infection with *N. gonorrhoeae* was associated with HPV infection at baseline ($p = 0.03$). HPV infection declined in the study area that had the more intensive educational programme ($p < 0.01$).

Strickler *et al*. (2003) examined the relationship between prevalence and incidence of specific HPV types in HIV-positive women who participated in the Women's Interagency HIV and HIV Epidemiology Research Studies. In a cross-sectional analysis of data from the first study, HPV 16 had a weaker association with more advanced immune status as measured by $CD4^+$ cell counts than other HPV types. This largely reflected the observation that the prevalence of HPV 16 was higher among women with higher $CD4^+$ cell levels than that of other HPV types, the proportional prevalence of which was increased at lower $CD4^+$ cell strata. A summary prevalence ratio and incidence hazard ratio were estimated for each HPV type. Using data from both studies, the prevalence ratio for HPV 16 was low compared with that of other HPV types at every visit in both populations. The prevalence ratio was smallest for HPV 16 compared with that of all HPV types measured (1.25; 95% CI, 0.97–1.62; $p = 0.01$). The association of $CD4^+$ T-cell stratum with incidence of HPV 16 was also among the smallest measured. HPV types that had small summary prevalence ratios also had small incidence hazard ratios. The investigators concluded that the prevalence and incidence of HPV 16 is more weakly associated with immune status as measured by $CD4^+$ cell level in HIV-positive women than that of other HPV types, which suggests that HPV 16 may be more resistant to the effects of immune surveillance.

Massad *et al*. (2004a) studied the natural history of histologically confirmed CIN1 in 202 HIV-positive and 21 HIV-negative women who participated in the Women's Interagency HIV Study. The prevalence of high-risk HPV in HIV-infected women was 49.5% compared with 19.1% in HIV-negative women. Progression occurred in eight (3.8%) HIV-

positive women (incidence density, 1.2/100 person–years) but not in HIV-negative women. Regression occurred more often in HIV-negative than in HIV-positive women (relative risk, 0.40; 95% CI, 0.25–0.66; $p < 0.001$). In a multivariate analysis, regression was associated with HPV infection (hazard ratio for low-risk HPV, 0.28; 95% CI, 0.13–0.61; hazard ratio for high-risk HPV, 0.34; 95% CI, 0.20–0.55 versus no HPV detected) and Hispanic ethnicity (hazard ratio, 0.48; 95% CI, 0.23–0.98). The lesions of HIV-positive women with HPV infection at the time of diagnosis of CIN were less likely to regress than those of HIV-negative women (hazard ratio, 0.18; 95% CI, 0.05–0.62).

Moscicki *et al*. (2004) studied the incidence of HSIL diagnosed cytologically among HIV-positive and HIV-negative adolescent girls who participated in the Reaching for Excellence in Adolescent Care and Health cohort. The incidence of HSIL at the end of follow-up was higher for HIV-positive girls (21.5%) than for HIV-negative girls (4.8%). In a multivariate analysis, use of hormonal contraceptives (hazard ratio, 2.60; 95% CI, 1.25–5.40), high concentrations of IL12 in cervical mucous (hazard ratio, 2.28; 95% CI, 1.17–4.43), persistent LSIL diagnosed cytologically (hazard ratio, 1.67; 95% CI, 1.29–2.18) and infection with HPV type 16 only (hazard ratio, 3.69; 95% CI, 1.06–12.80), HPV type 18 only (hazard ratio, 4.49; 95% CI, 1.16–17.42) and multiple high-risk HPV types (16, 18 and 56) (hazard ratio, 3.69; 95% CI, 1.07–12.71) were significantly associated with the development of HSIL.

(iii) *Effect of highly active antiretroviral therapy (HAART) on CIN* (Table 72)

Heard *et al*. (1998) examined the natural history of cervical lesions in 49 HIV-positive women in Paris, France, before and 5 months after initiation of HAART. They examined the prevalence of HPV using southern blot hybridization (to define high-level infection) and PCR (to identify low-level infection). The prevalence of SIL decreased from 69 to 53% during follow-up ($p < 0.0001$). Two women with HSIL regressed to LSIL and one regressed to normal, and nine of 21 (43%) women with LSIL regressed to normal. In all patients except one, the prevalence of HPV infection detected by southern blot hybridization and PCR did not change. There was a greater increase in the absolute number of $CD4^+$ cells in the subgroup of patients whose lesions regressed (99 versus 50×10^6/L; $p = 0.03$) compared with those whose lesions did not regress.

Delmas *et al*. (2000) evaluated the natural history of SIL in HIV-positive women in a cohort that was followed in 12 European countries. Women with lower $CD4^+$ cell counts ($< 200 \times 10^6$/L) had twice the prevalence and incidence of SIL than women with higher $CD4^+$ cell counts ($> 500 \times 10^6$/L), and fewer had regression of CIN1. Regression of SIL in women with a low $CD4^+$ cell count was lower (20.5%) in those who received antiretroviral therapy than in those who did not (31.4%), but the difference was not significant ($p = 0.30$).

Lillo *et al*. (2001) studied the effect of HAART on high-risk HPV infections and related cervical lesions in 163 HIV-positive women in Italy. High prevalences of both high-risk HPV infection (68%) and SIL diagnosed cytologically were found at baseline

Table 72. Effect of highly active antiretroviral therapy (HAART) on cervical intraepithelial neoplasia (CIN) in HIV-positive women

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p* value		
Heard *et al.* (1998), France	MY09/MY11 PCR primers (16, 18, 33) plus SBH (6/11/42, 16/18/33, 31/35/39, other types), sequencing of unidentifiable types	49		HIV+ *Before HAART* 81 by PCR, 54 by SBH *After HAART* 93 by PCR, 56 by SBH		9/21 women with LSIL at baseline regressed to normal cytology (3 with persistent colposcopic abnormalities); 2/13 women with HSIL at baseline regressed to LSIL and one regressed to normal.	$p < 0.0001$	Cytology, histology	HPV testing on cervical cotton swab and wooden spatula; women were followed prospectively at a median of 5 months after initiation of HAART.
Delmas *et al.* (2000), 12 European countries	SBH (6, 11, 42, 16, 18, 31, 33, 35, 39), PCR MY09/MY11 (16, 18, 33), GP1/GP2 primers to detect other types	467 (baseline), 229 (follow-up), 115 CIN1 (follow-up)				*SIL* 24.2 HIV+ at baseline, 23.6 at 1 year, 29.5 at 18 months *Progression of LSIL to HSIL* 8.1 at 1 year *Regression of LSIL to normal* 30.9 at 1 year *Cumulative regression rate among women with* $CD4^+ < 200$ 20.5 with HAART, 31.4 without HAART	$p = 0.30$	Cytology	Women were participants in a European cohort on natural history of HIV infection; HPV testing was performed on cervical brush specimens; women with normal cytology at baseline were followed every 6 months for a median of 2 years; women diagnosed with CIN1 were followed for a median of 18 months without treatment.
Lillo *et al.* (2001), Italy	HC 2 and MY09/MY11 PCR with E6 and L1 priming (6, 11, 16, 18, 31, 33, 35, 45)	163		68 HR HPV; persistent HPV infection in HAART-treated versus non-treated	1.18 (0.63–3.46)	6.2 HSIL, 20.2 LSIL; progression of cytological changes in HAART-treated versus non-treated	2.01 (0.44–9.20)	Cytology, histology	Participants followed at 6-month intervals; HPV infection defined as having the same type at baseline and follow-up, transitory as having a change in HPV status and HPV– as having consistently negative HPV tests; HPV samples obtained by brushing squamocolumnar junction

Table 72 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p* value		
Minkoff *et al*. (2001), USA	MY09/MY11/ HMB01 PCR (6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	507		NR		*Regression of cytological abnormalities* 35.3 with HAART, 29.5 without HAART *Progression of cytological abnormalities* 15.6 with HAART, 22.3 without HAART	1.4 (1.04–1.82) 0.68 (0.52–0.88)	Cytology	Women were participants in the WIHS; HPV testing performed on a cervicovaginal lavage specimen; women had measurement of serum retinol and were followed every 6 months; all had HR HPV infection; progression and regression were measured using consecutive Pap smear pairs.
Robinson *et al*. (2001), USA	No HPV DNA detection	56	62			*Disease persistence among women not treated within 6 months of diagnosis* 60 HIV+, 32 HIV– *Persistent or recurrent CIN after therapy* 17.6 with HAART, 70.3 without HAART	$p < 0.05$ $p < 0.05$		Retrospective chart review of women treated for CIN; cervical conization or loop electrosurgical excision therapy used
Heard *et al*. (2002), France	Regression to normal cytology in HAART-versus non-HAART-treated	168 CIN (96 HAART-treated)		NR		Regression to normal, 34.1 Progression to high-grade, 22.7	1.93 (1.14–3.29)	Cytology, histology	Women were followed at 6-month intervals; 37 were treated with HAART at the time of study initiation, 59 initiated HAART after the baseline visit.

Table 72 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p* value		
Moore, A.L. *et al*. (2002), United Kingdom	No HPV DNA detection	71				*Prevalence of CIN* 55 before HAART, 62 after HAART Regression, 13	$p = 0.20$	Cytology, histology	Women attended a gynaecology clinic, required cytology before HAART initiation and another at least 6 months after HAART initiation; median time between pre- and post-HAART smears, 10 months.
Schuman *et al*. (2003), USA	MY09/MY11 PCR primers (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, 68, 70, Pap 155, Pap 291, W13B)	774	391	64.0 HIV+, 28.7 HIV–				Cytology	Cervicovaginal lavage specimen used for HPV testing; women were participants in the HERS and were followed every 6 months.
Uberti-Foppa *et al.* (2003), Italy	HC2 PCR MY09/MY11 and SPF10	83 stable with no or standard HAART, 71 more potent HAART		NR		SIL baseline end-point *Standard HAART* LSIL, 30.1–13.3 HSIL, 14.5–1.2 *More potent HAART* LSIL, 15.5–24 HSIL, 12.7–2.8	$p < 0.0001$ $p = 0.06$	Histology	Mean age of patients was 32.3 ± 5.2 years (range, 21–45 years); histology classified according to the Bethesda system

Table 72 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence		Cervical abnormality		Pathology reading	Comments
				%	Odds ratio (95% CI)/ *p* value	%	Odds ratio (95% CI)/ *p* value		
Ahdieh-Grant *et al*. (2004), USA	MY09/MY11/ HMB01 PCR (6, 11, 13, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	141 with regression of SIL, 171 with no regression of SIL		NR		*Incidence of regression* 0 (95% CI, 0–2.4)/100 person–years before HAART, 12.5 (95% CI, 9.9–15.1)/ 100 person–years after HAART	$p = 0.002$	Cytology	Participants in the WIHS; HPV testing performed on a cervicovaginal lavage specimen; women had measurement of serum retinol and were followed every 6 months; all had HR HPV infection?; progression and regression were measured among women with a normal Pap smear at enrolment who developed SIL during at least 7 years of follow-up.
Massad *et al*. (2004b), USA	PCR MY09/MY11 and specific probes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and other low-risk types)	15 621	469	NR		HAART versus non-HAART Genital warts VIN1 VIN2–3	 0.76 (0.58–0.99) 0.65–0.49–0.88) 0.64 (0.40–1.04)	Cytology	Follow-up of 8 years

See Table 7 for a description of the primers used.

CI, confidence interval; HC, Hybrid Capture; HERS, HIV Epidemiology Research Study; HIV, human immunodeficiency virus; HIV+, HIV-positive; HIV–, HIV-negative; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; ISH, in-situ hybridization; LSIL, low-grade squamous intraepithelial lesion; NR, not reported; Pap, Papanicolaou test; PCR, polymerase chain reaction; SBH, southern blot hybridization; SIL, squamous intraepithelial lesion; VIN, vulvar intraepithelial neoplasia; WIHS, Women's Interagency HIV Study

(LSIL, 20.2%; HSIL, 6.2%). The women were followed every 6 months for a mean observation time of 15.4 months (range, 6–24 months). Although $CD4^+$ cell counts increased significantly in subjects who received HAART, persistence of high-risk HPV infection (adjusted odds ratio, 1.18; 95% CI, 0.63–3.46) and progression of SILs (adjusted odds ratio, 2.01; 95% CI, 0.44–9.20) were comparable among those who were and those who were not treated with HAART.

Minkoff *et al*. (2001) examined the relationship between the use of HAART and progression or regression of cervical cytology in the Women's Interagency HIV Study. Women with persistent HPV positivity were more likely to have lesions that progressed: 16.2% progressed with HPV positivity at one of three visits, 23.6% progressed with positivity at two of three visits and 24.6% progressed with positivity at all three visits ($p < 0.001$). After adjustment for $CD4^+$ cell count and cytological status, women who took HAART were more likely to experience regression (odds ratio, 1.4; 95% CI, 1.04–1.82) and less likely to show progression (odds ratio, 0.68; 95% CI, 0.52–0.88).

Robinson *et al*. (2001) performed a retrospective chart review of 56 HIV-positive and 62 HIV-negative women to determine the rates of recurrence, persistence and progression of CIN after excisional therapy with and without HAART. Among those who were not treated within 6 months of diagnosis, persistence occurred in 60% of HIV-positive and 32% of HIV-negative women ($p < 0.05$). Risk factors for recurrence among HIV-positive women after treatment included margin involvement of specimens obtained by loop electrosurgical excision ($p < 0.05$). Disease in women who took HAART that included protease inhibitors was less likely to recur or persist after treatment than that in HIV-positive women who did not take HAART ($p < 0.05$).

Heard *et al.* (2002) studied 168 HIV-positive women, of whom 37 were taking HAART at the time of study initiation and 59 began taking HAART after baseline, every 6 months. Overall, regression of CIN was observed in 39.9% of the women. The risk for regression of CIN was nearly twice as high in women who received HAART than in those who did not (relative hazard, 1.93; 95% CI, 1.14–3.29). HAART had a similar effect on the regression of low-grade CIN to normality (30/88 women; 1.99; 95% CI, 0.94–4.18) and on the reversion of high-grade to low-grade CIN (31/174 women).

Moore, A.L. *et al*. (2002) studied the prevalence of CIN in 71 HIV-positive women 10 months after initiation of HAART. The prevalence of CIN before HAART was 55%; a median of 10 months after treatment with HAART, this had increased to 62% ($p = 0.20$). Thirteen per cent of patients experienced regression of a CIN lesion, which was associated with a greater but non-statistically significant increase in $CD4^+$ cell count.

Schuman *et al*. (2003) examined risk factors for the progression and regression of cytological abnormalities in 774 HIV-positive and 391 HIV-negative women who participated in the HIV Epidemiology Research Study. HAART was not significantly associated with the probability of progression of cervical lesions.

Uberti-Foppa *et al*. (2003) assessed the long-term effect of HAART by comparing HPV and cytology/histology results at the beginning and end of a study of 83 women who were stable without HAART or who were taking HAART but did not change their therapy

(Group S), and 71 women who changed to a more potent HAART regimen due to failure of the treatment (Group W). Participants were followed at 6–12-month intervals for a mean of 36 months. HPV infection was defined as positivity for the same type at baseline and at follow-up; infection was described as transitory when a change in HPV status occurred; and HPV status was considered to be negative when HPV tests were consistently negative. Although treatment with HAART increased the $CD4^+$ cell level among women in both groups, HAART-associated increases in $CD4^+$ cell level did not affect the persistence of HPV. However, women who took HAART in Group S had fewer HPV-positive low-grade biopsies at the end of the study compared with baseline (30.1% versus 13.3%; $p = 0.00004$). No significant reduction was seen in Group W. The data suggest that long-term use of HAART may be beneficial in the regression of low-grade lesions.

Ahdieh-Grant *et al*. (2004) studied 312 HIV-positive women in the Women's Interagency HIV Study who had normal cervical cytology at baseline, who developed incidental SIL during 7 years of follow-up and who could be monitored for regression or progression of lesions in relation to the use of HAART. Of these, 141 (45.2%) had lesions that regressed to normal with a median time to regression of 2.7 years. The incidence of regression increased (p for trend = 0.002) after HAART was introduced. Women whose lesions did not regress had lower $CD4^+$ cell levels than those whose lesions regressed ($p < 0.01$). However, the majority of cervical lesions among HIV-positive women, whether they took HAART or not, did not regress to normal.

Massad *et al*. (2004b) followed the incidence and predictors of genital warts and VIN among HIV-positive and HIV-negative women at high risk who participated in the Women's Interagency HIV Study for up to 8 years. In a multivariate analysis, warts were associated with HAART (relative hazard, 0.76; 95% CI, 0.58–0.99), $CD4^+$ cell count (relative hazard, 0.91/100 cell/cm^2 increase; 95% CI, 0.86–0.96), history of AIDS (relative hazard, 1.25; 95% CI, 1.00–1.57), abnormal Pap test results (relative hazard, 2.18; 95% CI, 1.73–2.75), high- or medium-risk HPV types (relative hazard, 1.91; 95% CI, 1.48–2.47), low-risk HPV types (relative hazard, 1.48; 95% CI, 1.10–2.00), tobacco smoking (relative hazard, 1.43; 95% CI, 1.09–1.88), having one child (relative hazard, 1.54; 95% CI, 1.11–2.13) and age (relative hazard, 0.74/10 years; 95% CI, 0.64–0.86). VIN of any grade was linked to HAART (relative hazard, 0.65; 95% CI, 0.49–0.88), $CD4^+$ cell count (relative hazard, 0.92; 95% CI, 0.87–0.97), abnormal Pap test results (relative hazard, 16.03; 95% CI, 11.33–22.69), high- or medium-risk HPV types (relative hazard, 1.37; 95% CI, 1.06–1.77) and age (relative hazard, 0.85/10 years; 95% CI, 0.73–0.99). While HAART was associated with a reduced relative hazard for incidental genital warts and VIN of any grade, it was not significantly associated with a reduced relative hazard for VIN2–3 in a multivariate analysis (relative hazard, 0.64; 95% CI, 0.40–1.04).

Two studies analysed the incidence of cervical cancer before and after the introduction of HAART.

The International Collaboration on HIV and Cancer (2000) examined the relationship between HAART and incidence of cancer in HIV-positive adults. Rate ratios were estimated by comparing incidence rates from 1997 to 1999 with those from 1992 to 1996. The

rate ratio for cervical cancer was 1.87 (95% CI, 0.77–4.56), which indicated that there had been no significant change in the incidence of cervical cancer since the introduction of HAART.

Dorrucci *et al*. (2001) analysed the incidence of cervical cancer in Italian women before and after the introduction of HAART. They estimated the incidence per 1000 person–years of cervical cancer as a first AIDS-defining disease for the periods 1981–91, 1992–95 and 1996–98 in 483 women with a median follow-up of 7 years. Compared with 1981–95, the hazard ratio for cervical cancer for 1996–98 was 7.41 (95% CI, 1.21–45.44). After adjustment for age at HIV seroconversion, the hazard ratio decreased to 4.75 (95% CI, 0.80–28.24). The incidence of cervical cancer had not declined after the introduction of HAART in this population.

(*b*) *Studies of the anorectal region*

(i) *Prevalence of anal HPV infection and anal SIL* (Table 73)

Carter, P.S. *et al*. (1995) studied 90 HIV-positive men, 77 HIV-negative men and 43 men of unknown HIV status who attended a genitourinary medicine clinic in London, United Kingdom. The relative risk for AIN for HIV-positive men was 1.58 (95% CI, 1.01–2.48) compared with HIV-negative men. The relative risk for developing AIN for those with anal warts compared with those without anogenital warts was 4.70 (95% CI, 1.81–12.20).

Hillemans *et al*. (1996) examined the prevalence of anal HPV infection and anal disease in 102 HIV-positive and 96 HIV-negative women at high risk in the New York metropolitan area, USA. Using Hybrid Capture 2 on anal swabs, HPV DNA was found in 29.4% of HIV-positive and 2.1% of HIV-negative women. Anal cytological abnormalities were low-grade or atypical, and were found in 27.3% of HIV-positive and 6.4% of HIV-negative women with satisfactory smears. Of 33 women with anal cytological abnormalities, 19 (58%) had anal HPV DNA compared with 13 (8%) of 160 women without cytological abnormalities ($p < 0.001$). In a multivariate logistic regression analysis, HPV infection was the only risk factor associated with anal cytological abnormalities (adjusted odds ratio, 16.0; 95% CI, 8.9–3.2). In a logistic regression model of risk factors for anal cytological abnormalities that did not include HPV DNA positivity, HIV-positive women who had a $CD4^+$ cell count < 200/mm^3 were at higher risk than HIV-positive women who had a $CD4^+$ cell count > 200/mm^3 (adjusted odds ratio, 7.3; CI, 1.8–49.2). HIV positivity was found to be an independent risk factor for HPV infection and, similarly to anal cytological abnormalities, the strength of the association with anal HPV infection among HIV-positive women was greater in those who had $CD4^+$ T-lymphocyte counts < 200/mm^3 than in those who had $CD4^+$ counts > 200/mm^3 (adjusted odds ratio, 11.6; 95% CI, 2.1–64.5).

Melbye *et al*. (1996) studied 81 HIV-positive and 70 HIV-negative women in Copenhagen and Aarhus, Denmark. Using PCR, anal HPV was detected in 78% of HIV-positive women and 60% of HIV-negative women. Abnormal anorectal smears were found in 19% of HIV-positive women and in none of the HIV-negative women.

Table 73. Prevalence of anal HPV infection and anal squamous intraepithelial lesions (SIL) in HIV-positive and HIV-negative individuals

Reference, study location	Sex	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Carter, P.S. *et al.* (1995), United Kingdom	Men		90	77 HIV–, 43 unknown HIV status		*AIN* 41.1 HIV+, 26.0 HIV–, 39.5 unknown HIV status *Anogenital warts* 58.9 (HIV+), 79.2 (HIV–), 95.3 unknown HIV status	Histology	Men were recruited from a genitourinary medicine clinic.
Hillemans *et al.* (1996), USA	Women	HC2 with typing of HC2-positives using MY09/MY11 PCR	102	96	29.4 HIV+ 2.1 HIV–	27.3 HIV+, 6.4 HIV–	Cytology	HPV testing on anal swab material
Melbye *et al.* (1996), Denmark	Women	MY09/MY11 PCR (6, 11, 16, 18, 31, 33, 35, 45, 39, 51, 52) and HC	81	70	*PCR* 78 HIV+, 60 HIV– *HC* 38 HIV+, 7 HIV–	19 HIV+, 0 HIV–	Cytology	HPV testing on anal Dacron swab material and cervical cotton swab material; women were recruited from HIV screening clinics.
Palefsky *et al.* (1997a), USA	Men	MY09/MY11 PCR (39 different HPV types) and HC	129		PCR, 93.2 HIV+ HC, 84.5 HIV+	35 ASCUS, 12 LSIL, 1 HSIL	Cytology	Testing on anal swab material; study subjects were MSM with CDC group IV HIV disease.
Friedman *et al.* (1998), USA	Men	HC2 and MY09/ MY11/HMB01 PCR	184	79	90.4 HIV+, 69.6 HIV–	60.0 HIV+, 29.6 HIV–	Cytology	HPV testing on anal swab material; patients were MSM.

Table 73 (contd)

Reference, study location	Sex	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Palefsky *et al*. (1998a), USA	Men	No HPV DNA detection	346	262		*Abnormal cytology* 45.9 HIV+, 9.9 HIV– *AIN by cytology or histology* 35.8 HIV+, 7.3 HIV–	Cytology, histology	
Palefsky *et al*. (1998b), USA		MY09/MY11 PCR (39 different HPV types) and HC2	346	262	*PCR* 93.1 HIV+, 61.0 HIV– *HC* 87.3 HIV+, 37.3 HIV–	NR		Same cohort as above; testing on anal swab material; patients were MSM.
Sayers *et al*. (1998), United Kingdom	Men	PCR with E6 primers (6, 11, 16, 18)	66	232 (181 MSM), 51 hetero-sexual)	NR	*AIN in satisfactory smears* 30.0 HIV+ MSM, 4.7 HIV– MSM, 0 HIV– heterosexual		Anal cytology obtained with a cytobrush
Goldstone *et al*. (2001), USA	Men	No HPV DNA detection	131			*Biopsy* 60.0 high-grade AIN, 3 invasive anal cancer	Cytology, histology, biopsy	Patients referred for condyloma or other presumably benign anorectal disease
Holly *et al*. (2001), USA	Women	MY09/MY11 PCR (39 types)	251	68		*Anal cytology* 26.0 HIV+, 8.2 HIV–	Cytology, histology	WIHS study

Table 73 (contd)

Reference, study location	Sex	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Palefsky *et al.* (2001), USA	Women	MY09/ MY11 PCR (39 types and HC2)	251	68	76.2 HIV+, 42.1 HIV– *Anal HPV* 79 HIV+ 63 HIV–			HPV testing on anal swab specimens
Sobhani *et al.* (2001), France	Men and women	In-situ PCR (6, 11, 16/18, 31, 33)	116	60	*Oncogenic HPV* 27 HIV+, 1.3 HIV–	*Condyloma recurrence* 75 HIV+, 6 HIV–	Histology	Eight women were diagnosed with condyloma at entry; fixed anal biopsy specimens; ISH or in-situ PCR for EBV and HSV; immunohistochemistry for CMV
Drobacheff *et al.* (2003), France	Men and women	HC2	50	50	58.0 HIV+, 6.0 HIV–	4 HIV+, 0 HIV–		HPV testing on anal swab material; the risk for HPV infection in HIV+ compared with HIV– was 9.7 (95% CI, 3.2–29.7).
Moscicki *et al.* (2003), USA	Men and women	MY09/MY11 PCR for HPV LR (6/11/42/44) and HR (16/18/31/33/ 35/39/42/51/52/56/5 8)	241	107	*Boys* 48.3 HIV+, 36.0 HIV– *Girls* 32.2 HIV+, 13.4 HIV–	*Boys* 52.5 HIV+, 16.7 HIV– *Girls* 21.3 HIV+, 5.7 HIV–	Cytology	Subjects were adolescents participating in the REACH cohort; HPV testing on anal swab material
Piketty *et al.* (2003), France	Men	MY09/MY11 PCR (39 different HPV types)	50 with no history of RAI, 67 with history of RAI		*No RAI* 46.0 any infection *RAI* 85	*No RAI* 16 LSIL, 19 HSIL *RAI* 49 LSIL, 18 HSIL	Cytology, histology	HPV testing from anal swab specimen

Table 73 (contd)

Reference, study location	Sex	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Chin-Hong *et al*. (2004), USA	Men	MY09/ MY11 PCR (39 different HPV types)		1218	56.8, 25.6 HR types, 26.4 LR types	NR		Study of sexually active MSM in 4 US cities participating in the EXPLORE cohort

See Table 7 for a description of the primers used.

AIN, anal intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; CDC, Centers for Disease Control; CI, confidence interval; CMV, cytomegalovirus; EBV, Epstein–Barr virus; EXPLORE, collaborative study among homosexual men in six US cities; HC2, Hybrid Capture 2; HIV, human immunodeficiency virus; HIV–, HIV-negative; HIV+, HIV-positive; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; HSV, herpes simplex virus; ISH, in-situ hybridization; LR, low-risk; LSIL, low-grade squamous intraepithelial lesion; MSM, men who had sex with men; NR, not reported; PCR, polymerase chain reaction; RAI, receptive anal intercourse; REACH, Reaching for Excellence in Adolescent Care and Health

Palefsky *et al*. (1997a) characterized the prevalence of anal HPV infection and anal lesions in 129 HIV-positive men who had sex with men and had Centers for Disease Control group IV HIV disease. Abnormal anal cytology was detected in 39% of subjects and anal HPV infection, as measured by PCR, in 93%. Risk factors for abnormal cytology in a multivariate analysis included HPV 16/18 infection (relative risk, 2.1; 95% CI, 1.2–3.5) and intravenous drug use (relative risk, 1.8; 95% CI, 1.2–2.7). Infection with HPV 6/11 also had significantly elevated relative risks in a separate model. Anal cytological abnormalities and HPV infection are common among homosexual/bisexual men with group IV HIV disease.

Friedman *et al*. (1998) studied the prevalence of anal HPV infection, using Hybrid Capture 2 and PCR, and anal disease, using cytology, in a cohort of HIV-positive and HIV-negative men who had sex with men. HPV was found more commonly in HIV-positive men (90.4% versus 69.6%), among whom the number of HPV types detected and Hybrid Capture RLU ratios were higher. Among HIV-positive men, the quantity of HPV DNA as measured by Hybrid Capture RLU ratio was inversely associated with $CD4^+$ cell count. Anal cytological abnormalities were more common among HIV-positive than HIV-negative men (60% versus 29.6%) and, among HIV-positive men, AIN was found more frequently among those with a $CD4^+$ cell count < 200/μL. Men with a high Hybrid Capture RLU ratio were 2.8 times more likely to have AIN (95% CI, 1.1–7.3) than those with samples that were both PCR- and Hybrid Capture 2-negative.

Palefsky *et al*. (1998a) examined the prevalence of AIN in a cohort of 346 HIV-positive and 262 HIV-negative men who had sex with men. Abnormal cytology was detected in 45.9% of HIV-positive and 9.9% of HIV-negative men. Using cytology or histology, AIN was diagnosed in 36% of HIV-positive and 7% of HIV-negative men (relative risk, 5.7; 95% CI, 3.6–8.9). High-grade AIN was found in 17 HIV-positive men (5%) and in only one HIV-negative man. Among HIV-positive men, the relative risk for AIN increased with lower $CD4^+$ cell levels but was elevated even in men with $CD4^+$ cell levels > 500/mm^3 (relative risk, 3.8; 95% CI, 2.1–6.7) compared with HIV-negative men. High-level HPV infection, as measured by detection of both low-risk and high-risk types using Hybrid Capture, was another significant risk factor for AIN in both HIV-positive (relative risk, 8.8; 95% CI, 2.3–35) and HIV-negative men (relative risk, 20; 95% CI, 5.5–71) compared with HPV-negative men.

Palefsky *et al*. (1998b) also studied the prevalence of anal HPV infection in the cohort described above. Anal HPV DNA was detected by PCR in 93% of HIV-positive and 61% of HIV-negative men. The spectrum of HPV types was similar in HIV-positive and HIV-negative men, and HPV 16 was the most common type. Infection with multiple HPV types was found in 73% of HIV-positive and 23% of HIV-negative men. Among HIV-positive men who were positive by Hybrid Capture for low-risk and high-risk types, lower $CD4^+$ cell levels were associated with higher levels of high-risk HPV DNA ($p = 0.004$) but not low-risk HPV DNA. These data suggest increased replication of the higher-risk HPV types with more advanced immunosuppression. For HIV-positive men, risk factors for the presence or absence of HPV DNA could not be assessed because most were positive for

HPV DNA using PCR or Hybrid Capture. Among the risk factors examined in univariate analyses for the HIV-negative men, the relative risks for HPV infection detected by PCR were: lifetime rectal drug use, 1.4 (95% CI, 1.1–1.7); lifetime history of rectal discharge, 1.3 (95% CI, 1.0–1.7); and lifetime level of receptive anal intercourse compared with no receptive anal intercourse: low, 1.3 (95% CI, 0.97–1.7); medium or high, 1.5 (95% CI, 1.1–2.1; p for trend = 0.03). The relative risks for HPV DNA positivity using Hybrid Capture were similar to those using PCR.

Sayers *et al*. (1998) studied 66 HIV-positive and 181 HIV-negative men who had sex with men and 51 HIV-negative heterosexual men who attended a genitourinary medicine clinic in Edinburgh, United Kingdom. AIN was noted in 30% of satisfactory anal smears from HIV-positive men, in 4.7% of satisfactory smears from HIV-negative men who had sex with men and in no smear from HIV-negative heterosexual men. There was no significant difference in the detection of HPV types 6, 11, 16 and 18 between HIV-positive and HIV-negative men.

Goldstone *et al.* (2001) determined the prevalence of anal HSILs and anal squamous-cell cancer in 131 HIV-positive and 69 HIV-negative men who had sex with other men. Ninety-three per cent had abnormal anal cytology. Biopsy results revealed that 60% of patients had high-grade AIN and 3% had invasive anal cancer. Four of five men with anal squamous-cell cancer were HIV-positive.

Holly *et al*. (2001) examined the prevalence of anal lesions in 251 HIV-positive and 68 HIV-negative women at high risk who participated in the San Francisco section of the Women's Interagency HIV Study. Abnormal anal cytology was diagnosed in 26% of HIV-positive and 8% of HIV-negative women. High-grade AIN was detected by histology or cytology in 6% of HIV-positive and 2% of HIV-negative women. HIV-positive women had an increased risk for anal disease as their $CD4^+$ cell counts decreased ($p < 0.0001$) and as their plasma HIV RNA viral load increased ($p = 0.02$). HIV-positive women with abnormal cervical cytology had an increased risk for abnormal anal cytology at the same visit (relative risk, 2.2; 95% CI, 1.4–3.3). In a multivariate analysis, an HIV viral load of > 100 000 copies/mL (relative risk, 2.4; 95% CI, 1.1–3.9), history of anal intercourse (relative risk, 2.3; 95% CI, 1.2–3.6) and concurrent abnormal cervical cytology (relative risk, 2.1; 95% CI, 1.0–3.6) were significantly associated with abnormal anal cytology.

Palefsky *et al*. (2001) studied the same population of women for the presence of anal HPV using PCR and Hybrid Capture 2 (relative risk, 1.8; 95% CI, 1.3–2.5). Among 200 women for whom there were concurrent data on anal and cervical HPV, anal HPV was more common than cervical HPV in both HIV-positive (79% versus 53%) and HIV-negative (43% versus 24%) women. In a multivariate analysis of HIV-positive women, $CD4^+$ cell counts ≤ 200 cells/mm^3 compared with counts > 500 cells/mm^3 (relative risk, 1.4; 95% CI, 1.1–1.5) and cervical HPV infection (relative risk, 1.3; 95% CI, 1.1–1.4) were associated with anal HPV infection. Women over 45 years of age had a lower risk than women under 36 years of age (relative risk, 0.80; 95% CI, 0.50–0.99), and African-American women had a lower risk (relative risk, 0.86; 95% CI, 0.72–1.0) than Caucasian women.

Sobhani *et al.* (2001) determined the prevalence of anal dysplasia and cancer in patients with anal condyloma with respect to HIV status and HPV positivity. The most important factors that differed significantly between HIV-positive and HIV-negative patients were the prevalence of oncogenic HPV and other current infections (44% versus 0%). During follow-up, condylomas recurred in 75% of HIV-positive patients but in only 6% of HIV-negative patients.

Drobacheff *et al*. (2003) studied 50 HIV-positive and 50 HIV-negative men and women in France. Using Hybrid Capture 2 on anal swab material, HPV DNA was found in 58% and 6% of the samples, respectively. There was no difference in the prevalence of high-risk HPV types between men with and without a history of anal intercourse. Risk factors for HPV infection were $CD4^+$ cell counts < 500/μL (relative risk, 2.13; 95% CI, 1.0–4.7) and history of anogenital warts (relative risk, 2.36; 95% CI, 1.2–4.6). A very low prevalence of anal lesions was found in this study but the authors did not mention the use of high-resolution anoscopy to detect lesions. Using the Hybrid Capture RLU ratio, HPV load was greater in patients with $CD4^+$ cell counts ≤ 500/μL than in patients with $CD4^+$ cell counts > 500/μL ($p < 0.04$). Similar to the results of Palefsky *et al*. (2001), HIV-positive women in this study had a rate of anal HPV infection that was similar to that of HIV-positive men. Overall, the data showed that HIV-positive patients with low $CD4^+$ cell counts have anal HPV infection, regardless of the route of HIV transmission.

One study reported anal HPV and cytology data among HIV-positive and HIV-negative adolescent boys with a history of sex with men and girls who participated in the Reaching for Excellence in Adolescent Care and Health cohort (Moscicki *et al.*, 2003). The prevalence of anal HPV infection was similar in HIV-infected [28/58 (48%)] and uninfected [9/25 (36%)] boys ($p = 0.3$), but was greater in HIV-positive girls [59/183 (32%)] than in HIV-negative girls [11/82 (13%)] ($p < 0.001$). Abnormal anal cytology was more common among boys (41.6%) than girls (16.5%; $p < 0.001$). Independent risk factors for abnormal anal cytology in boys included infection with low-risk HPV types, infection with high-risk HPV types, infection with unknown HPV types and HIV positivity. Among girls, independent risk factors for abnormal anal cytology included infection with high-risk or unknown HPV types and number of sexual partners within the past 3 months. The results suggest that anal cytology screening should be considered in HIV-positive homosexual/bisexual boys and possibly HIV-positive girls.

Piketty *et al*. (2003) compared the prevalence of anal HPV infection and SIL among 67 HIV-positive men who had sex with men and 50 HIV-positive heterosexual male intravenous drug users with no history of receptive anal intercourse; 46% of the heterosexual intravenous drug users had anal HPV infection and 18% had anal HSIL. Among the 67 men who had sex with men, 85% had anal HPV infection and 18% had anal HSIL. The data showed that anal HPV infection and anal SIL may be acquired in the absence of anal intercourse in HIV-positive men.

Chin-Hong *et al*. (2004) studied the age-related prevalence of anal HPV infection among 1218 sexually active, HIV-negative men who had sex with men aged 18–89 years who participated in the EXPLORE study in four cities in the USA. HPV DNA was found

in the anal canal of 57% of the study participants. The prevalence of anal HPV infection did not change with age or geographical location. In a multivariate analysis, anal HPV infection was associated with receptive anal intercourse during the preceding 6 months (odds ratio, 2.0; 95% CI, 1.5–2.8; $p < 0.0001$) and with having 6–30 sexual partners during the preceding 6 months (odds ratio, 1.4; 95% CI, 1.1–1.9), and more than 30 partners (odds ratio, 2.3; 95% CI, 1.5–3.6).

(ii) *Natural history of anal HPV infection and anal SIL* (Table 74)

In a prospective study of 158 HIV-positive and 147 HIV-negative men who had sex with men and did not have anal disease at baseline, high-grade AIN developed in 15.2 and 5.4%, respectively (Critchlow *et al.*, 1995). High-grade AIN among HIV-positive men was associated with the detection of high levels of HPV 16 or 18, detection of HPV types other than 16 or 18, $CD4^+$ cell count $\leq 500 \times 10^6$/L and the number of positive HPV tests.

To characterize the natural history of anal HPV infection, Critchlow *et al*. (1998) followed 287 HIV-negative and 322 HIV-positive men who had sex with men who attended a community-based clinic. Anal HPV DNA was detected at study entry in 91.6% of HIV-positive and 65.9% of HIV-negative men. Detection of HPV was associated with lifetime number of sexual partners and recent receptive anal intercourse (HIV-negative men), lower $CD4^+$ lymphocyte count (HIV-positive men) and anal warts (all men). Among the men who were negative for HPV at study entry, detection of HPV during follow-up was associated with HIV, unprotected receptive anal intercourse and any sexual contact since the last visit. Becoming HPV-negative during follow-up was less common among men with HIV infection or high levels of HPV at study entry. Men with low-risk HPV types at entry were more likely to become HPV-negative than men with intermediate- or high-risk HPV types ($p \leq 0.05$).

In a prospective study of the incidence of anal HSIL among 346 HIV-positive and 262 HIV-negative men who had sex with men, Palefsky *et al*. (1998a) showed that HIV-positive men were more likely to develop HSIL than HIV-negative men (relative risk, 3.7; 95% CI, 2.6–5.7). A life-table estimate of the 4-year incidence of HSIL was 49% (95% CI, 41–56%) among HIV-positive and 17% (95% CI, 12–23%) among HIV-negative men. The incidence of high-grade AIN within 2 years of follow-up was 20% in HIV-positive and 8% in HIV-negative men who were normal at baseline; 62% of HIV-positive and 36% of HIV-negative men with low-grade AIN at baseline progressed to high-grade AIN. Of the HIV-positive men who had ASCUS at baseline, 70.3% were diagnosed with low- or high-grade AIN within 2 years, as were 30.8% of HIV-negative men. Overall, the relative risk for progression of anal disease in HIV-positive men was 2.4 (95% CI, 1.8–3.2) compared with HIV-negative men. The relative risk increased to 3.1 (95% CI, 2.3–4.1) in HIV-positive men with $CD4^+$ cell counts < 200/mm^3. Infection with multiple HPV types was a risk factor for progression of anal disease in both HIV-positive (relative risk, 2.0; 95% CI, 1.0–4.1) and HIV-negative (relative risk, 5.1; 95% CI, 2.3–11) men. The incidence of anal HSIL and progression of LSIL to HSIL within 2 years

Table 74. Anal HPV infection and anal squamous intraepithelial lesions (SIL) in HIV-positive and HIV-negative patients

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Critchlow *et al*. (1995), USA	SBH and MY09/ MY11 PCR	158	147	NR	High-grade AIN, 15.2 HIV+, 5.4 HIV–	Cytology, histology	Dacron anal swab specimens used for HPV testing. Participants had no anal disease at baseline. The study included MSM only.
Critchlow *et al*. (1998), USA	SBH, HC or MY09/MY11 PCR (LR 6/11/42/43/44, IR 31/ 33/35/39 or HR 16/18/45)	322	287	*All types* 91.6 HIV+, 65.9 HIV–, *LR types* 49.1 HIV+, 36.2 HIV–, *IR types* 39.1 HIV+, 14.6 HIV–, *HR types* 55.9 HIV+, 28.9 HIV–			Prospective cohort study of anal HPV infection. HPV testing on anal swab material; included MSM only

Table 74 (contd)

Reference, study location	Method of detection (types included)	No. of HIV-positive cases	No. of HIV-negative controls	HPV prevalence (%)	Anorectal abnormality (%)	Pathology reading	Comments
Palefsky *et al*. (1998a), USA	MY09/MY11/HMB 01 (6, 11, 16,18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, Pap 155, Pap 291, AE2)	346	262	NR	*4-year incidence of HSIL* 49 HIV+, 17 HIV– *2-year incidence of HSIL among men with no disease at baseline* 20 HIV+, 8 HIV– *2-year incidence of HSIL among men with LSIL at baseline* 62 HIV+, 36 HIV–	Cytology, histology	Dacron anal swab specimens used for HPV testing; HIV+ participants followed every 3–6 months; HIV– participants followed every 12 months; included MSM only
Lacey, H.B. *et al*. (1999) United Kingdom	GP5/GP6 PCR primers with type-specific primers for 6/11, 16, 18, 31, 33	57		84.2 HIV+	70 incident HSIL among men with no abnormality or LSIL at baseline	Cytology, histology	Dacron anal swab specimen used for HPV testing. Participants were followed every 4 months. The study included MSM only.
Sobhani *et al.* (2001), France	In-situ PCR (6, 11, 16/18, 31, 33)	114	60		*Recurrence during follow-up* 75 HIV+, 6 HIV–		Study included men and women who had been treated for anal canal condyloma.

See Table 7 for a description of the primers used.
AIN, anal intraepithelial neoplasia; CI, confidence interval; HC, Hybrid Capture; HIV, human immunodeficiency virus; HIV+, HIV-positive; HIV–, HIV-negative; HSIL, high-grade squamous intraepithelial lesion; IR, intermediate-risk; HR, high-risk; LR, low-risk; LSIL, low-grade squamous intraepithelial lesion; MSM, men who had sex with men; NR, not reported; PCR, polymerase chain reaction; SBH, Southern blot hybridization

of follow-up is high in HIV-positive homosexual or bisexual men and lower in HIV-negative men.

In a study in the United Kingdom, Lacey, H.B. *et al*. (1999) followed 57 HIV-positive men who had sex with men over an average of 17 months. High-risk HPV types were detected in 84% and high-grade disease in 10.5% at baseline; 70% of the men with no or low-grade disease at baseline developed high-grade disease during follow-up. The study was limited by the relatively small sample size and by the fact that no biopsies were taken at baseline, which raises the possibility of misclassification of disease in some participants.

Sobhani *et al*. (2001) followed 114 HIV-positive and 60 HIV-negative men and women who had been treated for anal canal condyloma. During follow-up, condylomata recurred in 75% of HIV-positive (19 high-grade AIN and one invasive carcinoma detected in the follow-up biopsy), but only 6% of HIV-negative patients (one high-grade AIN). Male sex (odds ratio, 2.9; 95% CI, 1.9–7.2) and HIV positivity (odds ratio, 10.3; 95% CI, 8.8–12.9) were among the independent risk factors for recurrence of condyloma. High serum HIV load was also associated with recurrence, whereas low $CD4^+$ T-lymphocyte counts were not. In-situ hybridization and in-situ PCR were also used to detect EBV, CMV, HSV and gonococcus in biopsy specimens at baseline. One or more of these agents was found in specimens from 21 HIV-positive but no HIV-negative patients.

(iii) *Effect of HAART on anal SIL*

Palefsky *et al*. (2001) studied the short-term effects of HAART on the natural history of AIN. AIN and the level of anal HPV DNA, measured using the Hybrid Capture RLU ratio, were evaluated among 98 HIV-positive men who had sex with men at least 6 months before initiation of treatment with HAART. The results were compared with those evaluated 6 months after initiation of HAART. Among men whose most severe pre-HAART diagnosis was ASCUS or low-grade AIN, 18% (95% CI, 6–31%) of the lesions progressed and 21% (95% CI, 8–34%) regressed 6 months after initiation of HAART. Seventeen per cent (95% CI, 0–38%) of men who had a normal diagnosis at the start of the study developed AIN. Only 4% (95% CI, 0–10%; 1/28) of men had high-grade AIN that regressed to normal. There was no reduction in the proportion of men who tested positive for HPV DNA or in the levels of HPV DNA after initiation of HAART. These results indicate that HAART has little effect on either AIN or HPV in the first 6 months after initiation of treatment.

Durante *et al*. (2003) studied the incidence of anal cytological abnormalities among 100 HIV-positive women who participated in the GRACE cohort. Fourteen had abnormal anal cytology at baseline; among the remaining 86 women, 40 (46.5%) had an HPV infection in both the anus and the cervix. Of these, 13 had at least one HPV type in common, while 27 had no HPV types in common. Cervical and anal HPV infection were compared and, as in earlier studies, anal HPV infection was found to be more common than cervical HPV infection. Anal infection was found in 57 women (66.3%) and cervical HPV infection was found in 45 women (52.3%). Among the 86 women who had normal baseline cytology, the incidence of an abnormality was 22 (95% CI, 14–33) per 100 person–years. In a

multivariate analysis, women were at increased risk if they had a baseline $CD4^+$ cell count of < 500 cells/mm^3 (relative hazard, 4.11; 95% CI, 1.18–14.25) or a high-risk anal HPV-type infection (relative hazard, 2.54; 95% CI, 0.91–7.14) or were current cigarette smokers at baseline (relative hazard, 3.88; 95% CI, 1.12–13.42). Use of HAART had no effect on incidental anal cytological abnormalities (relative hazard, 1.07; 95% CI, 0.40–2.85). The authors concluded that the incidence of anal cytological abnormalities was high among this cohort of HIV-infected women, which indicates that they are at high risk for anal SIL.

Horster *et al*. (2003) screened HIV-positive patients for visible anal condyloma between 1985–95 (before treatment with HAART) and 1996–2001 (after treatment with HAART). A total of 1472 patients were screened repeatedly for anal condyloma as a risk factor for AIN and anal cancer. The proportion of screens that were positive for anal condyloma was significantly higher after the treatment ($p < 0.001$) independent of $CD4^+$ cell counts.

Gonzales-Ruiz *et al*. (2004) compared 117 HIV-positive patients in 1994–95 with 109 HIV-positive patients in 2001–02 with respect to the prevalence and distribution of HIV-related anorectal pathologies, such as anal ulcer and anogenital condyloma, and non-HIV-related anorectal pathologies, including fissure, fistula *in ano*, haemorrhoids and perianal abscess. The prevalence and distribution of anorectal pathology seen in HIV patients did not change after the introduction of HAART.

Piketty *et al.* (2004) examined the effect of HAART-associated increases in $CD4^+$ cell count on the prevalence of AIN. Forty-five HIV-positive protease inhibitor-treated men who had sex with men were enrolled in a cross-sectional study in France. The patients had previously received HAART for a median of 32 months. Anal cytology was abnormal in 32 patients (71%), and HPV DNA was detected in 36 (80%). Baseline prevalence of anal HPV infection and AIN was not affected by baseline $CD4^+$ cell count. The prevalence of anal SIL and HPV infection was similar in patients who had a significant increase in $CD4^+$ cell count after initiation of HAART compared with those who did not. The results demonstrated a high prevalence of anal SIL, including HSIL, and anal HPV infection in HIV-positive men who had sex with men despite immunity associated with HAART.

Wilkin *et al*. (2004) assessed the association between HAART and the prevalence of anal HPV infection and AIN in 92 HIV-positive men, 40 of whom had no history of anal intercourse. High-risk HPV DNA was identified in 61%, and was associated with a history of receptive anal intercourse (78% versus 33%; $p < 0.001$); 47% had abnormal cytology and 40% had AIN on biopsy. Risk factors for anal HPV infection included history of receptive anal intercourse (odds ratio, 7.2; 95% CI, 2.8–18; $p < 0.001$) but not baseline $CD4^+$ cell count, age or use of HAART. Among men with HPV infection, in a multivariate analysis, higher baseline $CD4^+$ cell count was protective against AIN at biopsy (odds ratio, 0.5; 95% CI, 0.3–0.9; $p = 0.03$) as was current use of HAART (odds ratio, 0.09; 95% CI, 0.01–0.75; $p = 0.03$). The relationship between the use of HAART and AIN was apparent only after controlling for baseline $CD4^+$ cell count.

(*c*) *Invasive cervical and anal cancer among HIV-positive subjects*

Zanetta *et al*. (1995) evaluated retrospectively all patients referred for invasive cervical carcinoma from 1991 to 1994 at the San Gerardo Hospital, Milan, Italy. Six of 340 women (1.8%) were found to be HIV-positive. Five of the six HIV-positive patients with cervical cancer were known to have been infected 13–81 months before diagnosis of cancer, but none had undergone a Pap test in the previous year. HIV-positive women were younger than the general population ($p = 0.02$) and were more likely to have a history of intravenous drug use ($p = 0.000001$) and more advanced disease at presentation ($p = 0.04$).

In the USA and Puerto Rico, Goedert *et al*. (1998) matched people who had cancer, were under the age of 70 years and were taken from population-based cancer registries with people who had AIDS and were taken from population-based AIDS registries. AIDS-related cancers were defined as those that had a significantly increased incidence after diagnosis of AIDS and an increased prevalence between 5 years before and 2 years after diagnosis of AIDS. The relative risk for anal cancer after AIDS was 31.7 (95% CI, 11.6–69.2). However, the risk was also increased about 15-fold during the early pre-AIDS period. Thus, the *p* value for trend was not significant ($p = 0.085$). The relative risk for cervical carcinoma *in situ* was < 1 during the period before AIDS but increased significantly after AIDS to 1.7 (p for trend = 0.01); in contrast, invasive cervical cancer had a relative risk of 5.4 before AIDS that did not increase significantly after AIDS (relative risk, 2.9; 95% CI, 0.7–16.0).

Mayans *et al*. (1999) reported on the incidence of cervical cancer in Catalonia, Spain, as an AIDS-defining diagnosis using an AIDS surveillance system from 1994 to 1996. Age-specific incidence rates for invasive carcinoma of the cervix from a population-based cancer registry were used to calculate the population attributable risk per cent. Fifty-six women with cervical cancer were reported to the AIDS registry, with a mean age of 32 years. Cervical cancer was the sixth most common AIDS-defining illness. The age-specific rate among HIV-positive women aged 20–49 years was 186.7/100 000 and the attributable fraction of HIV among women in this age group was 94.5%. The incidence rate ratio among HIV-positive and HIV-negative women in this age group was 18.5 (95% CI, 11.2–29.2).

Serraino *et al*. (1999) studied the risk for cervical cancer in a longitudinal study of 1340 HIV-positive intravenous drug users, 811 HIV-negative intravenous drug users and 801 HIV-positive heterosexual women aged 15–49 years in northern Italy and south-eastern France. A total of 9070 person–years of observation were accumulated among HIV-positive women and 2310 among HIV-negative women. Overall, the standardized incidence ratio (SIR) was 12.8 (95% CI, 6.6–22.4) among HIV-positive women and was higher for HIV-positive intravenous drug users (SIR, 16.7; 95% CI, 5.2–28.2) than for HIV-positive heterosexual women (SIR, 6.7; 95% CI, 0.0–15.9). No case of invasive carcinoma of the cervix was diagnosed among HIV-negative intravenous drug users.

In the largest study on the relationship between HPV-related neoplasia and HIV infection, Frisch *et al*. (2000) performed an AIDS–cancer registry match to examine invasive HPV-associated cancers and carcinoma *in situ* among 257 605 men and 51 760 women with

HIV infection/AIDS from 5 years before the date of onset of AIDS to 5 years after this date. The incidences of all HPV-associated cancers in AIDS patients were significantly increased compared with the expected numbers of cancers. For invasive cancers, overall risks were significantly increased for cervical (relative risk, 5.4; 95% CI, 3.9–7.2), vulvovaginal (relative risk, 5.8; 95% CI, 3.0–10.2) and anal (relative risk, 6.8; 95% CI, 2.7–14.0) cancers in women. Among men, the risks for anal (relative risk, 37.9; 95% CI, 33.0–43.4), penile (relative risk, 3.7; 95% CI, 2.0–6.2), tonsillar (relative risk, 2.6; 95% CI, 1.8–3.8) and conjunctival (relative risk, 14.6; 95% CI, 5.8–30.0) cancers were significantly increased. The relative risks for these invasive cancers changed little during the 10 years spanning the AIDS onset. Relative risks were significantly increased for in-situ cervical (4.6; 95% CI, 4.3–5.0) and vulvovaginal (3.9; 95% CI, 2.0–7. 0) lesions in women. The relative risk for anal cancer among men was highest among those with a history of homosexual contact (59.5; 95% CI, 51.5–68.4), although men who were reported to have acquired HIV through intravenous drug use (relative risk, 5.9; 95% CI, 2.7–11.2) were also at a significantly increased risk. Women with a history of intravenous drug use were at higher risk for cervical cancer (relative risk, 7.0; 95% CI, 4.7–10.0) than those who acquired HIV through heterosexual contact (relative risk, 4.9; 95% CI, 2.7–8.2). The estimated incidence of anal cancer after AIDS was 18.2/100 000 person–years among men and 3.9/100 000 person–years among women. The estimated incidence of cervical cancer among women after AIDS was 85.7/100 000 person–years and that for vulvovaginal cancer was 7.9/100 000 person–years. Among men, the relative risk was increased for anal (60.1; 95% CI, 49.2–72.7) and penile (6.9; 95% CI, 4.2–10.6) in-situ lesions. In contrast to invasive cancers, relative risks for in-situ lesions increased during the 10 years spanning the AIDS onset for the cervix (p for trend < 0.001), vulvovaginal area (p for trend = 0.04) and penis (p for trend = 0.04).

In a case–control study, Sitas *et al*. (2000) examined the relationship between HIV and a number of cancer types or sites that are common in three tertiary referral hospitals in Johannesburg, South Africa. Significant excess risks associated with HIV infection were found for vulvar cancer (odds ratio, 4.8; 95% CI, 1.9–12.2) and cervical cancer (odds ratio, 1.6; 95% CI, 1.1–2.3).

Using a match between the New York State Cancer Registry and the New York City AIDS Registry, Gallagher *et al*. (2001) compared cancer incidence in patients who were diagnosed with AIDS between 1981 and 1994 and were 15–69 years of age in New York State with that in the New York State general population. Sex and HIV-risk group-specific SIRs, relative risks after AIDS and trends of relative risks were calculated to determine the risk for cancer. Among non-AIDS-related cancers, an elevated SIR for combined rectal, rectosigmoid and anal cancer was found for both men (SIR, 3.3; 95% CI, 2.60–4.15) and women (SIR, 3.0; 95% CI, 1.39–5.77). After the diagnosis of AIDS, the SIR for combined rectal, rectosigmoid and anal cancer was 4.0 (95% CI, 2.6–6.0) in men and 4.2 (95% CI, 0.9–12.2) in women. Among women, the SIR for invasive cervical cancer was 9.1 (95% CI, 6.9–10.8) and the relative risk after AIDS was 6.5 (95% CI, 4.1– 9.7). Among men, the SIR for rectal, rectosigmoid and anal cancer was significantly elevated only among men with a history of homosexual contact (SIR, 5.8; 95% CI, 4.4–7.4). Among women, a history of

intravenous drug use, heterosexual contact and unknown/other factors resulted in significantly increased SIRs for rectal, rectosigmoid and anal cancer as well as cervical cancer.

Newton *et al*. (2001) performed a case–control study of HIV infection and cancer risk in Kampala, Uganda. Of the 302 cases recruited, 190 had a cancer with a potential infectious etiology (cases); the remaining 112 adults who had a tumour not known to have an infectious etiology formed the control group. The odds ratios for HIV positivity among cases of specific cancers (other than Kaposi sarcoma in adults) were compared with those in controls, adjusted for age and sex and, in adults, for the number of lifetime sexual partners. In adults, HIV infection was associated with a significantly ($p < 0.05$) increased risk for non-Hodgkin lymphoma and conjunctival squamous-cell carcinoma but not for cervical cancer (odds ratio, 1.6; 95% CI, 0.7–3.6).

Gichangi *et al*. (2002) studied 3902 women who were diagnosed with reproductive tract malignancies at Kenyatta National Hospital from 1989 to 1998, a period when the Kenyan national prevalence of HIV rose from 5 to 15%; 85% of the women had invasive cervical cancer and the age at presentation and severity of cervical cancer were compared between HIV-positive and HIV-negative women. There was no significant difference in either age at presentation or severity of cervical cancer between HIV-positive and HIV-negative women. Of the 118 (5%) women who were tested for HIV, 36 (31%) were HIV-positive and were significantly younger (42 versus 47 years of age; $p < 0.001$) than HIV-negative women. Neither the proportion of women with cervical cancer under the age of 35 years nor the severity of cervical cancer changed during the study period. The authors concluded that HIV-positive women were younger at the time of diagnosis of cervical cancer than HIV-negative women, but that the profile of cervical cancer changed relatively little in Kenya despite the rapid rise in HIV prevalence in the population.

Serraino *et al*. (2002) analysed data from the national AIDS surveillance systems of 15 European countries that had 50 or more female AIDS cases and from population-based cancer registries of those countries. Female cases aged 20–49 years who were diagnosed between 1993 and 1999 were included in the study. The odds ratio for cervical cancer as an AIDS-defining illness increased with age, was significantly elevated in southern (odds ratio, 3.1; 95% CI, 1.8–5.4) and central Europe (odds ratio, 2.5; 95% CI, 1.4–4.4) compared with northern Europe and was also increased among intravenous drug users (odds ratio, 1.5; 95% CI, 1.2–1.9). The proportion of cervical cancer correlated directly with the proportion of intravenous drug users among female AIDS cases and was highest in areas where population-based cervical cancer screening programmes were less effective.

Cress and Holly (2003) reported on the age-adjusted incidence rates calculated by gender, race/ethnicity, county and year of diagnosis for over 2100 cases of cancer of the anus diagnosed between 1995 and 1999 in California, USA. Age-adjusted incidence rates by time period 1973–99 were calculated for San Francisco County, where HAART was introduced widely in 1996. For all of California, there was an average 2% annual increase in the incidence of anal cancer among non-Hispanic white men between 1988 and 1999. The incidence of anal cancer among white men who resided in San Francisco County more than doubled between 1984–90 and 1996–99 and, for men aged 40–64 years, rose

from 3.7 cases per 100 000 in 1973–78 and 8.6 cases per 100 000 in 1984–90 to 20.6 cases per 100 000 in 1996–99.

Dal Maso *et al*. (2003) performed a linkage study of people aged 15–69 years using records from the Italian Registry of AIDS and 19 cancer registries that covered 23% of the Italian population for the period 1995–98. Significantly increased SIRs were observed for cervical cancer (21.8; 95% CI, 12.9–34.6) and anal cancer (34; 95% CI, 12.1–73.6). SIRs were similar for both cervical and anal cancer among intravenous drug users and non-users.

In another case–control study, Gichangi *et al*. (2003) studied the association between HIV infection and cervical cancer in Kenyan women. Cases were 367 women who had invasive cervical cancer and controls were 226 women who had fibroids. HIV-positive women with cervical cancer were significantly younger than HIV-negative women with cervical cancer ($p < 0.001$) and were more likely to have poorly differentiated tumours (odds ratio, 3.1; 95% CI, 1.2–8.3) after adjustment for histological cell type and clinical stage. Risk factors for cervical cancer in a multivariate analysis included HIV positivity under the age of 35 years (adjusted odds ratio, 3.3; 95% CI, 1.0–10.8) and never having had a Pap smear (adjusted odds ratio, 5.1; 95% CI, 1.8–14.6).

Mbulaiteye *et al*. (2003) linked records from AIDS and cancer registries in 11 regions of the USA from 1990 to 1996 to examine the relationship between AIDS-related immunosuppression (measured by $CD4^+$ cell count) and the risk for cancer. The SIRs in AIDS patients were 8.8 (95% CI, 6.0–13.0) for invasive cervical cancer, 9.3 (95% CI, 7.4–11.6) for in-situ cervical cancer and 49.9 (95% CI, not reported) for anal cancer. These risks were not modified by $CD4^+$ cell count.

Bower *et al*. (2004) followed a cohort of 8640 HIV-positive individuals in London, United Kingdom, and found that the incidence of invasive anal cancer was 60/100 000 patient–years. In the period before the introduction of HAART (1984–95), the incidence of invasive anal cancer was 35 (95% CI, 15–72)/100 000 patient–years of follow-up. In the period after the introduction of HAART (1996–2003), the incidence was 92 (95% CI, 52–149)/100 000 patient–years of follow-up ($p > 0.05$). The relative risks for anal cancer in the HIV-positive cohort compared with the general population were 67 and 176 in the periods before and after the introduction of HAART, respectively. The data showed that, although the difference between the incidence of anal cancer in the periods before and after the introduction of HAART was not significant, the incidence of anal cancer has not declined since the introduction of HAART.

Sobhani *et al*. (2004) studied 164 French HIV-positive patients who had condylomata of the anus after treatment of their lesion from 1993 to 2002. At baseline, 16% of HIV-positive patients and 6% of HIV-negative patients had high-grade AIN. During follow-up, seven of 199 patients (3.5%; six HIV-positive, one HIV-negative) developed invasive anal cancer after 13–108 months. Six of seven patients who developed anal cancer had high-grade disease at baseline.