2. Studies of Cancer in Humans

In the following discussion of HPV infection as an exposure posing a risk of cancer, the importance of distinguishing 'transient' from 'persistent' infection is emphasized. Several lines of epidemiological evidence suggest that many incident HPV infections are transient, especially at younger ages, whereas less-common, persistent infections pose a higher risk for the development of subsequent cancer. These terms are admittedly ill-defined, as it is unclear whether persistent infection can include an undetectable 'latent' phase. Nevertheless, persistent cytological evidence of low-grade CIN has been shown to pose a higher risk of progression than the first occurrence of (incident) low-grade CIN (Richart & Barron, 1969; Nasiell *et al.*, 1986). Also, persistent HPV DNA detection accompanying cytological/histological diagnosis of CIN has been associated with a high absolute and attributable risk of progression (Remmink *et al.*, 1995).

In the absence of cytological evidence of CIN, 'high-risk' types of HPV (type 16 in particular) tend to persist longer than low-risk types (Hildesheim *et al.*, 1994), and continued viral DNA detection is associated cross-sectionally with greater risk of incident CIN (Wideroff *et al.*, 1995).

Persistence cannot be determined reliably by a single measurement of HPV infection. Moreover, it is not correct to assume that odds ratios or attributable proportion estimates from studies using cross-sectional assessment of past HPV infection are equivalent to prospective risk estimates. Typically, prospective risk estimates are lower than those from cross-sectional studies, because HPV infections appear to be lost more often in eventual 'controls' than in eventual 'cases', so that HPV positive cases in case–control studies are more likely to be persistent infections.

It is also worth noting here that there are too many anogenital HPV types to study each individually. For this reason, types are often grouped into putative 'high-risk' and 'low-risk' groups. These working definitions tend to be variable, as there are several possible sets of grouping criteria, based on either (i) biological behaviour *in vitro*, (ii) phylogeny imputed from DNA sequence homologies, or (iii) epidemiology. In epidemiological studies, the definition of 'high-risk' anogenital types can be based most liberally on finding type-specific HPV DNA (in the absence of other types) in cases of anogenital cancer. More strictly, 'high-risk' types have been defined as those associated with elevated relative risks of cancer in analytical studies.

Some authors have used the terms 'cancer-associated types' or 'oncogenic' types as synonyms for 'high-risk' types; all of these terms should be considered preliminary, given that the studies are designed to establish oncogenicity.

2.1 Descriptive studies of anogenital cancer

This section is limited to the descriptive epidemiological data addressing geographical and temporal correlations at the population level between cancer incidence and HPV prevalence at anogenital sites. To ensure comparability, the section includes only those studies in which the same HPV-testing methods were used for the populations being compared. There appear to be no relevant descriptive data relating rates of HPV infection to rates of cancer at non-anogenital sites.

Finally, because of the variability in exposure measurements of HPV and disease measurements of CIN, the results from different studies are only grossly comparable. Relative risks can be compared for general consistency and strength, but comparisons of absolute risk estimates are not reliable.

2.1.1 Cancer of the uterine cervix

Few studies have attempted to correlate rates (or time trends) of cervix cancer with rates (or time trends) of HPV infection in different parts of the world, but several studies have examined them separately.

Cervical cancer mortality trends in a number of countries have been surveyed by Cuzick and Boyle (1988), Beral *et al.* (1994) and others. Incidence and mortality trends are reported in a recent monograph (Coleman *et al.*, 1993a). The highest rates are found in South America, Asia and Africa, although there are few good data for the last. Very low rates are seen in Israel and the Middle East. The rates appear to be fairly stable in these areas and this is also true for southern and eastern Europe. However, marked declines have been observed in both incidence and mortality of cervical cancer in North America, western Europe and Japan. Much of the reduction has been attributed to an effect of screening (Hakama *et al.*, 1991; Aareleid *et al.*, 1993). An exception to the overall long-term decrease in these countries is a recent increase in rates among young women in the United Kingdom, Australia and New Zealand (Cuzick & Boyle, 1988). Even more recently, increases in cervical cancer rates among young women have been reported in Scandinavia and eastern Europe (Beral *et al.*, 1994). In the USA, blacks have approximately twice the cervical cancer rate of whites, but a study in Detroit of women aged 15–39 (Weiss *et al.*, 1994) found that the rates were decreasing more rapidly in blacks and were nearly equal to those of whites by 1991. The most recent incidence data from the USA suggest a stabilization or a slight increase in the rate of cervical cancer among white women under the age of 50 (Ries *et al.*, 1994). [Some of the international variation in rates of invasive cancer is likely to be due to the marked differences in the availability of screening and diagnosis and treatment of preinvasive lesions.]

No time-related trends in HPV DNA positivity have been identified in studies of archival CIN and cervical cancer specimens obtained from women residing in the USA and the United Kingdom (Collins *et al.*, 1988; Anderson *et al.*, 1993). Findings from these studies indicate that the association between specific types of HPV and cervical neoplasia has been consistent over the past 25–50 years. In each time period, high-risk types of HPV (HPV-16 and -18) were most frequently detected. Also, the more recently discovered HPV types (HPV-42, -45, -51, -52 and -56) were found in archival specimens, some of which were obtained 25 years earlier.

The early studies of the rates of HPV infection in normal women in high-risk and low-risk areas for cervical cancer yielded apparently paradoxical results (Table 7). Comparisons between Denmark and Greenland, the latter of which has a fivefold to sixfold higher cancer rate, did not show higher HPV rates in Greenland. In a population-based study of 1247 randomly selected women aged 20–39, cervical scrapes were analysed for HPV by filter in-situ hybridization (Kjaer *et al.*, 1988). The positivity rate for HPV-16/18 was higher in Denmark (13%) than in Greenland (8.8%). Rates for HPV-6/11 were also slightly higher (7.5% versus 6.7%). In a second study of 255 women (Kjaer *et al.*, 1993), similar prevalences were found in the two countries by dot blot (3.9% in Greenland versus 6.3% in Denmark) and PCR (43% versus 39%, respectively). [The Working Group noted that the apparent lower prevalence of HPV infection in the high-risk area may be explained by the earlier age at first intercourse among Greenlandic women causing an earlier peak and subsequent decline in the HPV–age curve. Consequently, studies covering only the age group 20–39 years may not be fully informative in this respect.]

In a study of 2330 women attending routine family planning and maternal and child health care clinics in Recife (high incidence) and São Paulo (lower incidence), Brazil, Villa and Franco (1989) found a threefold higher prevalence of HPV-16/18 by filter in-situ hybridization in women from the high-incidence area (5.9% versus 2.0% in the low-incidence area) and a 2.4-fold higher prevalence in women from the high-incidence area (5.1% versus 2.1%).

Muñoz *et al.* (1992) studied 228 population-based controls from a case–control study of invasive cancer and found a higher rate of HPV-positivity by PCR in Colombia compared to Spain (13.3% versus 4.6%), although the positivity rates were similar by Southern blot (4.2% versus 5.7%, respectively). In a comparison study in the same area (Bosch *et al.*, 1993), controls to CIN III cases had twice the positivity rate for any HPV by PCR in Colombia compared to Spain (10.5% versus 4.7%). Rates for HPV-16 were also much higher (3.3% versus 0.5%). Cervical cancer incidence rates are about eight times higher in Colombia.

Hildesheim and colleagues (Hildesheim *et al.*, 1993; Bauer *et al.*, 1993) compared HPV DNA prevalences in two populations of cytologically normal women in the USA, presumed to vary substantially in their risk of cervical neoplasia. Specifically, 404 poor, white, black and Hispanic women in Washington, DC (high prevalence of cervical neoplasia) were compared with 483 middle class, predominantly white women attending a pre-paid health plan in Portland, Oregon (low prevalence of cervical neoplasia) using the same *L1* consensus primer PCR test method. The prevalence of HPV DNA was 33.7% in the Washington population, compared to 17.7% among the Portland women.

2.1.2 Other anogenital cancers

Other anogenital cancers have been associated with HPV, notably vulvar, penile and anal cancer. Trends in the incidence of anal cancer have been studied in Denmark by Frisch *et al.* (1993) who found that they were stable between 1943 and 1957, but increased by 50% in men and tripled in women between 1957 and 1987. A similar pattern was observed in Connecticut, USA, where, on the basis of tumour registry data, the incidence of anal cancer increased 2.3-fold among women and 1.9-fold among men between 1960–88. An increase has also been observed in other parts of the USA, and anal cancer is now more frequent in women than men, in blacks than whites, and in residents of metropolitan rather than rural areas (Melbye *et al.*, 1994a).

Table 7. Comparison of HPV detection rates in populations at different risk of cervical cancer

Reference	Location	Cervical cancer incidence per 10^5 population	Source of study population	No. of participants	HPV type-specific detection rate (%)						Detection method (types included)
					6/11	16/18	16	18	6/11 + 16/18 + 31/33/35	HPV-positive	
Kjaer *et al.* (1988)	Greenland Denmark	1.98[a] 0.35[a]	Random samples of the general female population (1986)	586 661	6.7 7.5	8.8 13.0					Filter in-situ hybridization
Kjaer *et al.* (1993)	Greenland Denmark	1.98[a] 0.35[a]	Random samples of the general female population (1988)	129 126					3.9 6.3		ViraPap™/ ViraType™
	Greenland Denmark						20.2 24.6	14.7 19.8		43.4 38.9	PCR (11,16,18,33)
Villa & Franco (1989)	Recife São Paulo	96.5 35.1	Random samples of women attending 3 family-planning and maternal and child health clinics	Total: 2330	5.2 2.4	5.9 2.0					Filter in-situ hybridization
Muñoz *et al.* (1992)	Colombia Spain	48.2 5.7–6.1	Random samples of women in participating areas	98 130			9.2 3.1			13.3 4.6	PCR (6,11,16,18, 31,33,35)
Bosch *et al.* (1993)	Colombia Spain	48.2 5.7–6.1	Women attending screening programmes and family-planning clinics	181 193			3.3 0.5			10.5 4.7	PCR (6,11,16,18, 31,33,35)
Hildesheim *et al.* (1993); Bauer *et al.* (1993)	Washington, DC, USA Portland, OR, USA	High risk Low risk	Women attending screening programmes	404 483						33.7 17.7	PCR (*L1* consensus primers)

[a]Cumulative incidence per 100 women (20–39 years)

In contrast, the incidence of penile cancer was observed to decline during 1943–90 in uncircumsized populations despite the lack of screening procedures and changes in diagnostic criteria (Frisch *et al.*, 1995).

Correlation analyses based on data from 136 cancer registries around the world revealed statistically significant correlations between the rates of cervical cancer and cancers of the mouth and oesophagus and between cancer of the penis and cancers of the mouth and hypopharynx (Muñoz *et al.*, 1990). Bosch and Cardis (1990) used a similar approach to show a correlation between the incidence of cervical cancer and that of cancer of the penis, vagina and vulva. These reports are in line with previous studies in England and Wales (Smith *et al.*, 1980), China (Li *et al.*, 1982) and Brazil (Franco *et al.*, 1988).

Further support for an etiological parallel between anal and cervical intraepithelial neoplasia (Scholefield *et al.*, 1989, 1992) and between anal and cervical cancer has been substantiated in recent linkage studies of multiple primary cancers undertaken in Denmark and the USA (Melbye & Sprøgel, 1991; Rabkin *et al.*, 1992).

2.2 Case reports and case series

Nearly 20 years ago H. zur Hausen suggested that human papillomavirus (HPV) could be causally involved in cervical carcinogenesis (zur Hausen, 1976). Subsequently, HPV DNA has been found repeatedly in cervical cancers and has also been implicated in the genesis of several other human cancer types, particularly neoplasms of the anogenital area, as well as of the skin and the respiratory and digestive tracts.

2.2.1 Cancer of the uterine cervix

(a) Pre-invasive cervical lesions

Table 8 gives a summary of case series of cervical intraepithelial neoplasia (CIN) that included more than 70 cases and gave the HPV results by cytological/histological category. The range of HPV results is broad, but in general the HPV detection rate increased with the severity of the lesion independent of the detection method used. [Cytological/histological misclassification of less-severe diagnoses may explain much of this trend.] The prevalence of HPV in CIN III was reported to be more than 70% in 13 out of the 21 studies. The variation in detection rate between studies was lowest for the PCR method for all cytological/histological groups. The variety of HPV types found in less-severe lesions is greater than that in more-severe lesions, in which the cancer-associated types are typically found.

In the six case series including more than 10 patients with adenocarcinoma *in situ* (Table 9), HPV DNA was found in 25–97% of cases. [The diagnosis of glandular intraepithelial lesions is more problematic than squamous intraepithelial diagnoses.]

(b) Squamous-cell carcinoma

Microscopic evidence of HPV infection has been linked to adjacent cervical cancer for decades. Table 10 summarizes the prevalence of HPV DNA in series ($\geq$ 40 cases) of patients with cervical squamous-cell carcinoma recorded in studies published from 1988 to date. In studies using PCR-based tests designed to detect more than a single HPV type, the HPV

Table 8. Prevalence of HPV DNA in cervical intraepithelial neoplasia (CIN) case series[a] (≥ 70 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Fuchs *et al.* (1988)	Austria	Southern blot (6, 10, 11, 16, 18, 31, 33)	33 CIN I	36		27	9	3		Frozen tissue
			43 CIN II	40		9	35	2		
			140 CIN III	56		6	48	7		
Kurman *et al.* (1988)	USA/Brazil	Southern blot (6, 11, 16, 18, 31)	63 CIN I	71	11	5	19	2	35	Frozen tissue.
			61 CIN II	87	15		41	2	30	'Others' = HPV-31 and
			32 CIN III	97	3		66	6	22	uncharacterized types
Lim-Tan *et al.* (1988)	Singapore	Southern blot (11, 16, 18)	37 CIN I	22			3	3	16	Frozen tissue
			11 CIN II	55			9		45	
			41 CIN III	61		7	27	2	24	
McNicol *et al.* (1989)	Canada	Filter in-situ hybridization (6/11, 16/18)	44 CIN I	84	64			27	14	Cervical swabs
			18 CIN II	72	67			56		
			36 CIN III	81	56			33	19	
Amortegui *et al.* (1990)	USA	In-situ hybridization (6/11, 16, 18, 31)	312 CIN I	50	17		12	7	15	Fixed tissue.
			114 CIN II	54	5		25	7	17	'Others' = HPV-31 and
			46 CIN III	43			24	9	11	-16/18/31 unspecified
Billaudel *et al.* (1991)	France	Southern blot (6, 16, 18)	41 CIN I	27	9		9		22	Frozen tissue
			20 CIN II	50	5		15		30	
			32 CIN III	63			28	3	31	
Cooper *et al.* (1991a)	South Africa	In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	17 CIN II	53			6	6	41	Fixed tissue.
			55 CIN III	49			27	11	11	'Others' = HPV-31 and
	United Kingdom		24 CIN II	58			42	4	13	-33
			49 CIN III	73			49	14	10	
Nuovo *et al.* (1991b)	USA	In-situ hybridization (6/11, 16/18, 31/33/35, 42/43/44, 51/52, 45/56)	174 LSIL	89	18		29		41	Fixed tissue.
			70 HSIL	71			49		23	'Others' predominantly HPV-31, -33, -35
Cornelissen *et al.* (1992)	Netherlands	PCR (*L1* consensus primers and 6/11, 16, 18, 31, 33)	19 CIN I	68	5		21	5	37	Fixed tissue.
			16 CIN II	81			50	6	25	'Others' = HPV-31, -33
			73 CIN III	90			53	7	33	and unidentified
Meguenni *et al.* (1992)	Algeria	Southern blot (6/11, 16)	83 CIN I/II	42	7		35			Frozen tissue
			92 CIN III	54	3		51			
Saragoni *et al.* (1992)	Italy	In-situ hybridization (6/11, 16/18, 31/35/51)	26 LSIL	38				19	19	Fixed tissue
			45 HSIL	36				24	16	

Table 8 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Anderson *et al.* (1993)	USA	In-situ hybridization (6/11, 16/18, 31/33/35, 42/43/44, 45/56, 51/52)	159 HPV/CIN I 136 CIN II/III	43 55		9 4		14 27	23 27	Fixed tissue. 'Others' predominantly HPV-31/33/35
Cromme *et al.* (1993a)	Netherlands	PCR:GP 5/6, TS (6, 11, 16, 18, 31, 33)	34 CIN I 32 CIN II 28 CIN III	68 91 100			53* 75* 96*			Fixed tissue. *HPV types 16, 18, 31
Hellberg *et al.* (1993)	Sweden	In-situ hybridization (6, 11, 16, 18, 31, 33)	50 CIN I 54 CIN II 23 CIN III	60 81 100	2	24 2	12 54 70	4 9 9	18 17 22	Fixed tissue. HPV-positive cases younger than HPV-negative cases
Cuzick *et al.* (1994)	United Kingdom	PCR (semi quantitative) (6/11, 16, 18, 31, 33, 35)	13 CIN I 12 CIN II 61 CIN III	77 75 95			23 42 67	23 25 20		Cervical swabs
Delvenne *et al.* (1994)	Belgium	In-situ hybridization, immunohistochemistry, PCR (*L1* consensus primers)	90 LSIL 50 HSIL	64 86		24 10		16 50	36 36	Fixed tissue. 'Others' include HPV-31, -33, -35 (21% in LSIL, 20% in HSIL) plus cases positive only by immunohistochemistry or PCR.
Hippeläinen *et al.* (1994)	Finland	In-situ hybridization (6/11, 16/18, 31/33, 42)	127 CIN I/II 25 CIN III	69 96		20 4		30 80	19 12	Fixed tissue
de Roda Husman *et al.* (1994)	Netherlands	PCR GP-5/6 and TS-PCR (6, 11, 16, 18, 31, 33) Southern blot of GP-PCR products (21 types)	971 Pap IIIa 295 Pap IIIb 107 Pap IV	72 85 100	2 1 1	1 1	25 50 51	5 8 12	35 23 31	Cervical swabs. 'Others' primarily HPV-31 and -33 or unknown

Table 8 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Sebbelov *et al.* (1994)	Denmark	In-situ hybridization (16)	52 CIN I/II	21			31			Fixed tissue
			53 CIN III	25			25			
		PCR (in β-globin positive) (16, 18, 31, 33, 35, 45)	40 CIN I/II				53		15	
			34 CIN III				85		29	
	Greenland	In-situ hybridization (16)	49 CIN I/II	14			14			Fixed tissue
			46 CIN III	20			20			
		PCR (in β-globin positive) (16, 18, 31, 33, 35, 45)	24 CIN I/II				54		13	
			30 CIN III				70	3	17	
Burger *et al.* (1995)	Netherlands	PCR (6/11, 16, 18, 31, 33)	32 CIN I	44		3	16	19	16	Cervical scrapes. Multiple types found in many samples
			39 CIN II	69		3	31	8	36	
			49 CIN III	86		2	59	8	27	
Matsukura & Sugase (1995)	Japan	Dot blot (27 HPV types)	71 CIN I	94			10	3	82	Frozen tissue. 'Others' predominantly HPV-52, -58 (CIN I, II, III), -56 (CIN I), -31 (CIN III)
			56 CIN II	100			23	1	75	
			93 CIN III	95			45		49	

PCR, polymerase chain reaction; GP, general probe; TS, type-specific; Pap, Papanicolaou smear test result; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions

[a]Including studies that used hybridization methods

[b]Of those types tested

Table 9. Prevalence of HPV DNA in cervical adenocarcinoma *in situ* case series (≥ 10 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Farnsworth *et al.* (1989)	Australia	In-situ hybridization (6, 11, 16, 18, 31)	17	88			29	59		Fixed tissue
Okagaki *et al.* (1989)	USA	In-situ hybridization (6, 16, 18)	21	67			24	48		Fixed tissue
Nicklin *et al.* (1991)	Australia	ViraType™ *in-situ* (6/11, 16/18, 31/33/35)	28	25			25		4	Fixed tissue. HPV status and smoking had no significant effect on behaviour of the lesions.
Higgins *et al.* (1992a)	Australia	In-situ hybridization (6, 11, 16, 18, 31, 33)	37[b]	97			32	65		Fixed tissue
Duggan *et al.* (1993)	Canada	Dot blot (6, 11, 16, 18, 31, 33, 35)	37	27			14	14		Fixed tissue. HPV positive cases tended to be older than HPV negative cases.
Lee *et al.* (1993)	USA	PCR (16, 18)	36	42			22	22		Fixed tissue. No association between HPV status and age

[a]Of those types tested
[b]Cervical intraepithelial glandular neoplasia (CIGN) grade III. CIGN I/II also present in 27 of the women

Table 10. Prevalence of HPV DNA in squamous-cell cervical cancer case series[a] (≥ 40 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Fuchs *et al.* (1988)	Austria	Southern blot (6, 10, 11, 16, 18, 31, 33)	44	68	9		57	9	7	Frozen tissue. All lymph node metastases had HPV DNA of the same type as the primary tumour. 'Others' = HPV-10
Kurman *et al.* (1988)	USA/ Brazil	Southern blot (6, 11, 16, 18, 31)	58	86			41	22	22	Frozen tissue. 'Others' = HPV-31 and uncharacterized types
Meng *et al.* (1989)	China	Dot blot (11, 16, 18)	46	48		2	39	7		Frozen tissue
Walker *et al.* (1989)	USA	Southern blot (6, 11, 16, 18, 31)	62	71			63	6	2	Frozen tissue. HPV-18-positive cases had a worse prognosis than HPV-16-positive and HPV-negative cases.
Ji *et al.* (1990)	China	In-situ hybridization (6, 11, 16, 18, 31, 33)	43	44			44			Fixed tissue. No information on histological group
Low *et al.* (1990)	Singapore	Dot blot (16, 18)	75	72			63	16		Frozen tissue
		Southern blot (16, 18)	58	78			66	21		
Riou *et al.* (1990)	France (~50%) Africa (~50%)	Southern blot/PCR (6, 11, 16, 18, 31, 33, 35, 39, 42)	89	83			61	8	18	HPV-negative women had a worse prognosis than HPV-positive women. No association between HPV status and age
van den Brule *et al.* (1991a)	Netherlands	PCR:GP 5/6, TS PCR (6/11, 16, 18, 31, 33)	50	100			84	26	6	Frozen/fixed tissue. 'Others' = HPV-31 and -33
Cooper *et al.* (1991b)	South Africa	In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	69	64			42	22		Fixed tissue. No association between HPV status and age
Higgins *et al.* (1991a)	Australia	In-situ hybridization (6/11, 16, 18, 31/33)	171	83			63	11	9	Fixed tissue. HPV-negative cases were older and had a worse prognosis than HPV-positive cases. Survival was not related to HPV type. 'Others' = HPV-31/33

Table 10 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Sebbelov *et al.* (1991)	Norway	Southern blot (11, 16, 18)	50	62			54	8		Frozen tissue. No association between HPV status and age, stage or survival
Hørding *et al.* (1992)	Denmark	PCR (16, 18)	50	72			60	12		Fixed tissue. No association between HPV status and tumour grade
Czeglédy *et al.* (1992b)	Hungary	PCR (16)	75	48			48			No information on histology. Presence of HPV-16 DNA in the majority of metastasizing lymph nodes of HPV-16-positive cases
Meguenni *et al.* (1992)	Algeria	Southern blot (6/11, 16)	78	76	3		73			Frozen tissue. No information on histological groups
Sarkar *et al.* (1992a)	India	In-situ hybridization (16, 18)	49	86			86	6		Fixed tissue
Chen *et al.* (1993a)	Taiwan, China	PCR (6, 11, 16, 18, 31, 33, 42, 52, 58)	40	78		3	50	5	20	Frozen tissue. No association between HPV status and stage or differentiation
Falcinelli *et al.* (1993)	Italy	PCR (6/11, 16, 18)	42	69			64			Fixed tissue. No mutation of Ki-*ras* gene was found.
Kenter *et al.* (1993)	Netherlands	PCR (16)	69	49			49			Fixed tissue. HPV-positive cases older than HPV-negative cases. No association between HPV status and prognosis
Kristiansen *et al.* (1994a)	Norway	PCR/Southern blot (6, 11, 16, 18, 33)	105	69			53	11		Frozen tissue. No association between overall HPV and age or differentiation. HPV-18 positivity was associated with younger age and poorly differentiated tumours.
Matulic & Saric (1994)	Croatia	Southern blot (6, 16/18)	44	61			61			Frozen tissue
Ngan *et al.* (1994)	Hong Kong	PCR/Southern blot (*L1* consensus primers and 16, 18)	64	73			67	39		Frozen tissue
Pao *et al.* (1994a)	Taiwan, China	PCR (*L1* consensus primers)	61 49	89 57			75 43			Frozen or fixed tissue. No information on histological groups

Table 10 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Williamson *et al.* (1994)	South Africa	PCR (*L1* consensus primers and 6, 11, 16, 18, 31, 33, 45)	59	78			44	2	34	Frozen tissue. 'Others' predominantly unclassified types
Bosch *et al.* (1995)	22 countries[c]	PCR (*L1* consensus primers and type specific, 26 types)	881	93			51	12	33	Frozen tissue. β-globin-positive (see also Table 12) 'Others' predominantly types related to HPV-16 and -18

PCR, polymerase chain reaction; GP, general probe; TS, type-specific

[a]Including studies published ≥ 1988 having used hybridization methods

[b]Of those types tested

[c]Africa (Algeria, Benin, Guinea, Mali, Tanzania, Uganda), Central and South America (Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Panama, Paraguay), Southeast Asia (Indonesia, Philippines, Thailand), North America (Canada, USA) and Europe (Germany, Poland, Spain)

prevalence is in the range of 57–100% with most studies detecting HPV in more than 75% of cases. The prevalence from the largest study was 93% (Bosch *et al.*, 1995). In studies using Southern blot or in-situ hybridization, the ranges are 61–86% and 44–86%, respectively. HPV-16 is by far the most prevalent type. The data currently available do not support a relationship between HPV detection in cervical cancers and the age of the patients.

Figures 14 and 15 show the percentage positivity in squamous-cell cervical cancer case series of HPV and HPV-16, respectively.

(c) Adenocarcinoma and adenosquamous carcinoma

Table 11 summarizes cervical adenocarcinoma case series published since 1988 that included more than 15 cases and used hybridization methods for HPV detection. The prevalence of HPV ranged from 15 to 88% in nine studies using PCR or Southern blot. In six studies using in-situ hybridization, HPV was detected in 0–69% of cases. In the majority of investigations, HPV-18 was the predominant type; however in some studies, HPV-16 was as prevalent as HPV-18. HPV-positive cases tended to be younger than HPV-negative cases for unknown reasons.

A few studies of HPV in adenosquamous carcinomas have been conducted. The detection rate in series with more than 10 cases ranged from 18 to 94%. In the most recent study, HPV-16 and -18 were detected with about the same frequency (Tase *et al.*, 1988; Walker *et al.*, 1989; Leminen *et al.*, 1991; Duggan *et al.*, 1993; Bosch *et al.*, 1995).

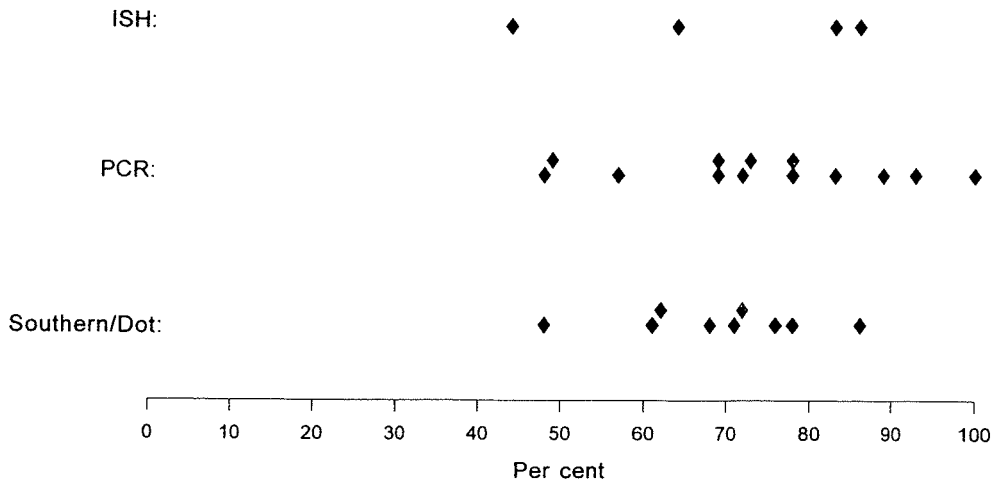
(d) Geographical and temporal variation

Little geographical variation is seen in the overall prevalence of HPV invasive cancers but there is some difference in the distribution of types. The best data come from a study by Bosch *et al.* (1995) in which nearly 1000 invasive cancer specimens, from 22 countries covering most parts of the world, were analysed in a single laboratory. The assay was based on PCR amplification using *L1* consensus primers, and samples were probed with different oligonucleotides capable of detecting and discriminating between 26 different anogenital HPV types. HPV DNA was detected in 93% of the tumours, with no significant geographic variation in overall positivity. HPV-16 was present in 50% of the specimens, HPV-18 in 14%, HPV-45 in 8% and HPV-31 in 5%. HPV-16 was the predominant type in all countries except Indonesia and Algeria, where HPV-18 was more common. There was significant geographical variation in the prevalence of some of the less-common virus types. A clustering of HPV-45 was apparent in western Africa, while HPV-39 and -59 were virtually confined to cancer cases from Latin America. In squamous-cell tumours, HPV-16 predominated, but HPV-18 predominated in adenocarcinomas and adenosquamous tumours. Further details on type-specific positivity by area are shown in Table 12.

In a series of smaller studies, Sebbelov *et al.* (1994) found similar rates of HPV-16 positivity by PCR in archival specimens from two additional countries, Greenland and Denmark (pre-invasive lesions, 63% and 68%, respectively; invasive cancers, 82% and 70%, respectively). Acs *et al.* (1989) found a slightly higher rate of HPV-16 positivity by Southern blot in 82 cancers in eastern Panama (43%) than in 69 from central Panama (29%). In a small study of 37 invasive cancers in two Mexican cities with high cervical cancer rates (Mexico City and Monterrey),

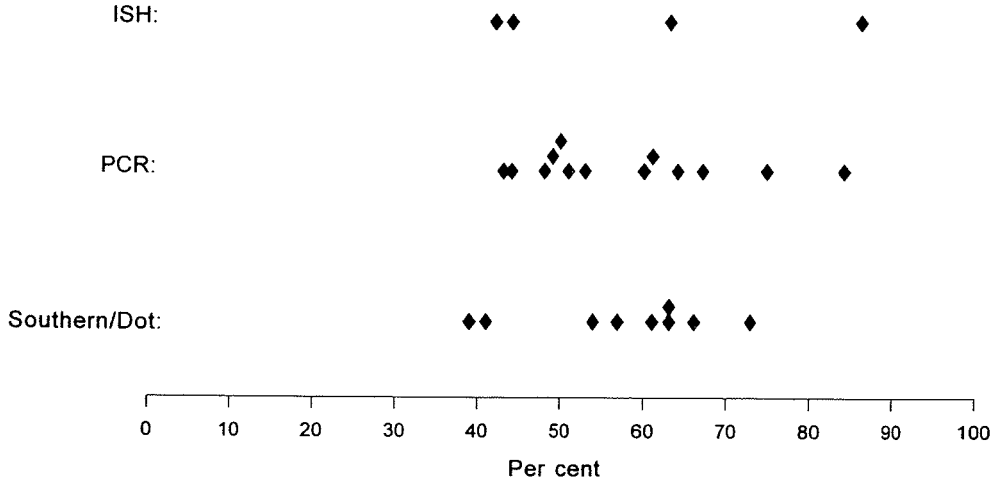
González-Garay *et al.* (1992) found similar positivity rates for HPV-16 (29% and 26%, respectively) and HPV-18 (7% and 10%, respectively) by Southern blot.

Figure 14. Detection of HPV DNA in sqamous-cell cervical cancer case series



ISH, in situ hybridization; PCR, polymerase chain reaction
Data are taken from Table 10.

Figure 15. Detection of HP-16 in squamous-cell cervical cancer case series



ISH, in situ hybridization; PCR, polymerase chain reaction
Data are taken from Table 10.

Two historical studies of biopsy material have yielded possibly conflicting data regarding changes in HPV positivity in neoplastic specimens over time. Anderson *et al.* (1993) compared 176 cervical tissues showing cervical intraepithelial neoplasia from 1964 to 1965 with 165 cases from 1988 to 1989 in Richmond, VA, USA, and found virtually the same positivity rate (43%

Table 11. Prevalence of HPV DNA in cervical adenocarcinoma case series[a] (≥ 15 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Tase *et al.* (1988)	USA	In-situ hybridization (2, 6, 16, 18)	40	43			3	40		Fixed tissue. No association between HPV and histological differentiation
Walker *et al.* (1989)	USA	Southern blot (6, 11, 16, 18, 31)	25	52			20	32		Frozen tissue. HPV-18-positive cases had a worse prognosis than HPV-16-positive and HPV-negative cases.
Riou *et al.* (1990)	France & Africa	Southern blot/PCR (6, 11, 16, 18, 31, 33, 35, 39, 42)	17	88			24	59	12	
Bjersing *et al.* (1991)	Sweden	PCR (16, 18)	26	42			15	27		Fixed tissue. HPV-positive cases were younger than HPV-negative cases.
Griffin *et al.* (1991)	United Kingdom	In-situ hybridization (6, 11, 16, 18, 31)	16	6				6		Fixed tissue
		PCR (11, 16, 18)	16	31			25	6		
Leminen *et al.* (1991)	Finland	In-situ hybridization (16, 18)	95	20			4*	16*		Fixed tissue. No correlation between HPV status and age, ploidy or survival, but correlation with both stage and tumour size. *Including 11 adenosquamous cancers
Young *et al.* (1991)	United Kingdom	In-situ hybridization (6, 11, 16, 18)	21	0						Fixed tissue
Hørding *et al.* (1992)	Denmark	PCR (16, 18)	50	70			18	52		Fixed tissue. No association between HPV status and tumour grade. Higher rate of HPV-18-positive carcinoma among younger women
Cooper *et al.* (1992)	United Kingdom	In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	16	69			25	44		Fixed tissue. No association between HPV status and tumour differentiation
	South Africa		22	41				41		

Table 11 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Johnson *et al.* (1992a)	USA	PCR (16, 18)	19	79			21	58		Fixed tissue. HPV-positive cases were younger than HPV-negative cases.
Duggan *et al.* (1993)	Canada	Dot blot (6, 11, 16, 18, 31, 33, 35)	60	42			15	25	2	Fixed tissue. HPV-positive cases tended to be younger than HPV-negative cases.
Fulcheri *et al.* (1993)	Italy	In-situ hybridization (6/11, 16/18, 31/35/51)	18	6			6			Fixed tissue
Lee *et al.* (1993)	USA	PCR (16, 18)	20	15			10	5		Fixed tissue
Matsuo *et al.* (1993)	Japan	PCR (*L1* consensus primers and 16, 18)	24	25			17	13		Fixed tissue. HPV-positive cases were younger than HPV-negative cases.
Milde-Langosch *et al.* (1993)	Germany	PCR (consensus primers) (6/11, 16, 18, 31)	24	63			38	29		Fixed tissue. HPV-positive cases were younger than HPV-negative cases.
Bosch *et al.* (1995)	22 countries[c]	PCR (*L1* consensus primers and type specific, 26 types)	25	96			28	56	12	Frozen tissue. 'Others' related to HPV-18

PCR, polymerase chain reaction

[a]Including studies published ≥ 1988 having used hybridization methods

[b]Of those types tested

[c]Africa (Algeria, Benin, Guinea, Mali, Tanzania, Uganda), Central and South America (Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Panama, Paraguay), Southeast Asia (Indonesia, Philippines, Thailand), North America (Canada, USA) and Europe (Germany, Poland, Spain)

versus 47%) by in-situ hybridization. However, Kock and Johansen (1987) compared 208 biopsy specimens from 1972 and 225 from 1983 in Denmark and found a large increase in the proportions showing condylomatous atypia (12% versus 50%, respectively). Within this group, immunoperoxidase staining for the HPV antigen was performed and an increase in positivity in the more recent samples was also found (12% versus 21%, respectively).

Table 12. Prevalence of individual HPV types by geographical region

HPV type	Region					
	Africa	Central and South America	Southeast Asia	Europe	North America	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
HPV-16 and related						
HPV-16	79 (42.5)	255 (50.5)	42 (42.9)	56 (65.1)	33 (57.9)	465 (49.9)
HPV-31	5 (2.7)	35 (6.9)	1 (1.0)	5 (5.8)	3 (5.3)	49 (5.3)
HPV-33	5 (2.7)	18 (3.6)	2 (2.0)	1 (1.2)	0	26 (2.8)
HPV-35	4 (2.2)	10 (2.0)	1 (1.0)	1 (1.2)	0	16 (1.7)
HPV-52	4 (2.2)	16 (3.2)	2 (2.0)	3 (3.5)	0	25 (2.7)
HPV-58	5 (2.7)	11 (2.2)	2 (2.0)	1 (1.2)	0	19 (2.0)
HPV-18 and related						
HPV-18	33 (17.7)	48 (9.5)	31 (31.6)	7 (8.1)	9 (15.8)	128 (13.7)
HPV-39	0	13 (2.6)	1 (1.0)	0	0	14 (1.5)
HPV-45	23 (12.4)	37 (7.3)	8 (8.2)	2 (2.3)	8 (14.0)	78 (8.4)
HPV-59	0	14 (2.8)	1 (1.0)	0	0	15 (1.6)
HPV-68	4 (2.2)	2 (0.4)	1 (1.0)	3 (3.5)	1 (1.8)	11 (1.2)
Other						
HPV-6	0	1 (0.2)	0	0	0	1 (0.1)
HPV-11	0	1 (0.2)	0	0	0	1 (0.1)
HPV-56	6 (3.2)	3 (0.6)	3 (3.1)	2 (2.3)	2 (3.5)	16 (1.7)
Miscellaneous	5 (2.7)	16 (3.2)	4 (4.1)	1 (1.2)	0	26 (2.8)
Undetermined	2 (1.1)	8 (1.6)	0	1 (1.2)	1 (1.8)	12 (1.3)
HPV negatives	19 (10.2)	36 (7.1)	3 (3.1)	4 (4.7)	4 (7.0)	66 (7.1)
Total specimens	186	505	98	86	57	932

Adapted from Bosch *et al.* (1995)

2.2.2 *Anogenital cancers*

(a) Cancer of the vulva and vagina

In Table 13, results from vulvar cancer case series (≥ 10 cases) are summarized. The HPV prevalence ranged from 0% to 100%. No HPV DNA was seen in 29 tumours with substantial keratinization and adjacent lichen sclerosus, but without adjacent vulvar intraepithelial neoplasia

Table 13. Prevalence of HPV DNA in vulvar cancer case series[a] (≥ 10 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Di Luca *et al.* (1986)	Italy	Southern blot (16)	10 SCC	20			20			Frozen tissue
Macnab *et al.* (1986)	United Kingdom	In-situ hybridization (16, 18)	10 SCC	80			80			Frozen tissue
Gupta *et al.* (1987)	Italy	In-situ hybridization (6/11, 16, 18)	10 early SCC	50		10		40		Fixed tissue. Among cases with histological evidence of viral infection, 78% were HPV positive. In those without signs of viral infection 33% were HPV positive.
			6 verrucous carcinomas	0						
Bender *et al.* (1988)	Germany	In-situ hybridization (6/11, 16/18)	27	59		19		41		No information on histological group
Carson *et al.* (1988)	USA	Southern blot/in-situ hybridization (1-6, 16, 18)	16 ASCC	6					6	Fixed tissue. The prognosis was worse for ASCC than for SCC.
			26 SCC	19	4		12		4	
Ikenberg *et al.* (1988)	Germany	Southern blot (16)	18	33			33			Frozen tissue. No correlation between HPV status and grade or stage of tumours
Venuti & Marcante (1989)	Italy	Dot blot/Southern blot (16, 18)	15 SCC	87			20	80		Frozen tissue
Neill *et al.* (1990)	United Kingdom	Southern blot (16, 18, 31, 33)	10 SCC with lichen sclerosus	0						
			10 SCC with VIN III	64*			55*			*11 lesions tested
Pilotti *et al.* (1990)	Italy	In-situ hybridization/ Southern blot (6, 11, 16, 18, 31)	10 SCC	10			10			Fixed/frozen tissue. Data only given for HPV-16
		PCR		75			75			

Table 13 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Andersen *et al.* (1991)	USA	In-situ hybridization (Vira type) (6/11, 16/18, 31/33/35)	30 SCC 12 intraepithelial like SCC	13 67						Fixed tissue. Cases with intraepithelial-like SCC were younger and more often smokers than cases with ordinary SCC.
Bloss *et al.* (1991)	USA	Southern blot/PCR (6, 11, 16, 18, 31)	21 SCC	48	10		48			Frozen tissue. No association between HPV and stage or prognosis. HPV-positive cases were younger than HPV-negative cases.
Hørding *et al.* (1991a)	Denmark	PCR (16)	24 SCC	58			58			Frozen tissue
Marcante & Venuti (1991)	Italy	PCR (11, 16, 18)	10	90			80	10		Fixed tissue. In all screened metastases, the same HPV type was found as in the primary tumour.
Nuovo *et al.* (1991c)	USA	PCR (*E6* and *L1* consensus primers and 6, 11, 16, 18, 31, 33, 35)	10 SCC (minimal keratinization) 13 SCC (substantial keratinization)	70 0			40		30	Fixed tissue. Adjacent VIN in 86% of HPV-positive cases compared to 6% of HPV-negative cases
Rusk *et al.* (1991)	USA	Dot blot/Southern blot (6/11, 16, 18)	31 SCC	23		6	10		6	Frozen tissue. No association between HPV and age or tumour grade
Toki *et al.* (1991)	USA	In-situ hybridization (6/11, 16, 18) PCR (6/11, 16, 18)	19 SCC 8 basaloid carcinomas 3 warty carcinomas	21 75 100			16 75 67	5 13 33		Fixed tissue. HPV-positive cases younger than HPV-negative cases
Brandenberger *et al.* (1992)	Switzerland	In-situ hybridization (6/11, 16, 18)	38 SCC	11		3	8			Fixed tissue. No association between HPV status and survival

Table 13 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Felix *et al.* (1993)	USA	PCR (6, 11, 16, 18, 33)	(Bartholin's gland) 7 SCC	86			86			Fixed tissue
			2 adenoid cystic carcinomas 1 AC	0						

SCC, squamous-cell carcinoma; ASCC, adenosquamous-cell carcinoma; VIN, vulvar intraepithelial neoplasia; PCR polymerase chain reaction; AC, adenocarcinoma
[a]Including studies having used hybridization methods
[b]Of those types tested

(VIN); however, HPV DNA was detected in 41% of tumours with minimal keratinization and/or adjacent VIN. HPV-16 was the predominant type detected, but cases containing HPV-6/11 have also been reported.

The prevalence of HPV in VIN III (≥ 20 cases) ranges from 30 to 95% with four of seven studies finding HPV in more than 80% of cases (Table 14). The prevalence tends to be consistently higher than in series of vulvar cancer.

In vaginal cancer case series (3–18 cases), the range of positivity was 21–67%, with HPV detected in 48% of cancers overall (see Table 15). HPV-6 and -18 were the predominant types.

(b) Cancer of the penis

Table 16 summarizes the prevalence studies of penile cancer that included more than five cases. Studies in which in-situ hybridization was used detected HPV in 5–40% of squamous-cell carcinoma, whereas Southern blot and PCR studies found HPV in 44–100% and 18–82%, respectively. It seems that HPV (predominantly HPV-16) is found in penile squamous-cell carcinomas in some regions of the world, but, curiously, HPV has not been found in the five small studies of 'verrucous' carcinomas conducted to date.

(c) Cancer of the anus

In Table 17 results from anal cancer case series (≥ 5 cases) are shown. In-situ hybridization studies found 24–73% of cases (squamous-cell carcinomas) to be HPV positive. The corresponding figures for Southern blot and PCR studies are 63–85% and 24–100%, respectively, and HPV-16 is the type most commonly observed. Scholefield *et al.* (1991) examined 173 anal squamous-cell cancers from fixed sections in six countries and found HPV-16 DNA in 29% of those using DNA-hybridization. Much lower positivity rates were found in India (3%) and South Africa (11%) than in Poland, Switzerland, Brazil and the United Kingdom, where the rates were similar (approximately 40%).

2.2.3 Other cancers

The presence of HPV at other cancer sites has been studied considerably less often and in far fewer patients compared to cancer of the cervix and anogenital areas. Some positive findings reported below may have been inflated by publication bias, as suggested by some especially high HPV prevalences in early reports that are refuted in more recent studies (e.g. prostatic cancer). From an interpretative viewpoint, it is also important to bear in mind that the presence of HPV according to any technique does not constitute a proof of causality *per se*, and the finding of HPV in cancer tissues frequently goes along with detection of several other viruses (e.g. Epstein–Barr virus, hepatitis C virus (see IARC, 1994a), etc.). Conversely, negative results also cannot be used confidently, since the type-specific methods developed for search of the genital tract (e.g. to detect HPV-6, -11, -16 and -18) may not be informative in cancers at different sites where other, perhaps not yet identified, site-specific types may play a role.

Table 14. Prevalence of HPV DNA in high-grade vulvar intraepithelial neoplasia (VIN III) case series (≥ 20 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Bornstein *et al.* (1988)	USA	In-situ hybridization (6/11, 16/18/31)	46	83	37		48		9	Frozen tissue. No difference in HPV status between cases with multicentric and unicentric disease
Buscema *et al.* (1988)	USA	Southern blot (6, 11, 16, 18, 31)	22 (37 lesions)	84			68	5	11	Frozen tissue
Kaufman *et al.* (1988)	USA	In-situ hybridization (6, 11, 16, 18, 31)	46	83	20	24	17	24	9	Frozen tissue. HPV DNA more often present in older cases. Prevalence of HPV DNA similar in multifocal and unifocal lesions
Jones *et al.* (1990a)	New Zealand	In-situ hybridization (6, 11, 16, 18)	29	52			52			Fixed tissue. HPV positive cases were younger than HPV negative cases. Five cases progressed to cancer. All five had multifocal anogenital neoplasia. In four cases, HPV-16 was found in both VIN III and cancer tissue.
Beckmann *et al.* (1991a)	USA	In-situ hybridization, PCR (6, 16)	21	95	29		76		14	Fixed tissue. All women had multicentric squamous-cell neoplasia of the anogenital region.
Park *et al.* (1991)	USA	PCR, in-situ hybridization (6, 11, 16, 18)	20 warty 10 basal	65 30			65 30			Fixed tissue. HPV positive cases were younger than HPV negative cases.
Nuovo *et al.* (1991c)	USA	PCR (*E6*, *L1* consensus primers and 6, 11, 16, 18, 31, 33, 35)	22	59			41		18	Fixed tissue

PCR, polymerase chain reaction

[a]Of those types tested

Table 15. Prevalence of HPV DNA in vaginal cancer case series[a] (≥ 3 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Mitrani-Rosenbaum *et al.* (1988)	Israel	Southern blot (6/11, 16/18)	3	33			33			Frozen tissue
Ostrow *et al.* (1988)	USA	In-situ hybridization/filter in-situ hybridization (2, 6, 16, 18)	14	21			14			Fixed tissue
Kiyabu *et al.* (1989)	USA	PCR (16, 18)	14	64			57	7		Fixed tissue
Ikenberg *et al.* (1990)	Germany	Southern blot (6/11, 16, 18)	18	56			44		11	Frozen tissue
Kulski *et al.* (1990)	Australia	Histological filter in-situ hybridization (6, 11, 16, 18)	3	67						Fixed tissue

PCR, polymerase chain reaction

[a]Including studies having used hybridization methods

[b]Of those types tested

Table 16. Prevalence of HPV DNA in penile cancer case series[a] (≥ 5 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity					Comments
					6	11	16	18	Others	
McCance *et al.* (1986)	Brazil	Southern blot (16, 18)	53	51			49	9		Frozen tissue
Villa & Lopes (1986)	Brazil	Southern blot (6, 11, 16, 18)	18 SCC	44		6		39		Frozen tissue. Presence of HPV DNA was not related to age, extent of disease or prognosis.
Kiyabu *et al.* (1989)	USA	PCR (16, 18)	5	40			40			Fixed tissue
Weaver *et al.* (1989)	USA	In-situ hybridization (6, 11, 16, 18, 31, 33)	9 SCC	11			11			Fixed tissue. HPV-6/11 was found in 25/30 penile condylomas.
Higgins *et al.* (1992b)	Australia Brazil	In-situ hybridization (6, 11, 16, 18, 31, 33)	20 SCC 6 SCC 5 verrucous carcinomas	35 33 0			35 33			Fixed tissue. Verrucous carcinomas from Australia (2) and Brazil (3)
Kulski *et al.* (1990)	Australia	Histochemical filter in-situ hybridization (6, 11, 16, 18)	10 SCC	20						Fixed tissue
Moriyama *et al.* (1990)	Japan	In-situ hybridization (6/11, 16/18, 31/33/35)	19	5				5		Fixed tissue. HPV-6/11 was found in 11/12 penile condylomas.
Varma *et al.* (1991)	USA	PCR (6/11, 16) In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	16 SCC 2 verrucous carcinomas	81 0	6*		56		19*	Fixed tissue. *Only positive by in-situ hybridization
Sarkar *et al.* (1992b)	USA	PCR (6/11, 16, 18)	11 SCC 1 verrucous carcinoma	82 0			82			Fixed tissue. Bowenoid CIS: 7/7 HPV-16 positive; non-Bowenoid CIS: 0/8 HPV positive
Tornesello *et al.* (1992)	Uganda	Dot blot/Southern blot (6, 11, 16, 18, 31, 33)	13 SCC	100				100		Frozen tissue. Samples contained DNA with low homology to HPV-16/18.

Table 16 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity					Comments
					6	11	16	18	Others	
Wiener *et al.* (1992a)	USA	PCR (16, 18)	29 SCC	31			28	3		Fixed tissue. No association between HPV status and age, grade or survival
Iwasawa *et al.* (1993)	Japan	PCR (16, 18, 33)	111 SCC (untreated)	63			61	2		Fixed tissue. HPV-positive cases younger than HPV-negative cases; lymph node metastases from HPV-16-positive cases were also HPV-16 positive. Lymph nodes from HPV-negative cases were HPV negative.
			12 SCC (treated)	17			17			
Malek *et al.* (1993)	USA	PCR (*L1* consensus primers)	7 SCC	71						Fixed tissue; 75% of cases were heavy smokers.
Masih *et al.* (1993)	USA	In-situ hybridization (6, 11, 16, 18, 31)	10 SCC	40			40			Fixed tissue
			10 verrucous carcinomas	0						
Chan *et al.* (1994b)	Hong Kong	PCR (16, 18)	34 SCC	18			12	12		Fixed tissue. HPV positive cases older than negative cases
			7 verrucous carcinomas	0						
Suzuki *et al.* (1994)	Japan	PCR (*L1*, *E6* consensus primers)	13 SCC	54			31		23	Frozen/fixed tissue. 'Others' = HPV-31 and -33. No *p53* mutation was found among HPV-positive or -negative cases.

SCC, squamous-cell carcinoma; PCR, polymerase chain reaction; CIS, carcinoma *in situ*
[a]Including studies having used hybridization methods
[b]Of those types tested

Table 17. Prevalence of HPV DNA in anal cancer case series[a] (≥ 5 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Löning *et al.* (1988)	Germany	In-situ hybridization (6, 11, 16, 18)	8 SCC	25			25	13		Fixed tissue
			2 verrucous carcinomas	100	100	50				
Taxy *et al.* (1989)	USA	In-situ hybridization (6/11, 16, 18, 31)	12 SCC (9 F, 3 M)	25		8	17			Fixed tissue
Kulski *et al.* (1990)	Australia	Histochemical filter in-situ hybridization (6, 11, 16, 18)	18 SCC	39						Fixed tissue
Scholefield *et al.* (1990a)	United Kingdom	In-situ hybridization (16)	207 SCC	24			24			Fixed tissue. 1948–77: 15% HPV-16-positive cases; 1978–82: 33% HPV-16-positive cases; 1983–87: 50% HPV-16-positive cases
Scholefield *et al.* (1990b)	United Kingdom	Southern blot (6/11, 16, 18)	67 SCC (37 F, 30 M)	63			60	3		Frozen tissue. HPV status not related to any of the clinico-pathological variables examined
Wolber *et al.* (1990)	Canada	In-situ hybridization (6/11, 16/18)	9 CIS	78		22		56		Fixed tissue
			12 SCC (F:M = 0.9)	58				58		
			14 cloacogenic carcinomas (F:M = 1.3)	0						
Aparicio-Duque *et al.* (1991)	USA/Spain	In-situ hybridization (6/11, 16/18, 31/35/51)	5 cloacogenic carcinomas (3 M, 2 F)	80				80		Fixed tissue
Crook *et al.* (1991a)	United Kingdom	PCR/Southern blot (16, 18)	50 SCC	NA			76	8		Amplification of c-*myc* was demonstrated in 15/50 cancers, of which 13 were HPV-16-positive and one also HPV-18-positive.
		Southern blot (16, 18)		NA			58	6		

Table 17 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Duggan *et al.* (1991)	Canada	In-situ hybridization with:	13 SCC							Fixed tissue
		Horseradish peroxidase	(9 F, 4 M)	0						
		Alkine phosphatase		62	8		54			
		Dot blot (6, 11, 16, 18, 33)		85	23	8	70			
Higgins *et al.* (1991b)	Australia	In-situ hybridization (6, 11, 16, 18, 31, 33)	41 SCC	73			68	2	2	Fixed tissue. HPV-positive cases were younger than HPV-negative cases.
			6 AC	0						
Koulos *et al.* (1991)	USA	PCR (16, 18, 31, 35)	6 AC	33				33		Fixed tissue
			2 SCC	100			100	50		
Palefsky *et al.* (1991)	USA	PCR (6/11, 16, 18, 31, 33)	13	85		15	77		31	Fixed tissue
Scholefield *et al.* (1991)	India, South Africa, Switzerland, Poland, Brazil, United Kingdom	In-situ hybridization (16)	173 SCC	29			29			Fixed tissue. HPV prevalence significantly lower in samples from India and South Africa compared to the samples from Switzerland, Poland, Brazil and United Kingdom
Zaki *et al.* (1992)	USA	PCR (*L1* consensus primers)	7 CIS	86			29	14	43	Fixed tissue
			11 SCC (7 F, 4 M)	73	9	9	18		36	

SCC, squamous-cell carcinoma; F, females; M, males; CIS, carcinoma *in situ*; PCR, polymerase chain reaction; AC, adenocarcinoma; NA, not available

[a]Including studies having used hybridization methods

[b]Of those types tested

(a) Oral cancer

In Table 18, results from case series of oral cancer (≥ 15 cases) are presented. Studies using in-situ hybridization detected HPV in 0–24% of squamous-cell carcinomas while HPV was detected by Southern blot in 12–70% and by PCR in 0–100%. In verrucous carcinomas, HPV was detected in 0–48% of cases. Generally, HPV-16 was the most frequent type observed, although HPV-18 and other types have been observed with equal frequency in some studies (e.g., Kashima *et al.*, 1990; Yeudall & Campo, 1991; Woods *et al.*, 1993). This area has recently been reviewed by Snijders *et al.* (1994a).

(b) Cancer of the pharynx and tonsil

Among patients with cancer of the pharynx, four studies found HPV DNA, of types 16 and 18, in 13–67% of cases (Brandsma & Abramson, 1989; Watts *et al.*, 1991; Arndt *et al.*, 1992; Ogura *et al.*, 1993), whereas Dickens *et al.* (1992) found no HPV in 16 cases using PCR to test for HPV-16/18. In two studies of tonsillar carcinoma, HPV-16 was detected by Southern blot in two of three cases (Brachman *et al.*, 1992) while in the other study, using general primer mediated PCR, HPV DNA was detected in 12 of 14 (86%) cases (Snijders *et al.*, 1994a). In this latter study, HPV-16 and -33 were detected most frequently (in eight and four carcinomas, respectively)

(c) Oesophageal cancer

Table 19 gives a summary of oesophageal cancer case series of more than 10 cases. The HPV prevalences found in studies using in-situ hybridization range between 0% and 43%. One study detected HPV in 45% using Southern blot. Four PCR studies found that 0–10% of cases were positive while another study detected HPV in 49% of oesophageal cancer patients. HPV-16 and -18 have been observed with similar frequency.

(d) Cancer of the colon and rectum

One study found one HPV-positive case of caecum cancer out of 27 malignant colon cancers (Ostrow *et al.*, 1987a). Several other studies detected no HPV in this type of neoplasia (Grimmel *et al.*, 1988; Kulski *et al.*, 1990; Higgins *et al.*, 1991b; Koulos *et al.*, 1991; Nuovo, 1991; Shah *et al.*, 1992; Shroyer *et al.*, 1992).

(e) Nasal/sinonasal cancer

In invasive nasal/sinonasal cancer series (≥ 5 cases), HPV has been detected in 0–25% of cases, with HPV-16 being the most common type (Klemi *et al.*, 1989; Ishibashi *et al.*, 1990; Furuta *et al.*, 1991; Judd *et al.*, 1991; Furuta *et al.*, 1992; Kashima *et al.*, 1992b; Ogura *et al.*, 1993).

(f) Laryngeal cancer

Table 20 summarizes laryngeal cancer series (≥ 25 squamous-cell carcinoma cases, ≥ 3 verrucous carcinoma cases). Among squamous-cell carcinomas and carcinomas of unknown

Table 18. Prevalence of HPV DNA in oral cancer case series[a] (≥ 15 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Maitland *et al.* (1987)	United Kingdom	Southern blot (16)	15 SCC	47			40		7	Frozen tissue
Gassenmaier & Hornstein (1988)	Germany	In-situ hybridization (2, 6, 11, 16)	68 SCC	24 9	HPV-11 and -16 (relaxed conditions) HPV-6, -11, -16 (stringent conditions)					Fixed tissue
Syrjänen *et al.* (1988)	Finland	In-situ hybridization (6, 11, 13, 16, 18, 30)	51 SCC	12			6	8		Fixed tissue
Chang *et al.* (1990a)	Finland	In-situ hybridization (6/11/16/18)	40 SCC	3						Fixed tissue
		PCR (6, 11, 16, 18)		28	3		23	3		
Greer *et al.* (1990)	USA	In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	50 SCC 20 verrucous carcinomas	6 20	5		2 15	2	2	Fixed tissue
Kashima *et al.* (1990)	USA	Southern blot/reverse blot (6, 11, 16, 18, 31)	29	17	3		3		10	Frozen tissue. 'Others' = HPV-3, -13 and -57 related types
Tsuchiya *et al.* (1991)	Japan	Southern blot (6/11, 16/18, 31/33/35)	25 SCC	12						Frozen tissue
Watts *et al.* (1991)	USA	Southern blot (2, 6/11, 13, 16/18, 32)	23 SCC	70	35		48			Frozen tissue
		PCR (6, 11, 16, 18)	14 SCC	100	7	50	79	14		
Yeudall & Campo (1991)	United Kingdom	Southern blot/PCR (4, 16, 18)	39 SCC	46			26	21		Frozen tissue
Young & Min (1991)	USA	In-situ hybridization (6/11, 16/18, 31/33/35)	17 SCC 10 verrucous carcinomas	0 0						Fixed tissue

Table 18 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Zeuss *et al.* (1991)	Spain	In-situ hybridization (6/11, 16/18, 31/33/35)	15 SCC 5 CIS	0 0						Fixed tissue
Shindoh *et al.* (1992)	Japan	PCR (16, 18, 33)	24 SCC (tongue only)	33			33	4		Fixed tissue. HPV-positive cases tended to be older than HPV-negative cases.
Holladay & Gerald (1993)	USA	PCR (*L1* consensus primers)	37 SCC 2 verrucous carcinomas	19 0			19	3		Fixed tissue
Noble-Topham *et al.* (1993)	Canada	PCR (6/11, 16, 18)	25 verrucous carcinomas	48	4		8	40		Fixed tissue
Ogura *et al.* (1993)	Japan	PCR (16, 18)	15 SCC	0						Frozen tissue
Shroyer *et al.* (1993)	USA	PCR (generic), in-situ hybridization (6/11, 16/18, 31/33/35)	17 verrucous carcinomas	41	18	29				Fixed tissue
Woods *et al.* (1993)	USA	PCR (*L1* consensus primers)	18 SCC	78	28		61	61		Fixed tissue. No association between HPV status and age, gender, tobacco usage or alcohol consumption

SCC, squamous-cell carcinoma; PCR, polymerase chain reaction; CIS, carcinoma *in situ*

[a]Including studies having used hybridization methods

[b]Of those types tested

Table 19. Prevalence of HPV DNA in oesophageal cancer case series[a] (≥ 10 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Kiyabu *et al.* (1989)	USA	PCR (16, 18)	13 SCC	0						Fixed tissue
Chang *et al.* (1990b)	China	In-situ hybridization (6, 11, 16, 18)	51 SCC (25 F, 26 M)	43	6	8	18	16		Fixed tissue. Koilocytosis present in 55% of cases
Kulski *et al.* (1990)	Australia	Histochemical filter in-situ hybridization (6/11/16/18)	39	23						Fixed tissue
Loke *et al.* (1990)	Hong Kong	In-situ hybridization Slot blot (6, 11, 16, 18)	37 SCC	0						Fixed tissue Frozen tissue
Chang *et al.* (1992)	China	Southern blot (11, 16, 18, 30)	20 SCC	45						Frozen tissue
		PCR (6, 11, 16, 18)	51 SCC* (25 F, 26 M)	49	6	14	18	14		Fixed tissue. Koilocytosis present in 49% of cases *Same material as Chang *et al.* (1990b)
Toh *et al.* (1992)	Japan	PCR (*L1* consensus primers) (16, 18, 31, 33, 52, 58)	45 SCC	7			2	4		Fixed tissue
Chang *et al.* (1993)	China	In-situ hybridization (6/11, 16, 18, 30, 53)	363 SCC (148 F, 215 M)	23	2		4	2	16	Fixed tissue. HPV DNA was found in 7/57 of the lymph node metastases. 'Other': unidentified types
Furihata *et al.* (1993a)	Japan	In-situ hybridization (16, 18, 31, 33)	71 SCC (9 F, 62 M)	34			14	20		Fixed tissue. HPV positivity and especially high-level expression of *p53* were both markers of poor prognosis.

Table 19 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Ogura *et al.* (1993)	Japan	PCR (16, 18)	14 SCC	7			7			Frozen tissue
Poljak & Cerar (1993)	Slovenia	In-situ hybridization (6/11, 16/18, 31/33/51)	20 SCC (M)	0						Fixed tissue
		PCR		10			10			
Togawa *et al.* (1994)	International	PCR (consensus primer) and RFLP (6, 11, 16, 18)	72	24	0	0	13	1	10	Fixed or frozen tissue. HPV-16, -18 not detected in adjacent normal tissue

SCC, squamous-cell carcinoma; PCR, polymerase chain reaction; M, males; F, females; RFLP, restriction fragment length polymorphism

[a]Including studies having used hybridization methods

[b]Of those types tested

Table 20. Prevalence of HPV DNA in laryngeal cancer case series[a] (SCC, ≥ 25 cases; verrucous carcinomas, ≥ 3 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Abramson *et al.* (1985)	USA	Southern blot (11, 16, 18)	5 verrucous carcinomas	100			100			Frozen tissue
Brandsma *et al.* (1986)	USA	Southern blot (6, 11, 16, 18)	6 verrucous carcinomas	100			100			Frozen tissue
Kahn *et al.* (1986)	Germany	Southern blot (30)	41	0						Frozen tissue
Scheurlen *et al.* (1986a)	Germany	Southern blot (16)	36	3			3			Frozen tissue
Syrjänen *et al.* (1987a)	Finland	In-situ hybridization (6, 11, 16, 30)	116 SCC	13	4	8	5			Fixed tissue
Hoshikawa *et al.* (1990)	Japan	PCR (6, 16)	34 SCC	21	3		18			Fixed tissue
Perez-Ayala *et al.* (1990)	Spain	PCR (11, 16)	48 SCC 3 verrucous carcinomas	54 100			54 100			Frozen tissue. HPV-16 prevalence increases with decreasing differentiation.
Somers *et al.* (1990)	USA	Southern blot (2, 6, 11, 16, 18, 30, 31, 33, 35)	25 SCC	4					4	Frozen tissue. 'Others' = HPV-35
Arndt *et al.* (1992)	Germany	In-situ hybridization (6/11, 16/18)	27 SCC	63		37	71			Fixed tissue. Most cases were heavy smokers.
Brandwein *et al.* (1993)	USA	PCR (*L1* consensus primers)	40 SCC	8			3		5	Fixed tissue. All but one HPV-negative cases were smokers.

Table 20 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
Kasperbauer *et al.* (1993)	USA	PCR (*L1* consensus primers)	20 verrucous carcinomas	85						Fixed tissue
		In-situ hybridization (6/11, 16/18, 31/33/35)		0						
Ogura *et al.* (1993)	Japan	PCR (16, 18)	31	20			16	3		Frozen tissue

SCC, squamous-cell carcinoma; PCR, polymerase chain reaction
[a]Including studies having used hybridization methods
[b]Of those types tested

histology, two studies using in-situ hybridization found 13% and 63% of cases to contain HPV, respectively. Southern blot studies found 0–4% HPV-positive patients and studies that used PCR detected HPV in 8–54% of the cases. In contrast, in four studies of laryngeal verrucous carcinoma, which is a variant of well-differentiated squamous-cell carcinoma that clinically resembles laryngeal papilloma, HPV DNA was detected in 85–100% of cases (a total of 31 cases out of 34 were positive). HPV-16 was by far the most frequent type detected in these tumours.

(g) Lung cancer

Development of HPV-11-positive lung cancer subsequent to (juvenile) recurrent laryngo-tracheo-bronchial papillomatosis has been reported (Byrne *et al.*, 1987; Bejui-Thivolet *et al.*, 1990a; Guillou *et al.*, 1991). In one report of a single patient, respiratory papillomatosis was associated with HPV-6/11 infection but HPV-16 was detected with increasing frequency as the atypia progressed to carcinoma over a period of nine years (Doyle *et al.*, 1994).

Four case series have reported HPV in primary lung cancers. Stremlau *et al.* (1985) found HPV-16 in one of 24 (4%) cases of various histologies; Ostrow *et al.* (1987a) found HPV-16 in one of 20 (5%) cases; Bejui-Thivolet *et al.* (1990b) found predominantly HPV-16 and -18 in six of 33 (18%) cases of squamous-cell cancer and Yousem *et al.* (1992) found HPV (predominantly HPV-16/18, -31/33 and -35) in six of 20 (30%) squamous-cell cancers. In other case series, no HPV DNA was detected (Carey *et al.*, 1990; Shamanin *et al.*, 1994b; Szabó *et al.*, 1994). In 131 bronchial squamous-cell carcinomas, Syrjänen *et al.* (1989) found 12 HPV-positive cases (9%) using in-situ hybridization.

In a series of 31 bronchial squamous-cell papillomas, Popper *et al.* (1994) found that HPV-11, detected by in-situ hybridization, was frequently associated with benign papillomas (9 of 16, 56%) while HPV-16 and -18 were found in 11 of 12 (92%) papillomas associated with squamous-cell carcinomas.

(h) Cancer of the skin

The evidence available to date suggests a very low prevalence of mucosal-associated HPV types in all non-genital skin cancers other than those occurring at periungual and palmoplantar sites. In these rare tumours [sometimes found in patients who also have HPV-16/18-positive cervical disease], the high prevalence of HPV-16/18 DNA suggests possible genital transmission of HPV infection from genital sites.

It should be noted that many studies employ methods suitable for the detection of mucosal-associated HPV types only. This may not be informative in determining the true prevalence of HPV in non-genital skin cancers. Recent studies, employing degenerate and nested primers designed to detect EV-related HPV types, have found a higher prevalence of HPV DNA in skin cancer, both in immunosuppressed patients and (in preliminary studies) in skin cancers in the general population (Berkhout *et al.*, 1995). These early data need further evaluation.

Squamous-cell carcinoma and keratoacanthoma

A very low prevalence of HPV (0–20%) is found in squamous-cell carcinoma and keratoacanthoma in most studies where the tumour site is not specified (Table 21). However, HPV-9 and -37 were found in a single keratoacanthoma in one case series of seven tumours and

Table 21. Prevalence of HPV DNA in skin cancer case series — Squamous-cell carcinoma and keratoacanthoma

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity (%)[a]	HPV type-specific positivity (%)			Comments
					6/11	16/18	Other types detected	
Scheurlen *et al.* (1986b)	Germany	Blot hybridization and cloning/recombination	7 KA	14			9, 37*	Frozen tissue. *In a single KA. Present at ~10 copies/cell. HPV-37 not found in any of 35 malignant melanomas or 190 other skin tumours
Grimmel *et al.* (1988)	Germany	Southern blot (41)	6 KA 10 SCC	0 20			41	Frozen tissue. HPV-41 not found in any of 44 melanomas or 47 non-malignant skin lesions
Eliezri *et al.* (1990)	USA	In-situ hybridization (NA)	16 SCC	0				Fixed tissue
Kawashima *et al.* (1990)	Poland	Southern blot/PCR (5/8/14, 17/20/23/24, 6/11, 16/18/33, 1–4/7, 10/28)	33 KA 51 SCC (location NA) 25 SCC (lip)	0 2 4		4*	untyped	Frozen tissue. *HPV-16. No HPV DNA found in any of 14 cutaneous horns
Pierceall *et al.* (1991)	USA	PCR (6/11, 16, 18)	21 SCC	19		19*		Frozen tissue. *All HPV-16. 0/7 normal biopsies contained HPV.

KA, keratoacanthoma; SCC, squamous-cell carcinoma; NA, not available; PCR, polymerase chain reaction
[a]Percentage of those types tested

HPV-41 was found in two (of 10) squamous-cell carcinomas in another. HPV-16, although not found in most studies other than those examining periungual tumours, was found in four (of 21) squamous-cell carcinomas in one case series and in one (of 25) in another.

HPV-16/18 was found in 60% of 21 periungual squamous-cell carcinomas (based on two case series). Individual case reports also document the presence of HPV-16/18 in periungual squamous-cell carcinoma (Table 22). It should be emphasized that tumours at this site are extremely rare.

Verrucous carcinoma (epithelioma cuniculatum)

Individual reports document HPV-1, -6/11 and -16/18 in single cases of verrucous carcinoma, a rare, indolent (typically non-metastasizing) tumour occurring at acral sites. However, HPV was not identified in any of 11 tumours in one case series (Table 23).

Premalignant cutaneous disease (Bowen's disease and actinic keratoses)

There is a very low prevalence of HPV in Bowen's disease and actinic keratoses where the tumour site is not specified. HPV-1 and -2 have been reported in single cases and HPV-2, -34, -36 and -41 have been found in occasional lesions in larger case series. HPV-16 was found in one (of three) Bowen's tumours in one study and in one (of 18) cases in another (Table 24). These data contrast with the high prevalence of HPV found in periungual and palmoplantar Bowen's disease, where HPV-16/18 is found in 57–70% of lesions in several case series (Table 25). Again, it should be emphasized that disease at this site is very rare.

Basal-cell carcinoma

There is a very low prevalence of HPV in basal-cell carcinoma in most case series (0–2%), but in one study HPV-16 was found in three (of 16) cases (Table 26).

Other skin lesions

HPV has not been identified in other benign skin lesions at non-genital sites, including seborrheic keratoses (Zhu *et al.*, 1991), trichilemmomas (Leonardi *et al.*, 1991a) or acanthomas (Leonardi *et al.*, 1991b) in single case series. HPV-38 has been found in a single malignant melanoma (out of 36) in one study (Scheurlen *et al.*, 1986b).

(i) Breast cancer

HPV DNA was not detected in one series of 25 breast cancer cases where low-stringency Southern blot was used (Ostrow *et al.*, 1987a) and in a study of 80 breast cancers (consensus PCR) (Wrede *et al.*, 1992). In contrast, one other PCR study found HPV-16 in 30% of 17 cases (Di Lonardo *et al.*, 1992).

(j) Ovarian cancer

Among ovarian cancer cases, HPV was not detected in five studies ($\geq$ 5 cases). However, in one study HPV-6 was detected in 10/12 cases using in-situ hybridization, and in another HPV-16 DNA was found in nine (50%) and HPV-18 DNA in three (17%) cases (of 18) using PCR (Table 27). One case contained both HPV-16 and -18.

Table 22. Prevalence of HPV DNA in skin cancer case series — Periungual/palmar squamous-cell carcinoma

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity (no. or %)[a]	HPV type-specific positivity (no. or %)			Comments
					6/11	16/18	Other types detected	
Moy *et al.* (1989)	USA	Dot blot (6/11, 16/18)	10 periungual SCC	60		60		Fixed tissue. Episomal HPV-16 found in 4/6 HPV-16-positive specimens
Ostrow *et al.* (1989a)	USA	Southern blot and two-dimensional gel electrophoresis (2, 6, 16, 18, 31)	1 SCC, finger	1/1		1/1		Episomal and integrated HPV-16 demonstrated in tumour tissue
Eliezri *et al.* (1990)	USA	In-situ hybridization (NA)	11 periungual SCC	81		63*	2 untyped	Fixed tissue. *HPV-16 also found in 25/40 anogenital SCC
Guitart *et al.* (1990)	USA	In-situ hybridization (6/11, 16/18)	1 SCC, nail bed	1/1		1/1		Clinicopathological study of 12 cases. Only one examined for HPV patient also had HPV-16 in cervical tissue.
Ashinoff *et al.* (1991)	USA	PCR (16/18) In-situ hybridization (6/11, 16/18, 31/35/51)	2 SCC, finger	2/2		2/2		Fixed tissue
Moy & Quan (1991)	USA	Dot blot (6/11, 16/18)	1 SCC, finger	1/1		1/1		Frozen tissue

SCC, squamous-cell carcinoma; NA, not available; PCR, polymerase chain reaction
[a]Percentage of those types tested

Table 23. Prevalence of HPV DNA in skin cancer case series — Verrucous carcinoma/epithelioma cuniculatum

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity	HPV type-specific positivity			Comments
					6/11	16/18	Other types detected	
Knobler *et al.* (1989)	Austria	Dot blot (6, 11, 16/18)	1 EC, lower leg	1/1	1/1			Frozen tissue
Garven *et al.* (1991)	USA	In-situ hybridization (11, 16, 18)	1 VC, leg	1/1	1/1	1/1		Fixed tissue. A single tumour contained HPV-11 and -18.
Noel *et al.* (1993)	Belgium	In-situ hybridization (1–5, 11, 16, 18)	1 VC, leg	1/1			1	Frozen tissue. Tumour contained HPV-1.
Petersen *et al.* (1994)	Denmark	PCR (consensus primer)	13 CC*, site not specified	0/11				Fixed tissue. *2 of 13 specimens did not amplify β-globin.

PCR, polymerase chain reaction; EC, epithelioma cuniculatum; VC, verrucous carcinoma; CC, carcinoma cuniculatum belonging to the group of VC

Table 24. Prevalence of HPV DNA in skin cancer case series — Bowen's disease and actinic keratoses

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity (no. or %)[a]	HPV type-specific positivity (%)			Comments
					6/11	16/18	Other types detected	
Ikenberg *et al.* (1983)	Germany	Southern blot (16)	3 BD	33*		33		Frozen tissue. *HPV containing BD at unspecified site. HPV-16 in 12/15 genital BD
Pfister & Haneke (1984)	Germany	Southern blot (1, 3, 6, 8, 11, 13)	1 non-genital BD	1/1			2	
Grimmel *et al.* (1988)	Germany	Southern blot (41)	6 AK	17			41	Frozen tissue
Guerin-Reverchon *et al.* (1990)	France	In-situ hybridization (1, 2, 5, 6/11, 16/18)	11 non-genital BD	45		18	2 and untyped	Fixed tissue. HPV-16/18 found in 1/6 bowenoid papulosis and in 0/10 control skin samples
Kawashima *et al.* (1990)	Poland	Southern blot/PCR (5/8/14, 17/20/23/24, 6/11, 16/18/33, 1–4/7, 10/28)	83 non-genital BD 55 AK	2.4 5.5			34 36	Frozen tissue. HPV-16 found in 9/23 genital BD and HPV-33 in a further 4/23 cases
Kettler *et al.* (1990)	USA	In-situ hybridization (1, 6/11, 16/18)	18 non-genital BD	6		6		Fixed tissue
Inaba *et al.* (1993)	Japan	In-situ hybridization (NA)	1 BD forearm (6-year-old boy)	1/1			1	Frozen tissue

BD, Bowen's disease; AK, actinic keratoses; PCR, polymerase chain reaction; NA, not available
[a]Percentage of those types tested

Table 25. Prevalence of HPV DNA in skin cancer case series — Periungual and palmoplantar Bowen's disease

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity (no. or %)	HPV type-specific positivity (no. or %)[a]			Comments
					6/11	16/18	Other types detected	
Ikenberg *et al.* (1983)	Germany	Southern blot (16)	1 periungual BD	1/1		1/1		Frozen tissue
Kawashima *et al.* (1986)	Poland	In-situ hybridization (16 followed by cloning and recombination)	1 periungual BD	1/1			34*	Frozen tissue. *HPV-34 also found in 1/36 genital bowenoid papulosis but in 0/13 non-genital SCC and 0/12 non-genital BD
Stone *et al.* (1987)	USA	In-situ hybridization (1, 4, 6/11, 16/18)	1 plantar BD	1/1		1/1		
Rüdlinger *et al.* (1989b)	USA	In-situ hybridization (NA)	1 periungual BD	1/1			35	
Kettler *et al.* (1990)	USA	In-situ hybridization (1, 6/11, 16/18)	7 palmoplantar BD	70		70		Fixed tissue
Ashinoff *et al.* (1991)	USA	PCR (16/18) In-situ hybridization (6/8, 16/18, 31/35/51)	5 periungual BD	60		60		Fixed tissue
McGrae *et al.* (1993)	USA	Dot blot hybridization and PCR (NA)	3 BD finger (1 patient)	100		100		
Nordin *et al.* (1994)	Netherlands	NA	1 BD finger	1/1		1/1		HPV-16 also found in vulvar and cervical dysplastic tissue from this patient
Sau *et al.* (1994)	USA	In-situ hybridization (6/11, 16/18, 31/33/51)	7 BD nail bed	57		57		Fixed tissue

BD, Bowen's disease; SCC, squamous-cell carcinoma; NA, not available; PCR, polymerase chain reaction
[a]Percentage of those types tested

Table 26. Prevalence of HPV DNA in skin cancer case series — Basal-cell carcinoma

Reference	Study area	Detection method (types included)	Number and type of lesions	Overall HPV positivity (%)[a]	HPV type-specific positivity (%)			Comments
					6/11	16/18	Other types detected	
Grimmel *et al.* (1988)	Germany	Southern blot (41)	13 BCC	0				Frozen tissue
Eliezri *et al.* (1990)	USA	In-situ hybridization (NA)	26 BCC	0				Fixed tissue
Kawashima *et al.* (1990)	Poland	Southern blot/PCR (5/8/14, 17/20/23/24, 6/11, 16/18/33, 1–4/7, 10/28)	53 BCC	2			20	Frozen tissue
Pierceall *et al.* (1991)	USA	PCR (6/11, 16,18)	16 BCC	19		19*		Frozen tissue. *All HPV-16. 0/7 normal skin biopsies contained HPV.
Nahass *et al.* (1992)	USA	PCR (*L1* consensus primers)	3 scrotal BCC	0				Fixed tissue
Zhu *et al.* (1993b)	USA	PCR and Southern blot	13 BCC	0				

BCC, basal-cell carcinoma; NA, not available; PCR, polymerase chain reaction
[a]Percentage of those types tested

Table 27. Prevalence of HPV DNA in ovarian cancer case series (≥ 5 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
de Villiers *et al.* (1986b)	Germany	Reverse Southern blot	7 malignantt cancers	0						Frozen tissue. No information on histology
Kaufman *et al.* (1987)	USA	In-situ hybridization (6, 11, 16, 18)	12 adenocarcinomas	83	83					Frozen tissue
Ostrow *et al.* (1987a)	USA	Filter in-situ hybridization (low stringency)	10 malignant cancers 4 metastatic cancers	0 0						Frozen tissue
Leake *et al.* (1989)	USA	Southern blot (6, 16, 18, 31, 35) PCR (6/11)	12 adenocarcinomas 3 tumours of low malignant potential	0 0						Frozen tissue. Histopathological changes suggestive of HPV infection (koilocytosis) in 5/15 tumours
McLellan *et al.* (1990)	USA	PCR (6, 11, 16, 18)	24 tumours of low malignant potential	0						Fixed tissue
Beckmann *et al.* (1991b)	USA	PCR (*L1* consensus primers)	18 malignant cancers 11 'borderline'	0 0						Fixed tissue. β-Globin was successfully amplified in each tissue sample.
Lai *et al.* (1994)	Taiwan, China	PCR (16, 18)	18 cases	61			50	17		Frozen tissue

PCR, polymerase chain reaction
[a]Of those types tested

(k) Cancer of the bladder and urethra

For these tumours, contamination during tissue acquisition is a particular concern. The range in prevalence of HPV detected in bladder cancer cases is wide. In six studies, HPV was detected in 0% (0/5), 2% (1/44), 5% (1/22), 16% (12/76), 20% (4/20) and 29% (26/90) of cases, respectively, with HPV-16 being the most common type (Ostrow *et al.*, 1987a; Bryant *et al.*, 1991; Kerley *et al.*, 1991; Chetsanga *et al.*, 1992; Shibutani *et al.*, 1992; Furihata *et al.*, 1993b). No HPV DNA was found in a total of 150 bladder cancers from three further studies using PCR (Knowles, 1992; Saltzstein *et al.*, 1993; Sinclair *et al.*, 1993).

In urethral carcinomas, HPV has been detected in 29–100% of the cases based on four case series including one, four, 14 and 18 cases, respectively (Grussendorf-Conen *et al.*, 1987; Mevorach *et al.*, 1990; Wiener *et al.*, 1992b; Wiener & Walther, 1994).

(l) Prostate cancer

Concerning HPV in prostatic cancer tissue (Table 28), four studies found HPV in 41–75% of cases using Southern blot or PCR. HPV-16 was the type most frequently detected. In contrast, three studies found no HPV DNA and two studies found 3/23 cases (13%) and 6/34 (25%) to be positive for HPV-16. Cuzick (1995) has recently reviewed the data on HPV and prostate cancer. He noted that the positivity rates were as high in benign prostatic hypertrophy as in invasive cancer, raising doubt about any direct role of HPV in prostatic cancer.

(m) Cancer of the eye

HPV has been found in both intraepithelial neoplasia of the conjunctiva (0–80%) and in 62–100% of invasive carcinomas of the conjunctiva, eyelid and lacrimal sac (Table 29).

2.3 Cohort studies

Virtually all prospective studies of HPV infection and cancer have focused on the cervix. The pathogenesis of HPV-related cervical cancer is usually pictured as a multistage process with at least three pathological stages: (i) HPV infection, (ii) increasingly severe grades of intra-epithelial neoplasia (CIN I–III) and (iii) invasive cancer. In practice, these postulated stages cannot be distinguished perfectly. The cytological/histological signs of HPV infection and CIN I in particular tend to be overlapping and transient. Despite the resultant methodological difficulties, prospective epidemiological studies have adopted this useful three-stage model.

Accordingly, two different types of cohort studies of cervical HPV infection have been conducted. The first type examines the postulated transition from HPV infection to development of CIN. The second focuses on the apparent 'progression' of CIN (including the cytological diagnosis of HPV) to cancer or, more commonly, to high-grade CIN as a surrogate or intermediate endpoint for cancer. These two complementary types of cohort studies will be discussed in turn.

Table 28. Prevalence of HPV DNA in prostatic cancer case series[a]

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[b]	HPV type-specific positivity (%)					Comments
					6	11	16	18	Others	
McNicol & Dodd (1990)	Canada	Southern blot (16, 18)	4	75						Frozen tissue
Masood *et al.* (1991)	USA	In-situ hybridization (6, 11, 16, 18, 31, 33, 35)	20	0						Fixed tissue
McNicol & Dodd (1991)	Canada	PCR (16, 18)	27	52			52	4		Frozen tissue. Specimens obtained by transuretheral resection and suprapubic prostatectomy
Anwar *et al.* (1992a)	Japan	PCR (16, 18, 33)	68	41			16	25	7	Fixed tissue
Effert *et al.* (1992)	USA	PCR (16, 18)	30	0						Fixed tissue. Specimens obtained by radical prostatectomy
Ibrahim *et al.* (1992)	USA	PCR/in situ hybridization	24	25			25			Frozen and fixed tissue
Rotola *et al.* (1992)	Italy	PCR (6/11, 16)	8	NA	50		75			Frozen tissue
Serfling *et al.* (1992)	USA	PCR (6, 11, 16, 18, 33)	30	0						Frozen tissue
Sarkar *et al.* (1993)	USA	PCR (6/11, 16, 18)	23	13			13			Fixed tissue
Moyret-Lalle *et al.* (1995)		PCR (16, 18)	17	53			53			

PCR, polymerase chain reaction; NA, not available
[a]Including studies having used hybridization methods
[b]Of those types tested

Table 29. Prevalence of HPV DNA in eye lesion case series[a] (≥ 2 cases)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (no. or %)[b]	HPV type-specific positivity (no. or %)					Comments
					6	11	16	18	Others	
Conjunctiva										
Lass *et al.* (1983)	USA	Southern blot (11)	2 papillomas	50		50				Frozen tissue
McDonnell *et al.* (1987)	USA	In-situ hybridization (2, 6, 16, 18)	28 dysplasias 23 papillomas	0 65	65					Fixed tissue. Of papilloma cases, 14 contained koilocytosis.
McDonnell *et al.* (1989a)	USA	PCR (16, 18)	5 dysplasias 1 carcinoma 1 melanoma	100 1/1 0			100 1/1			Fixed tissue
Lauer *et al.* (1990)	USA	PCR (16, 18)	5 intraepithelial neoplasias	80			80	20		Fixed tissue
Odrich *et al.* (1991)	USA	PCR (*E1* consensus primers and 6, 11, 16, 18, 33)	3 SCC (bilateral)	100			100			Frozen tissue
McDonnell *et al.* (1992)	USA	PCR (16, 18)	42 neoplasias	88			88			Fixed tissue. 11 invasive carcinomas, 12 severe dysplasias/CIS and 19 mild/moderate dysplasias
Eyelid										
McDonnell *et al.* (1989b)	USA	PCR (16/18)	1 SCC	1/1			1/1			Fixed tissue
Hayashi *et al.* (1994)	Japan	In-situ hybridization (6, 11, 16, 18, 31, 33)	21 sebaceous carcinomas (14 F, 7 M)	62	19	24	52	33	48	Fixed tissue. 12 cases had antibodies to *p53*. The presence of antibodies was more frequent in advanced cases.
Lacrimal sac										
Madreperla *et al.* (1993)	USA	PCR/in-situ hybridization (6, 11, 16, 18, 31, 33, 35)	3 carcinomas 3 papillomas	100 100		100		33	67	Fixed tissue

SCC, squamous-cell carcinoma; CIS, carcinoma *in situ*; PCR, polymerase chain reaction; F, female; M, male

[a]Including studies having used hybridization methods

[b]Percentage of those types tested

2.3.1 Following HPV DNA detection in normal women to cytological diagnosis of CIN

Cohort studies of HPV infection as a risk factor for incident CIN have enrolled apparently normal women, using DNA diagnostic assays to test for HPV infection. However, no examination of the cervix *in vivo* can rule out the presence of CIN in its most subtle form, when only a few cell clusters might be affected. Thus, no prospective study of HPV infection and 'incident' CIN can claim truly to have started with a completely CIN-free cohort. As a result, the strong cross-sectional association between HPV DNA detection and prevalent CIN unavoidably biases (upwards) estimates of absolute and relative risk for newly developed CIN, especially in the early months of follow-up.

At the practical (e.g. clinical) level, the importance of this bias is unclear. In effect, the cohort studies are determining whether the detection of HPV DNA precedes the diagnosis of CIN. In the published prospective studies, the diagnosis of the initial absence of CIN in the cervix has been based upon two common clinical techniques, exfoliative cytology and colposcopy. Most cohorts have been composed of women who are normal at enrollment and report no medical histories of abnormal cervical cytological (Papanicolaou smear) diagnoses except for benign inflammatory or reactive changes. For greater certainty of lack of disease, some groups have also subjected the key enrollment slides to review, or have examined all women colposcopically to rule out prevalent cervical lesions missed by cytology. The development of CIN within the cohorts has been diagnosed either by cytological or colposcopic screening at regular intervals, with the endpoint defined either as first cytological appearance of CIN, or more accurately a colposcopically directed biopsy diagnosed as CIN.

Using these available diagnostic techniques, the microscopic diagnoses of HPV infection and CIN overlap. Recognition by pathologists that the cytological diagnosis of HPV infection (koilocytotic atypia) is practically indistinguishable morphologically from the mildest grade of CIN (CIN I) has introduced logical circularity into the prospective studies of HPV infection and incident CIN I. If the diagnosis of CIN I includes the cytological evidence of HPV infection, then, of course, the relative risk of CIN I will be elevated following detection of HPV DNA. Some investigators have attempted to address this issue by presenting relative risks following HPV infection for koilocytotic atypia and various grades of CIN separately, even if the pathological distinctions are unreliable.

In addition, the Working Group was aware of several large unpublished studies of HPV infection in women with normal cytological diagnoses. The major (> 1000 women) ongoing cohorts include, at minimum, the work of the groups of R. Burk in the USA, J. Cuzick in the United Kingdom, E. Franco in Brazil, R. Herrero in Costa Rica, S. Kjaer in Denmark, L. Koutsky in the USA, C. Meijer in the Netherlands, A.B. Moscicki in the USA, J. Peto in the United Kingdom and India, M. Ronderos in Colombia and M. Schiffman in the USA.

Lörincz *et al.* (1990) followed a cohort of 215 cytologically normal women from a single gynaecologist's practice in Washington DC, USA, testing them for over 15 types of HPV DNA using low-stringency Southern blot. Mean follow-up was about two years, during which time three (15%) of the 20 women initially HPV-positive were diagnosed with cervical or vaginal intraepithelial neoplasia, compared to only nine (5%) of the 195 women who were initially HPV-negative. However, medical abstracting revealed that 10 of the 12 women developing

neoplasia had already had CIN prior to their cytological normalcy at enrollment. In other words, HPV positivity was, at least in part, predicting recurrent rather than incident disease.

Badaracco *et al.* (1992) followed 82 cytologically normal women with HPV-16 or -18 (by dot blot) for approximately 18 months. They observed 22 new cytological diagnoses of 'HPV effect' [koilocytotic atypia] and three new diagnoses of CIN among the initially infected women. However, they also observed seven new cytological diagnoses of 'HPV effect' among 20 women without HPV-16 or -18 at enrolment. Thus, the relative risk of cytological abnormality following detection of HPV DNA was not elevated. [The study population was not well described and no types other than HPV-16 or -18 were assayed.]

Koutsky *et al.* (1992) studied a cohort of 241 cytologically normal women who had no past medical history of CIN and had been recruited in a sexually transmitted disease clinic in Seattle, USA. HPV-6, -11, -16, -18, -31, -33 and -35 were assayed using dot blot or Southern blot hybridization. The population was followed every four months by repeated HPV testing and cytological and colposcopic examinations for an average of 25 months. Twenty-eight women developed histologically confirmed high-grade CIN. CIN I was not an endpoint. A Cox regression analysis, treating the repeated HPV test results as a time-dependent covariate, indicated that HPV DNA positivity was associated with an adjusted relative risk (RR) for high-grade CIN of 11 (95% CI, 3.7–31). The RR was highest for women with HPV-16 and -18 (RR, 11 (95% CI, 4.6–26)) and for those with repeated positive tests (for ≥ 3 tests: RR, 26 (95% CI, 6.5–112)). On the basis of survival analysis, the cumulative incidence of biopsy-confirmed high-grade CIN among HPV-positive women was 28% at two years, compared with 3% among HPV-negative women. Most of the incident high-grade CIN occurred within the first two years of follow-up.

In Helsinki, Finland, Stellato *et al.* (1992) followed a cohort of 214 cytologically normal women of whom 145 were HPV DNA positive and the remainder were age-matched HPV-DNA-negative patients from the same clinical setting. The commercial dot-blot system, ViraPap™/ViraType™, was used for HPV testing. The subjects were followed actively every four months for a mean of about one year, with cytological evidence of CIN triggering a colposcopically directed biopsy. During follow-up, cytological evidence of CIN was seen in 25 (17%) HPV-positive women compared with only one (1%) HPV-negative woman ($p = 0.005$ by χ^2 test). Of these 25 women, histological confirmation of CIN was reported for 10. The increased risk of incident CIN was restricted to women with the 'cancer-associated' or 'high-risk' types of HPV (HPV-16, -18, -31, -33 and -35).

de Villiers *et al.* (1992) used filter in-situ hybridization to assay for HPV-6/11 and -16/18 in a large cohort of cytologically normal women in south-western Germany. Two out of 13 women (15.4%) who developed carcinoma *in situ* or invasive cancer during five years of passive cytological follow-up were HPV-positive at enrolment, compared to 8.8% of all women who were cytologically normal at enrolment. [The study cohort was not well defined.]

Prospective data from a cohort study of HPV infection and incident CIN among 11 200 cytologically normal women with no past history of CIN, recruited in 1989–90 from cytological screening clinics at the Portland, Oregon, USA, Kaiser-Permanente prepaid health plan, have been reviewed by Shah and Howley (1996). The length of follow-up extended to four years. Incident cases of CIN were mainly low-grade or koilocytotic atypia, HPV DNA positivity at

enrolment (defined by Hybrid Capture™) was associated with an elevated risk of subsequent CIN. The risk peaked in the first two years following enrolment. Using life-table methods, the cumulative risk of new CIN following HPV DNA detection using Hybrid Capture™ approached 60% by four years of follow-up.

2.3.2 Following mild dysplasia/koilocytotic atypia to CIN III/invasive cancer

Prospective studies of the progression of HPV-associated mild dysplasia are designed to begin where the incidence studies end. In other words, they follow prospectively women with cytological or histological diagnoses of CIN to an endpoint of progression to more severe cervical neoplasia. The startpoint is usually mild dysplasia, and, for obvious ethical reasons, the endpoint in truly prospective (not historical cohort) studies is high-grade CIN and not invasive cancer, unless cases develop inadvertently despite surveillance.

Accurate assessment of disease states is difficult without interfering with the natural history of the disease. Cytological, histological and colposcopic assessments may all be inaccurate and, moreover, biopsy may lead to regression.

In the USA, CIN I and the cytological evidence of HPV are now formally combined as 'low-grade squamous intraepithelial lesions' (LSIL) (Solomon, 1989). Similarly in the forthcoming World Health Organization histopathology classification, changes associated with HPV infection are included under CIN I. There is a large literature dating back two decades regarding the natural history of mild dysplasia, which has also been called 'minimal dysplasia', 'slight dysplasia', 'mild dyskaryosis' or 'CIN I'. Correspondingly, previous terms for the cytological evidence of HPV infection in the absence of dysplasia were 'koilocytotic atypia', 'condylomatous atypia' or 'flat condyloma'. Prospective studies with any of these terms as an enrolment diagnosis were considered relevant for the evaluation of carcinogenicity of HPVs.

(a) Prospective studies of mild dysplasia/koilocytotic atypia without HPV DNA testing

The cytological/histological diagnosis of cervical HPV infection is neither sensitive nor specific (Sherman *et al.*, 1994; Kato *et al.*, 1995). Moreover, 'HPV effect' cannot reproducibly be distinguished from CIN I, which is associated with the same spectrum of HPV types. As mentioned above, the two diagnoses are combined as 'LSIL' in both the Bethesda system and the forthcoming WHO classification.

Nevertheless, it is useful to summarize briefly the many important studies that preceded the advent of HPV DNA testing, but which demonstrated the prospective risk of high-grade cervical cancer precursor lesions following the diagnosis of low-grade lesions now thought to be HPV-induced. Representative prospective studies of cohorts thought to have specifically cytologically or clinically defined HPV infections of women (koilocytotic atypia, condylomatous atypia, flat condyloma or venereal warts) will be reviewed individually. The many similar prospective studies of cohorts diagnosed with CIN I (mild, minimal or slight dysplasia or dyskaryosis) will be summarized in aggregate, because some groups continue to view CIN I as more severe (e.g. more of a cancer precursor) than the cytologic evidence of HPV infection alone.

(i) Follow-up of cohorts with HPV infection diagnosed microscopically or clinically

Meisels and Morin (1981) analysed the cytological records of 234 715 women participating in a mass screening programme in Quebec, Canada, from 1975 to 1979. Of these women, 3977 were diagnosed with 'flat condyloma'. Within the five-year study period, 4.7% of the 3670 patients with condyloma and 10% of the 307 patients with condyloma with nuclear atypia were subsequently diagnosed with dysplasia, carcinoma *in situ* or cancer.

Using a historical cohort approach, Franceschi *et al.* (1983) in Oxford, United Kingdom, compared the risk of diagnosis of CIN following an initial clinical diagnosis of genital warts with the risk following diagnosis of other sexually transmitted diseases. Genital warts are now known to be associated with HPV-6, -11 and related types (see section 1.6). Among the cohort of 489 women who had a smear three to four years after first attendance, the risk of CIN III or microinvasive carcinoma in women diagnosed with genital warts (7/206) appeared to be higher than among the women with other diseases (2/283), although the numbers of events were quite small.

Chuang *et al.* (1984) conducted a historical cohort study of cervical cancer risk in Minnesota, USA, following the diagnosis of genital warts. Among the 500 women, 11 cases of cervical carcinoma *in situ* were diagnosed after an average of four years giving a RR of 3.8 (95% CI, 1.9–6.8) compared to historical rates from the local area.

In the United Kingdom, Evans and Monaghan (1985) observed that, in a group of 51 patients with histological diagnoses of HPV infection, 16% progressed to high-grade cervical neoplasia within 12 months. This included one case of microinvasive carcinoma.

In Melbourne, Australia, Mitchell *et al.* (1986) followed 846 women, diagnosed in 1979 as having cytological evidence of HPV, and carried out repeated cervical examinations during 1980–85. Women with previous or concurrent diagnoses of dysplasia were excluded. Over these six years, carcinoma *in situ* developed in 30 women, compared to 1.9 cases expected from general population incidence rates, yielding an RR of 16 (95% CI, 11–22).

Pagano *et al.* (1987) followed 251 patients referred to a colposcopy clinic in Melbourne, Australia, for cytological diagnoses of HPV infection without CIN. Over up to three years of follow-up, 10% of women developed histologically confirmed CIN. Nearly all progressions occurred within the first 12 months following initial assessment.

To examine whether HPV predicted an increased risk of subsequent cytological or histological diagnosis of CIN, Boyle *et al.* (1989) established a historical cohort of women diagnosed in Connecticut, USA, in 1973–81, 631 with cytological evidence of HPV and 410 with *Trichomonas* infections. Average follow-up time was about two years. Thirteen women with HPV and two with *Trichomonas* had CIN diagnosed within six months of the identifying smear and were excluded from the analyses. The absolute risk of CIN following HPV infection was 8.7%, yielding an RR of 2.7 (95% CI, 1.4–4.9) when compared to the *Trichomonas*-infected cohort, after adjustment for age, nulliparity and frequency of cytological examination. The RR for CIN II–III was 3.2 (95% CI, 1.3–7.8).

Kataja *et al.* (1989) presented a life-table analysis of the best-known cohort study of cervical HPV infection defined cytologically (Syrjänen *et al.*, 1987b). A cohort composed of

256 women with cytological evidence of HPV infection without CIN and 106 women with HPV and CIN was established in Finland beginning in 1981. They were examined by colposcopy, cytology and/or punch biopsy every six months. From life-table analyses, the cumulative risk of progression to carcinoma *in situ* was estimated to be about 15% at 78 months for the group with HPV infection alone. The cumulative risk of progression among women with HPV/CIN I was 20–25% at 78 months. The two risk curves were not significantly different in the earlier years of follow-up when the person-years were greatest. However, longer follow-up of the group with HPV and CIN I indicated a continued substantial risk of progression up through the longest observation period of 96 months.

Sigurgeirsson *et al.* (1991) estimated the relative risks of cervical cancer and carcinoma *in situ* associated with a diagnosis of venereal warts by constructing a historical cohort from patients' records. They compared the incidence of cervical cancer among 711 Swedish women with venereal warts diagnosed during 1969–84 to national registry data. With a mean follow-up of eight years, the RR for cervical cancer was 1.8 (95% CI, 0–10; one case); that for carcinoma *in situ* was 1.5 (95% CI, 0.9–2.5; 17 cases). However, among a corresponding group of 2549 men with venereal warts, the RR of anogenital malignancy was 2.6 (95% CI, 1.2–5.0; nine cases).

Gram *et al.* (1992) linked hospital records to Norwegian cancer registry data to examine the temporal relationship between the cytological diagnosis of HPV or *Trichomonas* infection and subsequent risk of CIN III in northern Norway. From 1980 to 1989, HPV infection was noted in 678 of 43 016 women with negative Pap-smears, while *Trichomonas* was diagnosed in 988. The age-adjusted incidence rates of CIN III were as follows: 225 per 10^5 person-years follow-up among women with neither infection, 459 per 10^5 person-years among women with *Trichomonas* and 729 per 10^5 person-years among women with HPV. The RR for the development of CIN III among women with HPV, adjusted for several possible confounding variables, was 3.5 (95% CI, 1.9–6.6) compared to uninfected women.

Montz *et al.* (1992) followed 203 women in Los Angeles, USA, with cytological diagnoses of low-grade SIL. The presence of occult high-grade CIN missed by cytology was minimized by enrollment colposcopy. Seven women (3.4%) progressed to high-grade SIL: one at three months, one at six months and five at nine months.

(ii) Follow-up of cohorts with mild dysplasia

The many cohort studies of women diagnosed with CIN I were comprehensively reviewed recently by Östör (1993) who provided useful aggregate estimates by pooling the data from over 15 studies. In total, he reviewed the follow-up of about 4500 women with CIN I from published studies. The observed risk of progression to CIN III in the studies was 11%, with 1% observed progression to invasive cancer during follow-up (which was often short and truncated by treatment). The remaining cases persisted (32%) or regressed (57%).

Soutter and Fletcher (1994) reanalysed five studies (Robertson *et al.*, 1988; Fletcher *et al.*, 1990; Cooper *et al.*, 1992b; Hirschowitz *et al.*, 1992; Kirby *et al.*, 1992) of cytological follow-up of women with mildly abnormal cervical smears. The four latter studies were too recent to be included in Östör's pooled estimates (Östör, 1993). Soutter and Fletcher estimated that the annual incidence of invasive cancer among women with mild cytological abnormalities was

between 143 and 420 per 10^5 person-years, which is comparable to Östör's cumulative incidence of 1%. The magnitude of risk of cervical cancer following diagnosis of CIN I was calculated to be 16–47 times greater than the incidence of cervical cancer in women of similar age in the general population of England and Wales.

(b) Prospective studies of mild dysplasia/koilocytotic atypia with HPV DNA testing

In recent prospective studies of CIN I/koilocytotic atypia, HPV DNA typing has sometimes been included as an independent covariate. The aim of including HPV typing in progression studies has been to determine whether HPV type predicts the risk of progression independently of microscopic diagnosis.

Campion *et al.* (1986) in London, United Kingdom, used filter in-situ hybridization to test for HPV-6 and -16 among 100 women aged < 30 years with persistent CIN I (mild dyskaryosis on three consecutive smears taken within 16 weeks). No biopsies were taken at enrolment. The cohort was examined every four months by colposcopy and cytology for a range of 19 to 30 months. Of the 26 women who progressed to histologically confirmed CIN III, 22 (85%) had tested positive for HPV-16 DNA at enrolment; only 17 of the 74 women (23%) who did not progress to CIN III had tested positive for HPV-16 DNA. Thus, the presence of HPV-16 was significantly associated with risk of (and time to) progression; presence of HPV- 6 was not. [The HPV test method used in this study is now obsolete and possibly inaccurate with regard to typing. For example, the possibility of cross-reactivity between HPV-16 and related types not known at the time of the investigation should be considered.]

Schneider *et al.* (1987b) monitored 48 HPV-positive West German women cytologically over a period of three to 24 months. Filter in-situ hybridization had been used to detect HPV-6/11 and -16/18. Of the 17 women with an initial cytological diagnosis of 'condyloma', two progressed to histologically confirmed CIN III. Both were HPV-16/18-positive at enrolment (one was also positive for HPV-6/11), whereas there were no instances of progression among the seven women positive for HPV-6/11 only. The same association of risk of progression with HPV-16/18 positivity was seen among the six women who progressed to CIN III among the 26 who were initially diagnosed as 'CIN I/II'.

Caussy *et al.* (1990b) studied 47 cases of invasive cancer and 94 matched controls in British Columbia, Canada, using archival pathology specimens. The cases were women treated for invasive cancer from 1960 to 1986, and archival biopsies showing CIN, taken at least two years before the diagnosis of cancer, used to test for HPV DNA at the pre-invasive stage using tissue in-situ hybridization. HPV-6/11, -16/33 and -18 were assayed. The controls were women with previous, available CIN biopsies who had not progressed to invasive cancer. [An appreciably larger percentage of controls than cases had been treated at the CIN stage.] The controls were matched to cases on a variety of factors, including date of the biopsy showing CIN. The investigators observed non-significantly elevated HPV prevalence for all three type groups among the case group (6.4% for HPV-18; 8.5% for HPV-6/11; 19% for HPV-16/33) compared with their matched controls (2.1%, 3.2% and 11%, respectively).

Kataja *et al.* (1990) performed a survival analysis of enrolment HPV typing data from the long-term Finnish prospective study of women with cytological evidence of HPV infection (Syrjänen *et al.*, 1987b). Starting in 1981, the cohort was followed every six months by cytology

and colposcopy (with or without biopsy, depending on the study subgroup) for a mean of 50 months. For 458 women in the cohort, archival enrolment specimens were assayed for HPV-6, -11, -16, -18, -31 and -33 using in-situ hybridization. Clinical progression was defined as a change from no CIN to CIN or from lower to higher grade CIN. The overall rate of progression was 16.4%. The risk of progression was highest in 91 women positive for HPV-16 (35.2%) or 14 having multiple infections (28.6%), intermediate for 205 women with other HPV types (15%), and lowest in 48 HPV-negative women (6.1%). These differences were statistically significant in both contingency-table and survival-curve analyses. [The inconsistency of finding HPV DNA negativity in women diagnosed with cytological changes supposedly indicating HPV infection raises the possibility of either false positive cytological diagnoses or false negative DNA hybridization.]

Using a retrospective cohort design, Weaver *et al.* (1990) studied 32 patients in Ohio, USA, with histological diagnoses of CIN I 'with koilocytosis', testing archival paraffin-embedded specimens with in-situ hybridization for HPV-6/11, -16, -18, -31 and -33. The cytological follow-up, a minimum of two examinations over at least 1 year, ranged from 12 to 80 months with a mean of 27 months. The cumulative rate of progression to CIN II or greater was 9% (3 cases), with no apparent difference in rates of progression between the HPV-positive (7%) and HPV-negative (12%) subcohorts. [The Working Group noted the small sample size of this study.]

Byrne *et al.* (1990) followed 42 women in the United Kingdom with cytological evidence of CIN I or II at four-monthly intervals, using cytology and colposcopy, with an endpoint of histologically confirmed CIN III. Enrolment and follow-up specimens were tested for HPV-6, -11, -16 and -18 using a slot blot DNA hybridization method. The women were seen from three to 11 times over the 45-month follow-up period. Thirty of the 42 women (71%) were positive for HPV-16 or -18 DNA at some time during the study. In this subgroup, five of 30 (16.7%) progressed, compared to one of the 12 women (8.3%) who were HPV-16/18-negative throughout. [The HPV exposure measurement mixes enrolment and follow-up positivity into a cumulative positivity measurement that is not strictly prospective.]

Parry *et al.* (1990) reported on 44 women with CIN I–II, whom they followed by quarterly cytological and colposcopic examination over a two- to three-year period. The women were tested by dot blot at each visit for HPV-6, -11, -16 and -18. The detection of HPV-16 or -18 at any time during follow-up was associated with a significantly increased risk of progression to CIN III. [The enrolment DNA data are not reported separately to permit a strictly prospective analysis.]

Using cytology and colposcopy quarterly for up to 36 months, Hørding *et al.* (1991b) monitored 15 women in Copenhagen, Denmark, diagnosed histologically with mild dysplasia. Filter in-situ hybridization was used to test enrolment and follow-up specimens for HPV-11, -16 and -18. HPV positivity at any time was found in seven of the women, all of whom had progressive or persistent lesions. In comparison, only four of the eight persistently HPV-11/16/18-negative patients had persistent or progressive CIN. [When combined with similar results for women found initially to have CIN II, the differences by HPV positivity/negativity were statistically significant. However, this study mixes enrolment and follow-up HPV

positivity into a cumulative HPV positivity measurement that is not strictly prospective. The HPV test used is obsolete.]

Pich *et al.* (1992) followed 24 women in Italy, with CIN I or CIN II lesions immunohistochemically positive for HPV antigens. The biopsies were further analysed for HPV-6/11 and -16/18 DNA. Follow-up included quarterly cytological, colposcopic and histological examinations over a two-month to 65-month period. Four of 10 women with HPV-16/18 progressed to histologically diagnosed CIN III, compared with none of 14 HPV-16/18-negative women. [This investigation combines CIN I and II, and does not separate clearly enrolment versus follow-up HPV positivity.]

Hellberg *et al.* (1993) conducted a historical cohort study of 201 women with CIN lesions tested for HPV-6, -11, -16, -18, -31 and -33 by in-situ hybridization. The mean follow-up time was 17.3 years. In a multivariate analysis, HPV type was significantly associated with risk of progression, independent of grade of CIN. [Crude data permitting analyses specific for HPV type among women with low-grade CIN were not presented].

Downey *et al.* (1994) followed 92 women in the United Kingdom with cytologically/histologically diagnosed CIN I or 'wart virus infection' for up to 70 months of passive surveillance (medical record review and quarterly cytology with colposcopy following any abnormal cytological diagnoses). The endpoint, reached by 25 patients, was the histological diagnosis of CIN II or more-severe disease. Cervical DNA from samples taken at enrolment was tested for HPV-16 using 'semiquantitative' PCR only. The presence of HPV-16 DNA was non-significantly (but negatively) associated with risk of progression. Also, counter to the expectations raised by cross-sectional data from the same population (Bavin *et al.*, 1993), greater viral burden was not predictive of progression. [The Working Group noted that HPV testing was conducted for HPV-16 only, and that other high-risk types could be present in the 'HPV-negative' group. Also HPV-16 positivity was unusually prevalent among the low-grade cases (51/92), raising a concern about the accuracy of the viral typing.]

Gaarenstroom *et al.* (1994) performed a retrospective cohort study of HPV testing by general primer PCR among 227 patients with frozen cervical swabs taken at the time of first abnormal cervical cytological diagnosis. All women had colposcopically directed biopsies at entry. Cohort members were followed subsequently by colposcopy and cytology at 3-month intervals, without therapeutic interventions for at least 6 months. The presence of HPV-16 DNA at enrolment predicted the following: a significantly elevated 29% risk of progression to higher-grade neoplasia (defined histologically); a 10% risk of progression for HPV-18, -31, -33 and unknown types; and a 0% risk of progression among women positive only for HPV-6 or -11 or negative for HPV. [The cohort included 100 women with underlying CIN II–III at enrolment, as well as 101 with underlying CIN I and 26 with no CIN demonstrated histologically. Excluding the 24 women with CIN III did not alter the conclusions but further recalculations are not possible from the data presented.]

Remmink *et al.* (1995) prospectively followed a cohort of 342 women in Amsterdam, with a new cytological diagnosis of 'Pap IIIb' or lower, suggestive of mild, moderate or severe dysplasia. Surveillance every three to four months included cytology, colposcopy without biopsy and HPV DNA testing for 27 types by a well-validated PCR technique. The mean follow-up of the entire cohort was about 16 months. At the start of follow-up, 62% of women

were HPV-positive. Nineteen women (5.6%) progressed, defined as developing a lesion with a colposcopic impression of CIN III over more than two quadrants of the cervix or a Pap smear of class V (highly suggestive of malignancy). All 19 women subsequently had histologically confirmed CIN III and all were HPV-positive both at enrolment and continuously during follow-up.

2.3.3 Prospective studies of HPV infection at body sites other than the cervix

In Australia, Planner and Hobbs (1988) followed, without treatment, 103 women with colposcopic and histological evidence of HPV infection of the vulva, with no associated VIN. One patient experienced progression to a VIN III lesion after two years.

Arndt *et al.* (1993) studied the prospective risk of carcinoma of the larynx related to laryngeal infection with HPV-16 or -18 among 150 patients with chronic laryngeal inflammation, followed for up to 3.5 years. PCR was used to test for HPV-16 and -18 DNA in laryngeal biopsies. Fifteen (16.5%) of the 91 patients positive for HPV-16 or -18 developed laryngeal cancer, compared to only one patient (1.7%) among the 59 in the HPV-negative group.

2.4 Case–control studies

To date, case–control studies contribute the bulk of the epidemiological evidence linking HPV to cervical cancer and to CIN III. The initial studies were severely hampered by test validity and study design (for reviews see Koutsky *et al.*, 1988; Muñoz *et al.*, 1988; Bosch & Muñoz, 1989; zur Hausen, 1989; Franco, 1992; Bosch *et al.*, 1994b; Schiffman & Schatzkin, 1994). In addition to the general concern on the comparison of studies based on HPV DNA assays of different sensitivity and specificity, the interpretation of case–control studies is difficult because, in many cases, the published investigations were not based on cases and controls drawn from defined and comparable populations; rather, they consisted of a series of cases collected in a medical facility and compared to an undefined group of women without cervical abnormalities. In addition, many studies were based on small numbers of cases and controls, and potential confounders, such as age, were not controlled for.

This monograph includes a comprehensive compendium of published reports of different quality. The data on cervical cancer and CIN lesions are presented in an ordered fashion by stage of disease and by HPV detection method employed. Tables 30, 31 and 36 include studies of CIN lesions in which HPV was detected by non–hybridization methods (Table 30), hybridization methods not including amplification (Table 31) and PCR based methods (Table 36). In a similar manner, Tables 38, 39 and 40 summarize studies on invasive cervical cancer. Table 37 and 41 summarize studies of CIN and invasive cancer in which serological assays were used to assess HPV exposure.

Of the studies included, only a limited number fulfil the epidemiological requirements of a case–control study. These are the studies in which the following criteria are met:

(i) There is a recognizable study design aiming at avoiding bias in the recruitment of cases and the selection of controls.

(ii) The study subjects are representative of the general population.

(iii) There is a comprehensive effort to evaluate all known or suspected risk factors for cervical cancer.

(iv) The size of the study is sufficiently large to allow precise estimates of risk.

(v) The estimates of HPV exposure are based on state-of-the-art PCR methods.

(vi) The statistical analysis includes multivariate techniques.

The few studies that fulfilled these criteria were highly influencial in the Working Group's final evaluation and they are placed as first entries in Tables 36 and 40.

Of all the variables that may affect the results, test validity seems to be the main one. In this light, it is remarkable that hospital-based studies (Eluf-Neto *et al.*, 1994) produce similar risk estimates to population-based studies (Muñoz *et al.*, 1992). The increased detection level afforded by PCR-based methods over previous assays provided more accurate estimates of the HPV prevalence in cases and controls, but in many studies, quantitative information on the level of HPV DNA is not available, so the importance of high-level positivity cannot be investigated.

2.4.1 Cervical cancer

Reviews of the case–control studies relating HPV DNA detection and cervical neoplasia are largely consistent in showing that the association is strong with odds ratios greater than 10 in the majority of studies. The association is specific to a limited number of HPV types. HPV-16, -18, -31, -33 and -45 account for perhaps 80% of the types found in biopsies of invasive cervical cancer worldwide (Bosch *et al.*, 1995). The association is also consistent geographically in all countries in which studies have been conducted. The increased detection level afforded by PCR-based methods over previous assays has provided more-accurate estimates of the HPV prevalence in cases and controls. In general, odds ratios and attributable risks (ARs) are higher in PCR-based studies (although the association is consistently present and statistically significant irrespective of the HPV detection method used) (for reviews, see Bosch *et al.*, 1992; Muñoz & Bosch, 1992; Schiffman, 1992a,b).

(a) HPV and CIN III

Table 30 summarizes case–control studies in which exposure to HPV was assessed using morphological criteria of diagnosis in cells or biopsies or using immunoperoxidase staining to detect HPV capsid antigen in biopsies. It is known that the sensitivity of morphological changes such as koilocytosis is low and that the presence of markers associated with a productive HPV infection decreases as the severity of the intraepithelial process advances (see section 1.5). Many of these studies are crude by current standards but were of importance at the time when the association between HPV and cervical neoplasms was unknown. However, it soon became clear that DNA-based studies were capable of detecting HPV DNA in a large fraction of cervical cancer. The studies listed in Table 30 are therefore of limited value concerning the association between HPV and cervical cancer.

Reid *et al.* (1982) examined the margins of neoplastic lesions in women undergoing hysterectomy for cervical cancer, CIS or for reasons not related to cervical neoplasia. A semi-objective rating system of HPV-related morphological changes was used to evaluate specimens. Cases and controls were classified according to a wart score and a categorical division into

Table 30. Case–control studies of CIN I–III using HPV non-hybridization methods

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95% CI)[a]	HPV test/comments/adjustments
Reid *et al.* (1982) Detroit, MI, USA	All women with hysterectomy 20 CIN I 20 CIN III	40 age-, race- and SES-matched women with hysterectomy and no cervical pathology	Cases / Controls Negative 0 / 80.0 Suspect 12.5 / 10.0 Infected 87.5 / 10.0	Definite HPV versus negative or suspect [CIN I–III, 63 (13.4–345.5)]	Criteria for seven histological parameters scored in 3 levels: negative; suspect; infected
Grunebaum *et al.* (1983) New York, USA	251 patients referred to colposcopy with CIN I–III	90 normal cervices from same clinic	Control, 24.4 Mild dysplasia, 62.5 Moderate dysplasia, 59.8 Severe dysplasia, 22.9 CIS, 14.3	[CIN I–III, 2.5 (1.9–4.4)]	Koilocytosis, multinucleation, parakeratosis, dyskeratosis
Syrjänen (1983) Finland	345 dysplasias, CIS, invasive condylomatous lesions	275 dysplasias, CIS, invasive non-condylomatous lesions	Control, 0 (0/129) All, 56.2 (122/217) Papillomatous, 100 Inverted, 69.7 Flat, 52.5	[∞ (115–∞)] [∞ (187–∞)] [∞ (159–∞)] [∞ (98–∞)]	Staining, immunoperoxidase-PAP (paraffin sections), HPV Ag in cells
Syrjänen *et al.* (1983) Finland	79 dysplastic and/or CIS with condylomatous lesions	31 dysplastic and/or CIS without-condylomatous lesions	Control, 0 All cases, 70.8 Papillomatous, 100 Inverted, 83.3 Flat, 66.7	[∞ (44–∞)] [∞ (32–∞)] [∞ (43–∞)] [∞ (36–∞)]	Staining, immunoperoxidase-PAP (paraffin sections), HPV Ag in cells
Adam *et al.* (1985) Houston, USA	23 CIN	23 matched, no CIN	Control, 0 (0/10) CIN I–II, 27 (3/11) CIN III, 0 (0/2) All, 22.2 (4/18)	[∞ (0.4–∞)] Not computable Any lesion, [∞ (0.4–∞)]	Presence of structural antigen in biopsy specimens. Prospective cohort study of women exposed to diethylstilboestrol
Guijon *et al.* (1985) Manitoba, Canada	33 CIN I–III referred to colposcopy	54 women with no CIN attending family-planning clinic	Case, 45 Control, 3.7	[21.7 (4.1–152.8)]	Koilocytosis

Table 30 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test/comments/adjustments
Zaninetti *et al.* (1986) Italy	126 abnormal Pap smears in women less than 20 years of age	1914 normal cervices, same age and clinic		Ever having genital warts 9.15 (5.1–16.3)	History of genital warts. OR adjusted for number of sexual partners
Höckenström *et al.* (1987) Gothenburg, Sweden	49 women with a consort with genital warts	124 women age, parity, OC use and date-of-examination matched to the case attending family-planning clinics	Dysplasia or HPV infection: Case, 37 Control, 6	[8.4 (3.1–23.5)]	Koilocytosis, atypia, dysplasia
Alberico *et al.* (1988) Trieste, Italy	533 cases attending colposcopy with dyskaryosis to CIN III and CIS. Of these, 299 CIN I–III	533, age matched to the cases, with no CIN	Dyskeratosis, 26 CIN I, 39 CIN II, 51 CIN III–CIS, 25 Normal, 0.19	Any CIN versus normal [333 (45–6460)]	Detection of condylomatous cytohistological features
Cuzick *et al.* (1990) United Kingdom	110 CIN I 103 CIN II 284 CIN III	833 family-planning clinic and local GPs	Control, 5 CIN I, 33 CIN II, 28 CIN III, 16	CIN I, 8.4 CIN II, 7.1 CIN III, 3.4	History of genital warts. $p < 0.05$
Seshadri (1991) India	16 CIN I 29 CIN II 25 CIN III	50 controls	Control, 28.9 CIN I, 62.5 CIN II, 75.9 CIN III, 60.0	[4.2 (1.1–16.9)] [7.8 (2.6–27.0)] [3.8 (0.4–49.4)]	Histopathological evidence of HPV infection
Kjaer *et al.* (1992) Denmark	586 CIS 59 invasive cancers	614 population based		Ever genital warts 1.7 (1.2–2.5)	History of genital warts. OR increased with early age at first episode of genital warts. OR adjusted for age, smoking, number of partners, oral contraception, and parity
Thanapatra *et al.* (1992) Thailand	970 specimens with CIN I–III	22691 specimens with no CIN or carcinoma from screening programme	CIN I–III, 26 Control, 1.2	[28.8 (23.8–34.9)]	Koilocytosis, atypia

[ ] Calculated by the Working Group

CIN, cervical intraepithelial neoplasia; SES, socio-economic status; CIS, carcinoma *in situ;* OC, oral contraceptive; Ag, antigen; Pap, Papanicolaou; PAP, peroxidase-antiperoxidase; GP, general practitioner; OR, odds ratio

[a] ∞, zero cases in control group

definite HPV changes, suspected HPV changes and no HPV changes. Using as cases those that had definite HPV changes and comparing these to those that were negative or suspected, the odds ratios were [107 (95% CI, 17.6–877)] for invasive cervical cancer (see also Table 38) and [63 (95% CI, 13.4–345.5)] for CIN lesions.

Grunebaum *et al.* (1983) evaluated HPV signs in 348 patients referred to a colposcopy clinic. Of these, 251 women had histologically confirmed CIN I–III and they were compared to 90 women with normal cervices. The presence of HPV signs was 44.6% among cases and 24.4% among controls [OR, 2.5 (95% CI, 1.9–4.4)]. Of seven cases of invasive cancer diagnosed in this series, none had HPV signs.

In two studies, Syrjänen (1983) and Syrjänen *et al.* (1983) compared the presence of HPV signs in cervical lesions ranging from dysplasia to CIS in 79 and 345 patients with 31 and 275 patients without concomitant condylomatous lesions. HPV antigens were investigated using the immunoperoxidase–peroxidase–antiperoxidase method. Cases without associated condyloma (controls) were consistently negative and cases with associated condyloma were HPV antigen-positive in 70.8% and 56.2% of patients, respectively.

Adam *et al.* (1985) studied 23 cases of CIN lesions arising in a cohort of women exposed *in utero* to diethylstilboestrol. A control group of 23 cohort members without CIN were identified. The peroxidase–antiperoxidase method was used to detect HPV structural antigen and part of the biopsies were also reviewed for the presence of HPV signs. Of CIN I–II cases, 27% were immunoperoxidase-positive compared to none of the controls. None of the differences was statistically significant [$p > 0.05$]. Of 18 cases investigated (CIN I–III, HPV and squamous metaplasia), 15 had HPV-related changes histologically and four had HPV structural antigen reactivity.

Guijon *et al.* (1985) compared the presence of HPV signs in 33 women with colposcopically detected and biopsy-proven CIN I–III and 54 women with normal cervices; 45% of cases and 3.7% of the controls had HPV signs [OR, 21.7 (95% CI, 4.1–152.8)].

Zaninetti *et al.* (1986) conducted a similar study in Italy including 126 women below 20 years with abnormal Pap smears and 1914 women of the same age with a normal Pap smear. A history of warts was reported by 15 cases (11.9%) and 22 controls (1.1%). The estimated relative risk, adjusted for number of sexual partners, was 9.15 (95% CI, 5.1–16.3).

Höckenström *et al.* (1987) investigated the presence of dysplasia or HPV signs in 49 female consorts of men with condylomata acuminata and compared them to a group of 124 women from family-planning clinics matched to cases on age, oral contraceptive use, parity and date of examination. Thirty-seven percent of cases had HPV-related signs compared to 6% of controls [OR, 8.4 (95% CI, 3.1–23.5)].

Alberico *et al.* (1988) evaluated condylomatous signs in 533 women attending a colposcopy clinic with diagnoses ranging from dyskaryosis to carcinoma *in situ*. A control group of 533 women with normal cervices were also evaluated. The prevalence of HPV colposcopic signs in the 299 CIN I–III lesions was 38.5% compared with 0.19% in controls [OR, 333 (95% CI, 45–6460)].

Cuzick *et al.* (1990) investigated 497 women under the age of 40 with CIN I–III and 833 controls from general practitioners' files or family-planning clinics. A history of genital warts

was reported by 5% of the controls, 33% of the CIN I cases, 28% of the CIN II cases and 16% of the CIN III cases ($p < 0.05$ for each CIN stage as compared to controls).

Seshadri *et al.* (1991) compared 70 women with CIN I–III to 50 controls with normal cervices. HPV exposure was assessed using the standard histopathological criteria. The presence of HPV changes was reported in 28.9% of the controls, 62.5% of CIN I cases, 75.9% of CIN II cases and 60.0% of CIN III cases. The estimated odds ratio for the group of CIN I–III was [OR, 5.9 (95% CI, 2.3–15.4)].

Kjaer *et al.* (1992) compared the history of genital warts in 586 cases of carcinoma *in situ* of the cervix, 59 cases of invasive carcinoma and 614 controls drawn from the general population that generated the cases. One-hundred-and-two cases reported having had episodes of warts (17.9%) as well as 62 controls (10.3%). The odds ratio adjusted for age, smoking, number of partners, oral contraception and parity was 1.7 (95% CI, 1.2–2.5). The risk increased with earlier age at first episode of genital warts.

Thanapatra *et al.* (1992) compared the presence of HPV signs and a cytological diagnosis of CIN in women participating in a national screening programme in Thailand. Of 970 women diagnosed as CIN I–III, 26.0% had HPV signs compared to 1.2% of the controls [OR, 28.8 (95% CI, 23.8–34.9)].

Evans *et al.* (1992) investigated the history of anogenital warts in relation to the presence of CIN I–III in the United Kingdom. The reported odds ratio for a history of vulvar warts was 0.34 (95% CI, 0.13–0.84).

In China, Su (1987) used the peroxidase stain to explore HPV genus specific antigen in 244 cervical tissue specimens. Six of 52 cases of carcinoma (12%) were HPV positive as compared to none of the 20 controls tested.

In the USA, Amburgey *et al.* (1993) conducted a case–control study of 102 cases of CIN I–III matched to an equal number of controls. No HPV detection method was used but cases reported genital warts more often than controls (OR, 2.5 (95% CI, 1.0–6.4)).

In Durban, Kharsany *et al.* (1993) examined a series of patients attending an STD clinic. HPV exposure was found cytologically in 22/48 (46.0%) cases, colposcopically in 28/48 (58.3%) and histologically in 26/45 (57.8%). Women with CIN had clinical HPV signs in 13/28 (46.4%).

Kjaer *et al.* (1991) investigated the history of genital warts in husbands of monogamous women with CIN and in the husbands of a control group in Denmark. The study group included 41 case couples and 90 control couples. Genital warts were reported in 9/41 (22.0%) husbands of cases and in 2/90 (2.2%) husbands of controls. The odds ratio, adjusted for age and use of a condom with the partner, was 17.9 (95% CI, 3.3–98.3). Cell specimens from the male genital warts were analysed using ViraPap™ and ViraType™ and only two husbands of cases were found to be HPV-positive (one had HPV-6/11 and the other HPV-16/18). [The Working Group noted that ViraPap™ assays in specimens from the male genitalia may be inaccurate in detecting HPV DNA prevalence.]

Table 31 contains a summary of case–control studies investigating preinvasive lesions (CIN I–III) using hybridization assays without amplification techniques, including Southern blot, dot blot, filter in-situ hybridization (FISH) and variants of these methods.

Table 31. Case–control studies of CIN I–III lesions using HPV hybridization assays without amplification

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
McCance *et al.* (1985) London, United Kingdom	Colposcopy clinic 20 CIN I 30 CIN II 28 CIN III	17 without cervical abnormality	*HPV-16* CIN I, 55 CIN II, 66 CIN III, 71 Control, 18	*HPV-16* [5.7 (1.1–35.6)] [9.3 (1.8–53.6)] [11.7 (2.2–70.5)]	DNA hybridization Probes: 6, 16 and low stringency	
Demeter *et al.* (1987) Australia	6 minor cell atypias 29 CIN I 35 CIN II 62 CIN III	23 normal	*Any HPV* Minor cell atypia, 33.3 CIN I, 72.4 CIN II, 77.1 CIN III, 71.0 Normal, 30.4 *HPV-16/18* Normal, 21.8 Minor cell atypia, 16.6 CIN I, 51.7 CIN II, 51.4 CIN III, 53.2	*Any HPV* [1.1 (0.1–10.5)] [6.0 (1.5–24.4)] [7.7 (2.0–30.8)] [5.6 (1.8–18.2)] *HPV-16/18* [0.7 (0.03–9.9)] [3.9 (1.0–16.0)] [3.8 (1.0–15.0)] [4.1 (1.2–14.5)]	Filter in-situ hybridization Probes: 6/11 and/or 16/18	Colposcopy clinic attendees
Pao *et al.* (1989) Taipei, Taiwan, China	276 urogenital condylomata 47 cervical dysplasias	39 symptom free	Symptom free, 15.4 Urogenic condylomata, 84.4 CIN or CIS, 72.3	*Any HPV* [29.8 (11.0–84.8)] [14.4 (4.4–49.7)]	In-situ DNA hybridization 6/11, 16, 18, 31, 33	Detection in exfoliated cervicovaginal cells
Duggan *et al.* (1990) Canada	300 CIN/condylomata	90 normal	CIN/condylomata, 60 Normal, 26.6	*Any HPV* [4.1 (2.4–7.2)] *High-risk HPVs* [18.8 (7.6–49.4)]		
Kataoka & Yakushiji (1990) Japan	37 clinical findings	71 symptom free	Case, 32.4 Control, 5.6	*Any HPV* [8.0 (2.1–33.0)]	Non-isotopic subgenomic probes on Southern blot hybridization	Young women (14–27)
Becker *et al.* (1991) New Mexico, USA	52 atypias 77 slight dysplasias 27 moderate–severe dysplasias	1447 Pap negative	Pap negative, 5.6 Atypia, 21.2 Slight dysplasia, 45.5 Moderate–severe dysplasia, 66.7%	*HPV* [4.5 (2.1–9.5)] [14.0 (8.3–23.9)] [33.7 (13.8–84.1)] Any Pap abnormalities, [11.7 (7.8–17.6)]	Dot-blot hybridization assay (ViraPap™)	Random sample of patients undergoing a pelvic examination. Prevalence varies with ethnicity.

Table 31 (contd)

Reference and study area	Cases (type and number)	Controls (type and number)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
Lindh *et al.* (1992) Sweden	52 CIN I 23 CIN II–III	416 with no CIN	No CIN, 14 CIN I, 50 CIN II–III, 39	*HPV* [6.3 (3.3–12.1)] [4.8 (1.9–12.5)] All CIN, [5.5 (3.1–9.7)] *HPV-16* All CIN, [3.4 (1.1–9.8)] *HPV-18* All CIN, [12.3 (4.3–36.1)]	Southern blot, probes 6, 11, 16, 18, 31, 33, 35	Women attending outpatient clinics
Lörincz *et al.* (1992) USA	270 borderline atypias 638 definite cervical diseases: LSIL & HSIL	1566 normal cervices	Normal, 6.4 Borderline atypia, 23.7 Cervical disease, 79.3 LSIL, 69.5 HSIL, 87.4	*HPV* Any cervical disease, 27.1 ($p < 0.0001$) Definite cervical disease, [55.4] *LSIL* HPV-16, 36.9 (25.0–54.5) HPV-18, -45, -56, 32.7 (19.2–55.8) *HSIL* HPV-16, 235.7 (198.5–279.5) HPV-18, -45, -56, 65.1 (50.2–84.5)	Southern hybridization	Pooled analysis and HPV testing of subjects from 8 studies. No adjustments, crude odds ratios
Manavi *et al.* (1992) Austria	411 dysplasias	240 normal cytologies	Prevalence given by Pap grade	*HPV* II, [27.9 (11.2–73.5)] III, [52.9 (20.3–146.4)] IV, [136.5 (44.3–447.7)] V, [468.0 (130.9–1852.7)]	In-situ nucleic acid hybridization	
Meekin *et al.* (1992) New Zealand	87 dysplasias 84 atypias 495 infections/benign atypias	1347 normal cytologies	Control, 8.3 Dysplasia, 48.3 Atypia, 20.2 Infection/benign atypia, 10.1	Odds ratio for atypia or dysplasia: HPV, 5.8 (4.0–8.6) HPV-16/18, 6.4 (3.3–5.9)	Dot-blot DNA hybridization	Attendees of family planning clinics
Tanaka *et al.* (1992) Japan	145 abnormal cytologies	100 normal cytologies	Normal, 2 Benign, 2 Condyloma, 100 Mild–moderate dysplasia, 39 Severe dysplasia/CIS, 44 Invasive, 70 Any abnormal, 30	*HPV* [$p < 0.001$] [31.8(6.8–204.1)] [38.1(5.8–318.5)] [114.3 (12.9 –1463.2)] [20.7 (4.7–126.8)]	Southern blot, probes 6, 11, 16, 18, 31, 33, 35	HPV and HPV type specific prevalence. No multivariate analysis. HPV prevalence by age and type of lesion

Table 31 (contd)

Reference and study area	Cases (type and number)	Controls (type and number)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
Levine *et al.* (1993) USA	34 cytological SIL 25 equivocal atypias 19 histological HSIL	147 cytological controls 150 histological controls	Cytological control, 16 Histological control, 19 Cytological SIL, 58 Equivocal atypia, 40 Histological HSIL, 68	 7.3 (3.3–17.0) 3.4 (1.4–8.5) 10.3 (3.3–32.0)	ViraPap™	Population-based case–control study of college students. Age: 18–35. Covariates: age, multiple lifetime sexual partners, oral contraceptive use
Meyer *et al.* (1993) USA	61 LSIL 16 HSIL	72 cytologically normal women	Normal, 6.9 LSIL, 29.5 HSIL, 68.7	*HPV* [5.6 (1.8–18.8)] [29.5 (6.2–158.1)]	ViraPap™ combined with ViraType™ in-situ hybridization	Patients: high risk for HPV infection. Type-specific information also available. No odds ratios, no multivariate analysis
Becker *et al.* (1994) New Mexico, USA	201 H dysplasias	337 hospital controls	Case, 66.5 Control, 13.9	12.8 (8.2–20.0)	ViraPap™	
Brisson *et al.* (1994) Canada	548 H CIN 338 L CIN	612 hospital controls	H CIN, 42.5 L CIN, 12.0 Control, 6.02	8.7 (5.1–15.0) 1.6 (0.9–3.0)	Southern blot for HPV-16	
Davidson *et al.* (1994) Alaska, USA	74 atypias 68 LSIL 96 HSIL	723 normal cervices	Normal, 14.1 Atypia, 29.7 LSIL, 64.7 HSIL, 89.6	1.0 2.7 (0.8–9.5) 10.4 (6.1–17.8) 14.4 (9.6–21.8)	ViraPap™, ViraType™	Alaska native women. 3 groups: routine care clinics (n = 492), referral colposcopy clinic (n = 385) and population-based (n = 249). Odds ratio combined for HPV-16/18 and -31/33/35
Marin *et al.* (1994) Slovenia	109 abnormal smears 22 LSIL 7 CIN I 14 CIN II–III	42 normal smears	*HPV-16 / HPV-18* Normal, 11.9/4.8 LSIL, 13.6/13.6 CIN I, 14.3/0.0 CIN II–III, 21.4/14.3	*HPV-16* [1.2 (0.2–6.5)] [1.2 (0.0–15.0)] [2.0 (0.3–12.2)] *Any CIN/HPV-16/18* [1.9 (0.6–6.3)]	In-situ hybridization	Age: 17–51

[ ] Calculated by the Working Group

CIN, cervical intraepithelial neoplasia; CIS, carcinoma *in situ*; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; H, high grade; Pap, Papanicolaou smear

McCance *et al.* (1985) investigated 78 cases of CIN I–III with FISH using probes for HPV-6 and -16. The CIN cases were compared to 17 controls. HPV-16 positivity was found in 18% of the controls and 55%, 66% and 71% of CIN I–III, respectively [ORs, 5.7 (95% CI, 1.1–35.6); 9.3 (95% CI, 1.8–53.6); 11.7 (95% CI, 2.2–70.5)].

Demeter *et al.* (1987) conducted a FISH-based study of 132 cases of CIN I–III using probes for HPV-6/11 and -16/18. The CIN cases were compared to 23 controls. HPV-16/18 positivity was 21.8% among controls and 51.7%, 51.4% and 53.2% for CIN I–III respectively [ORs, 3.9 (95% CI, 1.0–16.0); 3.8 (95% CI, 1.0–15.0); 4.1 (95% CI, 1.2–14.5)].

Pao *et al.* (1989), using in-situ hybridization, investigated 47 cases of dysplasia and 39 controls. Prevalence rates of HPV DNA positivity were 15.4% among controls and 72.3% among cases [estimated OR, 14.4 (95% CI, 4.4–49.7)].

In Venezuela, Azocar *et al.* (1990) investigated 119 non-monogamous women using ViraPap™ and cytological criteria (normal/abnormal). HPV DNA positivity rates among cases with abnormal cytology were 88% (16/18) and 26% (26/101) among women with normal cytology [OR, 23 (95% CI, 4.6–157)].

In Canada, Duggan *et al.* (1990) used the dot blot system to investigate a series of 401 patients attending a colposcopy clinic in which a cytological/histological diagnosis was available. Of the 300 subjects classified as CIN/condyloma, 60% were HPV DNA positive. In the group with normal cytology, 26.6% had evidence of HPV DNA [OR, 4.1 (95% CI, 2.4–7.2)]. The HPV types identified were largely high-risk types (HPV-16/18/33) or HPV-6/11. HPV-16 was the predominant virus detected [OR for high-risk types, 18.8 (95% CI, 7.6–49.4)]. There was a strong trend of increasing prevalence of high-risk types in relation to the severity of the CIN lesion ($p < 0.001$).

Kataoka and Yakushiji (1990) used Southern blot hybridization to investigate 37 cases of dysplasia and 71 controls. HPV prevalence rates were 5.6% among controls and 32.4% among cases [estimated OR, 8.0 (95% CI, 2.1–33.0)].

In the USA, Becker *et al.* (1991) studied 1603 randomly selected Hispanic, native American and non-Hispanic white women to determine the prevalence of cervical HPV infection according to Pap smear results. These results included atypia (52 cases), slight dysplasia (77 cases) and moderate to severe dysplasia (27 cases). Women with normal Pap smears (1447 cases) served as controls. The method used to detect HPV was dot-blot hybridization (ViraPap™). The HPV DNA prevalence in the group with negative Pap smears was 5.6%, 21.2% for atypia, 45.5% for slight dysplasia and 66.7% for moderate–severe dysplasia [estimated crude ORs, 4.5 (95% CI, 2.1–9.5) for atypia; 14.0 (95% CI, 8.3–23.9) for slight dysplasia; and 33.7 (95% CI, 13.8–84.1) for moderate–severe dysplasia; 11.7 (95% CI, 7.8–17.6) for any Pap abnormality].

In northern Italy, Garuti *et al.* (1991) investigated HPV DNA prevalence in 276 biopsies using Southern blot hybridization. They reported an increased trend in the prevalence of HPV-16 from specimens with normal cytology to invasive carcinoma. The opposite trend was observed for HPV-6 and -11.

In Sweden, Lindh *et al.* (1992) compared the HPV DNA prevalence using Southern blot in 416 women with no clinical signs of HPV-related disease with that of 75 women with CIN (52

with CIN I and 23 with CIN II–III). A mixture of subgenomic probes were targeted at HPV-6, -11, -16, -18, -31, -33 and -35. HPV prevalences were 14% for asymptomatic women, 50% for women with CIN I lesions, and 39% for women with CIN II–III lesions [estimated crude ORs for any HPV: 6.3 (95% CI, 3.3–12.1) for CIN I; 4.8 (95% CI, 1.9–12.5) for CIN II–III; and 5.5 (95% CI, 3.1–9.7) for all CIN]. The type-specific prevalences for cases (all CIN) and controls were, respectively: 8% versus 3% for HPV-6, 4% versus 1% for HPV-11, 8% versus 4% for HPV-16, 15% versus 2% for HPV-18, 12% versus 4% for HPV-31, 8% versus 3% for HPV-33, 1% versus 1% for HPV-35, and 1% versus 0.5% for undetermined HPV [ORs for CIN: 3.4 (95% CI, 1.1–9.8) for HPV-16, and 12.3 (95% CI, 4.3–36.1) for HPV-18].

In the USA, Lörincz *et al.* (1992), in a pooled analysis of eight studies of the relationship between HPV infection and cervical neoplasia, compared 1061 cases of cervical disease (atypia (270), LSIL (377), HSIL (261) and carcinoma (153)) with 1566 women with a normal cervix. The method used to detect HPV was low-stringency and high-stringency Southern blot hybridization with specific probes for HPV-6/11, -16, -18, -31, -33, -35, -42, -43, -44, -45, -51, -52, -56 and -58. The overall HPV DNA prevalence among cases was 23.7% for borderline atypia and 79.3% for cervical disease. The HPV DNA prevalence for controls was 6.4%. Risk estimates (not adjusted for other risk factors) were 27.1 for any cervical disease and [55.4] for definite cervical disease. Type specific HPV prevalences are shown in Table 32.

Table 32. Distribution of HPV types by diagnosis

HPV type	Normal (%) (n = 1566)	Atypia (%) (n = 270)	LSIL (%) (n = 377)	HSIL (%) (n = 261)
Negative	93.6	76.3	30.5	12.6
6/11	0.5	2.2	16.7	3.1
16	1.0	4.4	16.2	47.1
18	0.3	1.9	4.0	5.0
31/33/35	0.8	3.0	11.1	19.2
42/43/44/45	0.5	2.6	4.5	1.5
51/52/56/58	1.2	2.2	7.7	5.7
Unclassified	2.1	7.4	9.3	5.7

From Lörincz *et al.* (1992)

The crude odds ratios (95% CI) for type-specific HPVs are shown in Table 33.

In Austria, Manavi *et al.* (1992) compared the HPV DNA prevalence using in-situ hybridization in 411 patients with cytological dysplasia with that of 240 cytologically normal women. HPV prevalences according to the Papanicolaou classification were: 2.5% for Pap grade I, 41.7% for Pap grade II, 57.6% for Pap grade III, 77.8% for Pap grade IV and 92.3% for Pap grade V. The corresponding crude ORs as compared to women with a Pap grade I were: [27.9 (95% CI, 11.2–73.5)] for Pap grade II, [52.9 (95% CI, 20.3–146.4)] for Pap grade III, [136.5 (95% CI, 44.3–447.7)] for Pap grade IV and [468.0 (130.9–1852.7)] for Pap grade V.

Table 33. Crude odds ratios for HPV types in relation to atypia, LSIL and HSIL

HPV type	Atypia	LSIL	HSIL
6/11, 42, 43, 44	6.1 (3.1–12.1)	52.6 (36.0–76.9)	24.1 (13.4–43.4)
31,33,35,51,52	2.6 (1.2–5.2)	21.6 (17.9–26.1)	71.9 (51.0–101.6)
16	5.0 (2.5–9.9)	36.9 (25.0–54.5)	235.7 (198.5–279.5)
18,45,56	6.6 (2.8–15.7)	32.7 (19.2–55.8)	65.1 (50.2–84.5)

From Lörincz *et al.* (1992)

In New Zealand, Meekin *et al.* (1992) compared the HPV DNA prevalence using dot blot in 1347 cytologically normal women with that of 666 women with dysplasia (n = 87), atypia (n = 84) or a cytology showing the presence of infection or benign atypia (n = 495). HPV prevalences were as follows: 8.3% for control women, 48.3% for dysplasia, 20.3% for atypia and 10.1% for infection or benign atypia. The OR associated with any HPV was 5.8 (95% CI, 4.0–8.6) for atypia or dysplasia. The corresponding OR for HPV-16/18 was 6.4 (95% CI, 3.3–5.9).

In Japan, Tanaka *et al.* (1992) compared the HPV DNA prevalence using Southern blot in 100 women with normal cytology with that in 145 women with an abnormal cytology. Probes for HPV-6, -11, -16, -18, -31, -33 and -35 were used. The HPV DNA prevalence in the group with normal cytology was 2%. The overall HPV prevalence in women with abnormal cytology was 30% — 2% for benign lesions, 100% for condylomas, 39% for mild–moderate dysplasia, 44% for severe dysplasia/CIS and 70% for invasive cancer. [Estimated crude ORs were 31.8 (95% CI, 6.8–204.1) for mild–moderate dysplasia, 38.1 (95% CI, 5.8–318.5) for severe dysplasia/CIS, 114.3 (95% CI, 12.9–1463.2) for invasive carcinoma and 20.7 (95% CI, 4.7–126.8) for any abnormality.]

In the USA, Goff *et al.* (1993) examined 360 biopsies of attendees of a colposcopy clinic with ViraPap™. Of 71 CIN I–III cases, 35 (49.3%) were HPV positive as were 31 of 225 controls (13.8%) [OR, 6.1 (95% CI, 3.2–11.6)].

Levine *et al.* (1993) conducted a population-based case–control study of US college students aged 18–35 years to estimate the effect of HPV infection on SIL of the uterine cervix. Cases included 34 cytological SIL and 25 equivocal atypia as well as 19 histologically confirmed HSIL. The control group consisted of 147 subjects with a normal cervical cytology. The method used to detect HPV was ViraPap™. The HPV prevalence among cases was 58% among cytological SIL, 40% among cases of equivocal atypia and 68% among histological HSIL. The HPV prevalence among cytological controls was 16% and among histological controls 19%. Risk estimates were adjusted for age, multiple lifetime sexual partners and oral contraceptive use. The adjusted odds ratios for HPV DNA presence were as follows: for cytological SIL, 7.3 (95% CI, 3.3–17.0); for equivocal atypia, 3.4 (95% CI, 1.4–8.5); and for histological HSIL, 10.3 (95% CI, 3.3–32.0). The distribution of HPV types among cases and controls are shown in Table 34.

Table 34. Distribution of HPV types by diagnosis

HPV type	Cytological diagnosis			Histological diagnosis	
	Control (%)	Equivocal (%)	SIL (%)	Control (%)	HSIL (%)
Negative	85.4	60.0	42.4	82.4	33.3
6/11	0.0	16.0	6.1	0.7	5.6
16/18	1.4	8.0	18.2	2.7	33.3
31/33/35	4.2	0.0	12.1	4.7	5.6
6/11 + 31/33/35	0.0	0.0	3.0	0.0	5.6
16/18 + 31/33/35	0.0	8.0	0.0	0.0	0.0
Indeterminate	9.0	8.0	18.2	9.4	16.7

From Levine *et al.* (1993)

The type-specific crude odds ratios (95% CI) for a larger sample selected subsequently from the same population are shown in Table 35.

Table 35. Odds ratios for HPV-types in relation to LSIL and HSIL

HPV type	Cytological LSIL	Cytological HSIL
6/11	6.9 (1.6–29.0)	–
16/18	12.9 (4.7–35.0)	12.4 (3.3–46.0)
31/33/35	5.2 (1.7–16.0)	10.8 (3.0–39.0)

From Levine *et al.* (1993)

In the USA, Meyer *et al.* (1993) estimated the HPV DNA prevalence in 61 patients with LSIL, 16 patients with HSIL and 72 cytologically normal women. The method used to detect HPV was ViraPap™ combined with ViraType™ in-situ hybridization. HPV prevalences were: 6.9% for cytologically normal women, 29.5% for LSIL and 68.7% for HSIL. Crude ORs were [5.6 (95% CI, 1.8–18.8)] for LSIL and [29.5 (95% CI, 6.2–158.1)] for HSIL.

In a young population in the USA, Moscicki *et al.* (1993) compared HPV-positive and HPV-negative women who were also studied with colposcopy. Detailed criteria to evaluate colposcopy findings were used. Women who were HPV-16/18-positive by ViraPap™ had a higher mean number of lesions (1.7 versus 0.7; $p < 0.001$) and higher lesional scores (3.4 versus 1.0; $p < 0.001$) than women who were HPV-negative or HPV-positive for HPV-6, -11, -31, -33 or -35.

Becker *et al.* (1994) conducted a case–control study in New Mexico, USA, including 201 cases of high-grade dysplasia and 337 hospital controls. Scraped specimens from the cervix were investigated using ViraPap™ and ViraType™ and PCR. The corresponding HPV DNA prevalences for ViraPap™ were as follows: cases 66.5%, controls 13.9% (OR, 12.8 (95% CI, 8.2–20.0)).

In Canada, Brisson *et al.* (1994) investigated the presence of HPV DNA using Southern blot in 548 cases of high-grade CIN, 338 cases of low-grade CIN and 612 hospital controls. The corresponding HPV DNA prevalences were 42.5%, 12.0% and 6.0%, respectively, and the ORs adjusted for age, number of sexual partners, age at first intercourse, smoking, oral contraceptive use, DNA source and DNA load were 1.6 (95% CI, 0.9–3.0) for low-grade CIN and 8.7 (95% CI, 5.1–15.0) for high-grade CIN. A dose-response was observed with increasing load of HPV-16 DNA. The association with low-grade CIN was not statistically significant.

In the USA, Davidson *et al.* (1994) estimated HPV prevalences in 961 Alaska native women, of which 723 had a normal cytology, 74 had atypia, 68 LSIL and 96 HSIL. ViraPap™ and ViraType™ were used to detect HPV DNA. The HPV prevalences were 14.1% for normal women, 29.7% for atypia, 64.7% for LSIL and 89.6% for HSIL. As compared to normalcy, the crude ORs were: 2.7 (95% CI, 0.8–9.5) for atypia, 10.4 (95% CI, 6.1–17.8) for LSIL and 14.4 (95% CI, 9.6–21.8) for HSIL. The corresponding odds ratios for HPV types 16/18 were 1.5 (95% CI, 0.7–3.4) for atypia, 2.6 (95% CI, 1.2–5.6) for LSIL and 7.1 (95% CI, 4.5–11.3) for HSIL. Odds ratios for HPV-31/33/35 were: 2.4 (95% CI, 1.3–4.5) for atypia, 3.9 (95% CI, 2.1–7.4) for LSIL and 6.3 (95% CI, 3.7–10.5) for HSIL.

In Slovenia, Marin *et al.* (1994), using in-situ hybridization, investigated 109 cases of abnormal smears, 22 with LSIL, 7 with CIN I and 14 with CIN II and 42 controls. Among controls, the prevalence rate of HPV-16 was 11.9% and of HPV-18 4.8%. Among cases the prevalence was 32.1% for HPV-16 and 10.4% for HPV-18. The estimated OR for any CIN and HPV-16/18 combined was [1.9 (95% CI, 0.6–6.3)].

Table 36 summarizes the results of case–control studies that investigated preinvasive lesions (CIN I–III) using hybridization assays with amplification techniques. There are two PCR techniques that were used by most of the studies. One uses consensus primers based on approximately 450 bp of the *L1* region of HPV-16/18, and utilizes type-specific and generic probes. The other amplifies a smaller region of *L1*, detects a broader range of types, and is often followed by a separate type-specific PCR (see section 1.3.3). The two tests have not been formally validated against each other. During the period in which they have been used, the number of HPV-specific probes has increased and the strategies to collect, store and analyse specimens have improved considerably. Therefore, any variation in the prevalences observed may be an artefact, partially due to differences in the methodology employed.

In Spain, Bosch *et al.* (1993) compared 157 cases of severe dysplasia, carcinoma *in situ* or CIN III with 193 controls having normal cytology, nonspecific inflammatory changes or Pap grades I and II. The method used to detect HPV was PCR based on consensus primers of the *L1* region with probes for HPV-6, -11, -16, -18, -31, -33 and -35. The HPV DNA prevalence was 70.7% among cases and 4.7% among controls. Risk estimates were adjusted for age, geographical area, number of sexual partners, age at first sexual intercourse, *Chlamydia trachomatis* and husband's sexual partners. The adjusted OR for HPV DNA presence was 56.9 (95% CI, 24.8–130.6; attributable fraction, 72.4%). The distribution of HPV types in cases was HPV-16 (69.4%), HPV-18 (0.9%), HPV-31 (1.8%), HPV-33 (8.1%), HPV-35 (0.9%) and HPV unknown (18.9%). The distribution of HPV types in controls was as follows: HPV-16 (11.1%), HPV-18 (0.0%), HPV-31 (11.1%), HPV-33 (11.1%), HPV-35 (0.0%) and HPV unknown (66.7%). The adjusted

Table 36. Case–control studies of CIN I–III lesions using HPV hybridization assays, including amplification (PCR) methods

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)			Odds ratios (95% CI)	HPV test	Comments/adjustments
			Type	Cases	Controls			
Bosch *et al.* (1993) Spain	157 severe dysplasias, carcinomas *in situ*, CIN III	193 normal cytology, nonspecific inflammatory changes or Pap I and II	HPV	70.7	4.7	56.9 (24.8–130.6)	PCR 6, 11, 16, 18, 31, 33, 35	Attributable fraction, 72.4%. Age, geographical area, number of sexual partners, age at first intercourse, *Chlamydia trachomatis*, husband's sexual partners
			16	69.4	11.1	295.5 (44.8–1946.4)		
			18	0.9	0			
			31	1.8	11.1	28.9 (5.5–152.8)		
			33	8.1	11.1			
			35	0.9	0			
Colombia	125 severe dysplasias, carcinomas *in situ*, CIN III	181 normal cytology, nonspecific inflammatory changes or Pap I and II	HPV	63.2	10.5	15.5 (8.2–29.4)	PCR 6, 11, 16, 18, 31, 33, 35	Attributable fraction, 60.3%
			16	51.9	31.6	27.1 (10.6–69.5)		
			18	0	0			
			31	3.8	0	23.4 (2.8–190.6)		
			33	3.8	5.3			
			35	2.5	0			
Coker *et al.* (1993) South Carolina	114 CIN I 28 CIN II/III 115 atypias 140 inflammatory	223 normal cytology	*HPV-16/18/33*				PCR 6b, 11, 16, 18, 33	Age, race, smoking, sexual behaviour, use of oral contraceptives. 60% were black and of low socioeconomic level.
			CIN II/III	35.7		21.9 (6.4–74.5)		
			CIN I	24.5		11.7 (4.3–32)		
			Atypia	6.1		3.0 (0.9–9.8)		
			Inflammatory	10.7		2.6 (0.8–8.4)		
			Normal	2.7		1		
Schiffman *et al.* (1993) Portland, OR, USA	319 condylomatous atypias 131 CIN I 50 CIN II/III	453 randomly selected from 17 654 women with normal cytology and no known history of CIN	*CIN I*				PCR 6/11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 57, 59	Age, HPV test results and lifetime numbers of sex partners
			HPV-16/18	36.0	2.9	200 (68.0–570.0)		
			31, 33, 35, 39, 45, 51, 52	34.4	3.3	130 (47.0–370.0)		
			6/11, 42, other or unknown	21.6	11.5	24 (9.3–60.0)		
			CIN II–III					
			HPV-16/18	62	2.9	180 (49.0–630.0)		
			31, 33, 35, 39, 45, 51, 52	10	3.3	22 (4.8–97)		
			6/11, 42 other or unknown	18	11.5	10 (3–36)		

Table 36 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)			Odds ratios (95% CI)	HPV test	Comments/adjustments
			Type	Cases	Controls			
Van der Brule *et al.* (1991a) Amsterdam, Netherlands	124 Pap IIIa 31 Pap IIIb 22 Pap IV	(a) 1346 symptom-free population (b) 239 gynaecological outpatient population without history of cervical pathology, (c) 177 gynaecological outpatient population having history of cervical pathology	*HPV overall* Pap IIIa Pap IIIb Pap IV (a) (b) (c) *HPV-16/18* Pap IIIa Pap IIIb Pap IV (a) (b) (c)	 70 84 100 41 58 70 	 3.5 9.2 21.5 0.9 2.5 12	*HPV overall* [68.1 (45.4–102.4)] *HPV-16/18* [135.8 (82.5–225.9)]	PCR 6/11, 16, 18, 31, 33	No adjustments. Cases: Pap IIIa + Pap IIIb + Pap IV Controls: (a) + (b) + (c)
Morrison *et al.* (1991) Bronx, NY, USA	Hospital 65 non-pregnant with histopathological documentation of SIL	Family-planning clinic 59 women with normal cytology	HPV One HPV type > one HPV type	84.6 53.8 30.8	39.0 35.6 3.4	10.4 (3.6–30.4) 7.2 (2.4–21.9) 43 (6.9–266.6)	PCR 16, 18, 33	Age, ethnicity, education, number of cigarettes daily, current use of oral contraceptives, age at first coitus, HPV infection determined by PCR. SIL: including atypia, CIN I, CIN II and CIN III
Pasetto *et al.* (1992) Urbino, Italy	Hospital 23 CIN II–III cytology specimens	Hospital 148 normal cytology specimens	HPV-16	32.4	8.9	[12.8 (4.4–38.8)]	PCR 16	
Nakazawa *et al.* (1992) Osaka, Japan	Hospital 37 CIN III	Hospital 69 normal	HPV-16 HPV-18 HPV-16 and -18 HPV	24.3 2.7 0 27	8.7 5.8 1.4 16	[2.8 (0.8–9.4)] [0.4 (0.02–4.1)] [1.95 (0.67–5.73)]	PCR 16, 18	No odds ratios given in the paper. No adjustments
Bavin *et al.* (1993) London, United Kingdom	Hospital 35 CIN III	Hospital 54 normal	HPV-16	74.3	63	[1.7 (0.61–4.84)]	PCR 16	Use of semiquantitative PCR for HPV-16

Table 36 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)			Odds ratios (95% CI)	HPV test	Comments/adjustments
			Type	Cases	Controls			
Margall *et al.* (1993) Barcelona, Spain	Hospital 66 biopsies/cervical scrapes of patients with cervical lesions:	Hospital: 64 women with normal Papanicolaou test	*16 and/or 18*	Biopsy (cases)	Scrape (cases)	HPV-16 and/or -18 Odds ratios for CIN using cervical scrapes [1.7 (0.5–5.8)]	PCR 16, 18	The prevalence of HPV-16 in controls was 11%.
	14 chronic inflammations (ChI)		ChI	43	0			
	5 cervical condylomas (CC)		CC	80	20			
	24 CIN I							
	12 CIN II		CIN I	50	17			
	10 CIN III		CIN II	50	17			
	1 invasive carcinoma (IC)		CIN III	70	20			
			IC	0	0			
Becker *et al.* (1994) New Mexico	201 H dysplasias	337 hospital controls		93.8	42.1	20.8 (10.8–40.2)	PCR *L1* consensus primers	
Cuzick *et al.* (1995) London, United Kingdom	81 CIN II–III	1904 negative cytology or CIN I	HPV	75	4.5	[65 (33–113)]	Type-specific PCR (16, 18, 31, 33)	Cases and controls from women undergoing routine screening
			16	44	1.6	[50 (28–88)]		
			18	6	0.8	[13 (5.4–31)]		
			31	25	1.4	[24 (13–45)]		
			33	9	0.8	[12 (4.7–30)]		
Liaw *et al.* (1995) Taiwan	40 CIN I 48 CIN II–III, invasive cervical cancers	261 normal cytology		92	9	CIN I, 14.0 (6.1–32) CIN II–III, invasive, 122.3 (38.5–388.9)	PCR	HPV-16 predominant in CIN II–III and invasive cancers; HPV-52 and -58 predominant in CIN I and controls
Strickler *et al.* (1995) Jamaica	49 CIN III, cervical cancers	40 women with normal cervix or CIN I		92.1	25.7	[2.7 (1.1–7.0)] p for trend with HPV grade < 0.001	PCR *L1* consensus primers	HTLV-1 was also identified as a risk factor for CIN III/-cervical cancer.

[ ] Calculated by the Working Group

Pap, Papanicolaou smear test result; CIN, cervical intraepithelial neoplasia; H, high-grade; PCR, polymerase chain reaction; HTLV-1, human T-lymphotropic virus; SIL, squamous intraepithelial lesion

ORs for specific HPV types were: for HPV-16, 295.5 (95% CI, 44.8–1946.4); for HPV-31, -33 and -35, 28.9 (95% CI, 5.5–152.8); and HPV unknown, 18.7 (95% CI, 6.6– 54.8).

In Colombia, Bosch *et al.* (1993) compared 125 cases of severe dysplasia, carcinoma *in situ* or CIN III with 181 controls having normal cytology, nonspecific inflammatory changes or Pap grades I and II. The method used to detect HPV was PCR with probes for HPV-6, -11, -16, -18, -31, -33 and -35. The HPV DNA prevalence was 63.2% among cases and 10.5% among controls. Risk estimates were adjusted for age, geographical area, number of sexual partners, age at first sexual intercourse, *C. trachomatis* and smoking. The adjusted OR for HPV DNA presence was 15.5 (95% CI, 8.2–29.4; attributable fraction, 60.3%). The distribution of HPV types in cases was HPV-16 (51.9%), HPV-18 (0.0%), HPV-31 (3.8%), HPV-33 (3.8%), HPV-35 (2.5%) and HPV of unknown type (38%). The distribution of HPV types in controls was HPV-16 (31.6%), HPV-18 (0.0%), HPV-31 (0.0%), HPV-33 (5.3%), HPV-35 (0.0%) and HPV of unknown type (47.4%). The adjusted ORs for HPV type specific were as follows: HPV-16, 27.1 (95% CI, 10.6–69.5); HPV-31, -33 or -35, 23.4 (95% CI, 2.8–190.6); HPV of unknown type, 12 (95% CI, 5.1–28.6).

In the same study (Bosch *et al.*, 1993), 852 specimens were tested using the commercial dot blot system, ViraPap™. The results were as follows: in Spain the study included 207 CIN III cases and 209 controls, and HPV DNA prevalences were 33.8% and 3.8% and the OR was 13.4 (95% CI, 5.9–30.6); in Colombia, the subjects included were 187 cases and 249 controls and HPV DNA prevalences were 28.9% and 10.0% and the OR was 8.7 (95% CI, 4.9–15.3). These risk estimates were adjusted for all variables that showed an association with cervical cancer in the study.

The comparison of results when testing the same specimens with ViraPap™, Southern hybridization and PCR showed concordant results for the presence or absence of HPV DNA in 65% of the cases. PCR and Southern blot hybridization were concordant in the type specific result in 86% of the specimens (see also section 1.3; Guerrero *et al.* 1992).

In South Carolina, USA, Coker *et al.* (1993) compared 114 cases of CIN I, 28 cases of CIN II–III, 115 cases of atypia and 140 cases of infection or inflammatory changes with 223 controls selected from normal cytology. The method used to detect HPV DNA was PCR and specific probes were used for HPV-6b, -11, -16, -18 and -33. The HPV DNA (HPV-16, -18 or -33) prevalence was 35.7% among cases with CIN II or III and 2.7% among controls. Risk estimates were adjusted for age, race, smoking, number of sexual partners and current use of oral contraceptives. The adjusted OR for HPV DNA (type HPV-16, -18 or -33) was 21.9 (95% CI, 6.4–74.5) for CIN II/III.

In Portland, Oregon, USA, Schiffman *et al.* (1993) compared 50 cases of CIN II–III with 453 controls selected randomly from 17 654 women with normal cytology and no known history of CIN (not matched). Cervico-vaginal lavages were used to collect cytological specimens. The method used to detect HPV was PCR and specific probes were used for HPV-6/11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -45, -51, -52, -53, -54, -55, -57 and -59. Risk estimates were adjusted for age, HPV test results, and lifetime numbers of sexual partners. The distribution of HPV types in cases of CIN II-III was as follows: HPV-16 or -18: 62%; HPV-6, -11, -42, other or unknown: 18%; and HPV-31, -33, -35, -39, -45, -51 or -52: 10%. The distribution of HPV types in controls was: HPV-16 or -18: 2.9%; HPV-6, -11, -42, other or unknown: 11.5%; and HPV-31, -33, -35, -39, -45, -51 or -52: 3.3%. The adjusted ORs for type-specific HPV in CIN II–III were: HPV-16 or -18, 180 (95% CI, 49-630); HPV-6, -11, -42, other or unknown, 10 (95% CI, 3–36);

HPV-31, -33, -35, -39, -45, -51 or -52, 22 (95% CI, 4.8–97). The study also included 319 women with condylomatous atypia, randomly chosen among the 492 women diagnosed in the screening programme. Taken together, the 492 cases with lesions classified as condylomatous atypia or above and the 453 controls, the odds ratios were: HPV-6, -11, -42, other or unknown, 8.7 (95% CI, 5.8–13.0); HPV-31, -33, -35, -39, -45, -51 or -52, 33.0 (95% CI, 18.0–59.0); HPV-16 or -18, 51.0 (95% CI, 28.0–94.0).

Further analyses of this study were reported following correspondence by Luthi and Burk (1993). This association of CIN I and CIN II–III was not modified by age.

In Amsterdam (Netherlands), van den Brule *et al.* (1991a) compared 124 cases of Pap IIIa (mild and moderate dysplasia), 31 cases of Pap IIIb (severe dysplasia) and 22 cases of Pap IV (carcinoma *in situ*) with 1346 controls selected from a symptom-free population of women, 239 female gynaecological outpatients without a history of cervical pathology and 177 gynaecological outpatients having a history of cervical pathology. The method used to detect HPV was PCR testing for HPV-6/11, -16, -18, -31 and -33. The HPV DNA prevalence among cases was 70% in Pap IIIa, 84% in Pap IIIb, 100% in Pap IV, 3.5% among symptom-free controls, 9.2% in women without a history of cervical pathology and 21.5% in women having a history of cervical pathology. The distribution of HPV-16 and -18 in cases was: Pap IIIa, 41%; Pap IIIb, 58%; and Pap IV, 70%. The distribution of HPV types 16 and 18 in controls was 0.9% in the symptom-free population, 2.5% in the gynaecological outpatient population without a history of cervical pathology and 12% in the gynaecological outpatients having a history of cervical pathology.

In New York (USA), Morrison *et al.* (1991) compared 65 nonpregnant woman who had histopathological documentation of a cervical SIL (including atypia, CIN I, II, III), with 59 controls selected from family-planning or other gynaecology clinics in order to obtain cytologically normal specimens. The method used to detect HPV was PCR for HPV-16, -18 and -33. The HPV DNA prevalence was 84.6% among cases and 39% among controls. Risk estimates were adjusted for age, ethnicity, education, number of cigarettes smoked daily, current use of oral contraceptives, age at first coitus and HPV infection determined by PCR. The adjusted ORs for HPV DNA presence was 10.4 (95% CI, 3.6–30.4). One HPV-type was found in 53.8% of cases, and more than one type was found in 30.8%. One HPV type was found in 35.6% of controls, and more than one type in only 3.4%. The adjusted ORs for HPV type specific were: one HPV type, 7.2 (95% CI, 2.4–21.9); more than one HPV type, 43 (95% CI, 6.9–266.6). In this study there was an attempt to quantify the intensity of the HPV viral load by visual inspection of the signal and the size of the band. The dose–response relationship was then assessed by stratifying the results of the Southern blot and the PCR assays into three categories (negative, weak, strong). The ORs were as follows: Southern blot — weak signal, 15.7 (95% CI, 4.4–56.3); strong signal, 21.1 (95% CI, 4.9–91.0; p for trend < 0.01); PCR — weak signal, 8.0 (95% CI, 2.3–27.5); strong signal, 12.7 (95% CI, 3.8–42.4; p for trend < 0.01).

In Urbino (Italy), Pasetto *et al.* (1992) compared 23 cases of CIN II and CIN III with 148 controls selected from women with a normal colposcopic examination, i.e. the absence of vaginal or vulvar lesions. The method used to detect HPV was PCR, HPV-16. The HPV DNA prevalence was 32.4% among cases and 8.9% among controls. [The crude OR for HPV-16 DNA was 12.8 (95% CI, 4.4–38.8).]

In Japan, Nakazawa *et al.* (1992) compared 37 cases of CIN III with 69 controls from the outpatient clinic of the department of obstetrics and gynaecology of Osaka University Medical School. The method used to detect HPV was PCR, for types 16 and 18. The HPV DNA prevalence was 27% among cases and 16% among controls. [The crude OR for HPV DNA presence was 1.95 (95% CI, 0.67–5.73)]. The distribution of HPV types in cases was: HPV-16, 24.3%; HPV-18, 2.7%; HPV-16 and -18, 0.0%. The distribution of HPV types in controls was: HPV-16, 8.7%; HPV-18, 5.8%; HPV-16 and -18, 1.4%. [Crude odds ratios for HPV-16, 2.8 (95% CI, 0.8–9.4); for HPV-18, 0.4 (95% CI, 0.02–4.1).]

In London, Bavin *et al.* (1993) studied 179 women sequentially referred to the Royal Free Hospital Colposcopy Clinic because of a smear suggesting mild dyskaryosis. The method used to detect HPV was PCR and the probe used was type 16. All women were explored with colposcopy and, when required, with biopsy, and a final diagnosis was reached. Of 179 women investigated, 35 were considered CIN III and 54 were considered normal. The HPV-16 DNA prevalence was 74.3% among cases and 63% among controls. [The crude OR for HPV-16 DNA presence was 1.7 (95% CI, 0.61–4.84)].

In Barcelona (Spain), Margall *et al.* (1993) compared 66 women who had had either abnormal Papanicolaou smears or abnormal biopsies (14 chronic inflammatory lesions, 5 cervical condylomata, 24 CIN I , 12 CIN II, 10 CIN III and 1 invasive carcinoma) with 64 controls with normal Papanicolaou tests. The method used to detect HPV was PCR, testing HPV-16 and -18 in the biopsies and cervical scrapes. The HPV-16 and/or -18 DNA prevalence in biopsies among cases was: chronic inflammatory lesions, 43%; cervical condyloma, 80%; CIN I, 50%; CIN II, 50%; CIN III, 70%; and invasive carcinoma, 0%; that in cervical scrapes was: chronic inflammatory lesions, 0%; cervical condyloma, 20%; CIN I, 16.6%; CIN II, 16.6%; CIN III, 20%; and invasive carcinoma, 0%. The HPV-16 prevalence in cervical scrapes among controls was 11%. The crude OR for HPV DNA presence in cervical scrapes for CIN I–III was [1.7 (95% CI, 0.5–5.8)]. This study showed that among cases, the observed HPV DNA prevalence varies according to the sampling method. PCR performed on biopsies yielded systematically higher positivity rates than PCR performed on cytological specimens (see Table 36). Comparing the HPV DNA prevalence rates in cytological specimens from cases and controls, there is no significant association between HPV and CIN. [Using the HPV results obtained using the biopsies in cases and the cytology among controls, the estimated OR for CIN I–III was [9.7 (95% CI, 3.3–29.2)].]

Becker *et al.* (1994), in a study described on p. 153, analysed 201 high-grade dysplasia patients and 337 controls. Using PCR and common primers, HPV prevalences were 93.8% in cases and 42.1% in controls (OR, 20.8 (95% CI, 10.8–40.2)).

In the United Kingdom, Cuzick *et al.* (1995) compared the use of cytology and HPV testing for the detection of cervical abnormalities in 1985 women undergoing routine screening. Semiquantitative PCR was used to detect HPV-16, -18, -31 and -33 and only 'high-level' infections were called positive. Women who were either HPV-positive or who had some degree of dyskaryosis on cytology were referred for colposcopy. Of 81 women found to have CIN II–III on colposcopy, 61 (75%) were HPV-positive in comparison with 85 of 1904 (4.5%) women with negative cytology or biopsies showing CIN I or less (control group) [OR, 65 (95% CI, 38–113)]. HPV-16, -18, -31 and -33 were found in 44%, 6%, 25% and 9%, respectively, of women with CIN

II–III and in 1.6%, 0.8%, 1.4% and 0.8%, respectively, of control women. Some women were infected with more than one HPV type. In comparison with HPV-negative women, the OR associated with infection with different HPV types were [50 (95% CI, 28–88)] for HPV-16, [13 (95% CI, 5.4–31)] for HPV-18, [24 (95% CI, 13–45)] for HPV-31 and [12 (95% CI, 4.7–30)] for HPV-33.

In Taiwan, Liaw *et al.* (1995) conducted a study including 88 patients with biopsy-confirmed CIN I–III and invasive cervical cancer (three cases). PCR was used to detect HPV DNA. The prevalence of HPV DNA was 92% among high-grade cases (CIN II–III, invasive cervical cancer) and 9% among controls (OR, 122.3 (95% CI, 38.5–388.9)). The viral types identified differed between high-grade cases (HPV-16 was predominant) and low-grade CIN and controls (HPV-52 and -58 were predominant).

Strickler *et al.* (1995) investigated a series of cases in Kingston, Jamaica, an area where human T-cell lymphotropic virus type 1 (HTLV-1) is prevalent. The HPV DNA prevalence, as assessed by PCR was as follows: benign (n = 40), 25.7%; ASCUS (atypical squamous cells of unknown significance) (n = 11), 50.0%; koilocytosis atypia/CIN I (n = 60), 49.2%; CIN II (n = 29), 63.0%; and CIN III/carcinoma (n = 49), 92.1%. The trend in HPV prevalence increased significantly with severity of the lesion ($p < 0.001$). The study also identified HTLV-1 seroprevalence as an independent risk factor for cervical cancer (OR, 3.82 (95% CI, 1.03–14.2)).

In Belgium, Vandenvelde and Van Beers (1993) used a PCR system to screen 71 dysplastic or neoplastic specimens and 323 normal specimens. HPV-33 was found to be highly prevalent in this population and HPV-16 and -18 were the types more strongly related to advanced CIN.

Table 37 summarizes the results of case–control studies that investigated preinvasive lesions (CIN I–III) using serological assays. In aggregate, the studies demonstrate that even relatively minor cervical abnormalities elicit a systemic immune response. Antigen choice was observed to influence greatly the epidemiological associations, suggesting that various stages of HPV natural history may be marked by different immune responses.

Serum IgA antibodies to a carboxyl-terminal 19mer peptide of the HPV-16 E2 protein was present in 20/30 CIN patients but only in 6/27 controls without CIN (Dillner *et al.*, 1989a).

Local IgA antibodies to BPV virions were detected more frequently in cervical secretions of 18 women presenting with an abnormal smear (with or without CIN) than in 24 controls ($p < 0.005$) (Dillner *et al.*, 1989b). Baird (1983) described an increased prevalence of serum IgG antibodies to disrupted BPV virions in sera of patients with CIN and cervical cancer (included in Table 37). This result was not confirmed in a subsequent study (Dillner *et al.*, 1990b), but an increase in serum IgA antibodies to disrupted BPV virions was found by these investigators.

Cason *et al.* (1992) investigated sera of 52 patients with CIN and of 21 children by ELISA using an HPV-16 L1-specific peptide (aa 473–492). Ninety-one percent of the 23 patients with HPV-16 DNA-positive CIN compared to only 66% of 29 HPV-16 DNA-negative patients ($p < 0.05$) and only 24% of the 21 children ($p < 0.001$) had measurable IgG antibodies.

Strickler *et al.* (1994) compared 21 women with incident SIL (16 koilocytotic atypia, 3 CIN I and 2 CIN III) to 56 matched controls with regard to HPV seropositivity using a panel of synthetic peptides. Many of the cases had only very slight cytological abnormalities (koilocytotic atypia), yet an elevated percentage (86%) of case sera was seroreactive to HPV-16 for IgG and/or IgA

Table 37. Case–control studies of preinvasive CIN lesions using serological assays for HPV antigens

Reference and country	Cases (number and type)	Controls (number and type)	HPV serological prevalence (%)	Odds ratio (95 % CI)	Serological tests/comments/-adjustments
Dillner *et al.* (1989a) USA	30 CIN	27 without CIN	Case, 66.7 Control, 22.2	[6.7 (1.9–27.5)]	Serum IgA antibodies to a HPV-16 E2 peptide
Dillner *et al.* (1989b) Sweden	18 abnormal smears, with or without CIN	24 normal smears	Case, 61 Control, 25	[4.5 (1.1–21.9)]	Local IgA antibodies to BPV virions
Cason *et al.* (1992) United Kingdom	23 HPV-16 DNA positive CIN	29 HPV-16 DNA negative CIN	Case, 91 Control: Adult, 66 Child, 24	[5.4 (1.0–56.5)]	ELISA, using HPV-16 L1-special peptide
Stricker *et al.* (1994) USA	21 SIL	56 matched	Case, 86 Control, 54	HPV-16 5.76 (1.24–26.8)	
Wideroff *et al.* (1995) USA	152 pathologically confirmed squamous intraepithelial lesions: 76 low-grade 21 high-grade 55 ASCUS	688 normal cytology	Case: Low-grade, 34.2 High-grade, 52.4 ASCUS, 14.5 Control, 16.1	 [2.7 (1.5–4.6)] [5.7 (2.1–15.4)] [0.9 (0.4–2.0)]	Serum IgG antibodies to HPV-16 virus-like particles

[ ] Calculated by the Working Group

CIN, cervical intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesion

(adjusted OR, 5.76 (95% CI, 1.24–26.8)). In contrast, seroreactivity to HPV-6 was negatively associated with risk of incident SIL (OR, 0.13 (95% CI, 0.02–0.77)) for IgG.

Wideroff *et al.* (1995) used an enzyme-linked immunosorbent assay to detect serum IgG antibody response to HPV-16 virus-like particles in a nested case–control study of cervical neoplasia. One hundred-and-fifty-two cases with pathologically confirmed squamous intra-epithelial lesions and 688 controls with normal cytology were tested. Of cases with low-grade and high-grade lesions, 34.2% and 52.4% were seropositive, respectively, compared to 16.1% of the controls. However, in multivariate analyses, seropositivity was associated more with HPV-16 DNA status (especially HPV-16 DNA persistence) than with pathology grade *per se*.

(b) HPV and invasive cancer of the uterine cervix

Table 38 summarizes the findings of five studies that investigated the association of HPV infections with invasive cervical cancer using non-hybridization methods.

A study from Detroit, MI, USA, described in detail on p. 142, used morphology to assess HPV exposure and included women undergoing hysterectomy for cervical cancer (40 cases) or for other reasons and who showed a normal cervix (40 controls). Using the women who clearly presented HPV signs as exposed and comparing them to women who were negative or suspect, [the OR was 107 (95% CI, 17.6–877)] (Reid *et al.*, 1982).

One study used history of genital warts and number of episodes of genital warts as a surrogate measure of exposure to HPV. The study found an excess risk for two or more episodes of warts and a trend with increasing number of episodes (Peters *et al.*, 1986). [The Working Group noted that genital warts is likely to be a surrogate of HPV-6/11 infection.]

Su (1987) used peroxidase–antiperoxidase staining and found no association of HPV signs with invasive cancer.

In a study from Thailand, described p. 146, 417 cases of cervical carcinoma were identified of which 19 showed HPV signs (4.6%). Among 22 691 controls, the prevalence was 1.2% [OR, 3.9 (95% CI, 2.3–6.4)] (Thanapatra *et al.*, 1992).

In a study from India (Thankamani *et al.* (1992a), using indirect immunofluorescence staining, HPV DNA was found in 41% of invasive cervical carcinomas compared to none in oral cancers.

Table 39 summarizes studies that used hybridization methods without amplification procedures to assess exposure to HPV. Most are listed below.

McCance *et al.* (1985) investigated, in London, UK, the prevalence of HPV-16 among 13 cases of invasive carcinoma with DNA hybridization with HPV probes 6 and 16. These were compared to 17 controls. HPV-16 positivity was 18% among controls and 92% among cases [OR, 45.2 (95% CI, 4.3–2555.9)].

Lörincz *et al.* (1987) examined 190 biopsies with southern hybridization for HPV-6/11, -18, -31 and others. Cases of CIN I–III were HPV positive in 77% of instances, invasive cervical cancer biopsies in 89% and controls in 9% [OR for CIN I–III, 35.0 (95% CI, 6.9–239.9); OR for invasive cervical cancer, ∞ (95% CI, 5.9–∞)]. The study noted the predominance of HPV-18 in cervical adenocarcinomas and suggested that HPV-16 was more common in specimens from Brazil and Peru than in specimens from the USA.

Table 38. Case–control studies of invasive cervical cancer using HPV non-hybridization assays

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test/comments/adjustments.
Reid *et al.* (1982) Detroit, MI, USA	40 ICC All women with hysterectomy	40 age-, race- and SES-matched women with hysterectomy and no cervical pathology	*Negative* Case, 0 Control, 70.0 *Suspect* Case, 5.0 Control, 15.0 *Infected* Case, 95.0 Control, 15.0	[107 (17.6–877)]	Criteria for seven histological parameters scored in 3 levels: negative, suspect and infected
Peters *et al.* (1986) USA	200 population-based registry ICC	200 matched neighbourhood controls	*One episode* Case, 5.5 Control, 2.5 *More than one episode* Case, 4.5 Control, 1.0	2.5 (0.8–7.3) 5.0 (1.1–23.5)	Self-reported genital warts. Adjusted odds ratios (genital warts)
Su (1987) China	30 ICC 22 ICC with condyloma	20 of unknown origin	ICC, 0 ICC + condyloma, 27 Control, 0		Peroxidase. Paraffin blocks (n = 244) classified by diagnosis
Thanapatra *et al.* (1992) Thailand	417 ICC	22 691 specimens with no CIN or carcinoma	Case, 4.6 Control, 1.2	[3.9 (2.3–6.4)]	Koilocytosis, atypia. Description of HPV signs in all smears (first visit and follow-up) attending a national screening program for a period of 1 year
Thankamani *et al.* (1992a) India	64 ICC 10 oral cancers	5 normal cervices	Normal, 0 ICC, 41 Oral cancer, 0	[∞ (0.6–∞)]	Indirect immunofluorescence staining and peroxide antiperoxidase

[ ] Calculated by the Working Group
ICC, invasive cervical cancer; SES, socioeconomic status

Table 39. Case–control studies of invasive cervical cancer using HPV hybridization assays without amplification

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
McCance *et al.* (1985) London, United Kingdom	13 ICC	17 with no cervical abnormality	HPV-16 ICC, 92 Control, 18	[45.2 (4.3–2555.9)]	DNA hybridization Probes: 6, 16 and low stringency	
Kulski *et al.* (1987) Australia	54 ICC (45 SCC) 11 CIN I–II CIN III	5 biopsies from healthy women	CIN, 74 ICC, 73 HPV-16, 65 HPV-18, 7 Control, 0	[∞ (1.9–∞)] [∞ (3.0–∞)] [∞ (1.5–∞)] [∞ (0.1–∞)]	Southern blot 11, 16, 18	Cross-sectional; 82 biopsies classified by diagnosis
Lörincz *et al.* (1987) USA, Brazil, Peru	78 CIN I–III 64 SCC	23 biopsies	CIN I–III, 77 SCC, 89 Control, 9	[35.0 (6.9–239.9)] [∞ (5.9–∞)]	Southern hybridization 6/11, 18, 31, others	Study in biopsies for the USA, Peru and Brazil
Meanwell *et al.* (1987) United Kingdom	47 ICC	26 benign gynaecologically	ICC, 66 Control, 35	HPV-16 [3.7 (1.3–10.0)]	Southern hybridization	Also risk factors but no adjustment
Schneider *et al.* (1987b) Germany	73 ICC 47 CIN	442 hysterectomies	ICC, 26 CIN, 45 Control, 14	HPV, [2.2 (1.2–3.9)] HPV-16/18, [2.8 (1.4–5.5)]	Filter in-situ hybridization 16, 18, 6/11	Hysterectomy cases classified by reason: cancer/benign
Zhang *et al.* (1987) China	29 ICC 2 dysplasias	9	*Cases* HPV-16, 52 HPV-18, 9 *Controls* HPV-16, 11	HPV, [13.1 (1.4–119)] HPV-16, [8.2 (0.9–405.3)]	Hybridization 16, 18	Cross-sectional; biopsy specimens
Choo *et al.*, 1988 Taiwan, China	31 ICC (biopsy) 7 ICC (cytology)	190 (cytology)	*ICC (biopsy)* HPV-16, 51 HPV-18, 5 HPV-31, 0 HPV-33, 3 *ICC (cytology)* HPV-16, 43 *Controls* HPV-16, 2.6	HPV, [28 (5–158)] HPV-16, [39 (13–123)]	Southern blot 16, 18, 31, 33	Controls not comparable to cases. Different series with only 7 ICC cases. No real case–control
Fuchs *et al.* (1988) Austria	216 CIN I–III 44 ICC	31 hospital controls	CIN I–III, 50.0 ICC, 68.2 Control, 3.2	[30.0 (4.3–601.9)] [37.7 (5.3–1673.1)]	Southern hybridization	
Hsieh *et al.* (1988) China	77 ICC	16 other tissues, including normal cervix	ICC, 63.6 Control, 6.3	HPV, [∞ (2.9–∞)]	Southern hybridization	

Table 39 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
Wilczynski *et al.* (1988a) USA	11 adenocarcinomas	11 other cancers 6 hysterectomies	ICC HPV-16, 18.2 HPV-18, 45.5 Control, 0	[∞ (0.3–∞)] [∞ (1.9–∞)]	Southern blot 16, 18, 31, 6/11	Adenocarcinomas only
Zhang *et al.* (1988) Australia	24 ICC 15 CIN III 20 CIN I–II	33 with non-cancer gynaecological problems	ICC, 96 CIN III, 80 CIN I–II, 65 Control, 9	HPV, [230 (22–2359)]	Dot hybridization/-Southern blot 6/11, 16/18	Cross-sectional study in gynaecologic clinic. Data from biopsy tabulated. Also good correlation with scrapes
Colgan *et al.* (1989) Canada	7 ICC 30 CIN/condylomata	12 hysterectomies	CIN, 60 ICC, 57 Control, 0	[∞ (1.5–∞)]	Southern blot HPV–16/31	Cross-sectional; only biopsy
Czeglédy *et al.* (1989) Hungary	41 ICC 12 CIN III 7 CIN I–II 3 vaginal cancers 18 condylomata	22 scrapes	ICC, 60 HPV-16, 46 HPV-18, 15 CIN III, 66 CIN I–II, 43 Control, 14	[9.9 (2.5–39.0)] [40.1 (4.3–937)] [∞ (0.7–∞)] [12.7(2.3 – 70)] [11.1 (2.0–6.2)]	Southern blot/dot blot/filter in-situ hybridization 4, 11, 16, 18	Cross-sectional; 336 samples tested, 22 of them were normal
Donnan *et al.* (1989) China	68 ICC 48 dysplasias HPV: subsample of 58	116 hospital: surgical and gynaecological outpatient controls matched by age. 17 controls for HPV, different from previous ones	ICC, 11/30, 37 Dysplasia, 5/28, 18 Controls, 1/17, 6	HPV-16 ICC: 9.3 (1.0–84.1) Dysplasia: 3.5 (0.3–118)	Filter in-situ hybridization	HPV determined only in subsample
Reeves *et al.* (1989) Panama, Costa Rica, Colombia, and Mexico	759 hospital-based cases	1467 (age-matched) community and hospital controls	*HPV* Case, 67.1 Control, 35.4 *HPV-16/18* Case, 62 Control, 32 *HPV-6/11* Case, 17 Control, 7	HPV [3.7 (3.0–4.5)] HPV–16/18 signal +/–: 2.1 (1.6–2.8) 1+: 4.1 (3.2–5.4) 2–4+: 9.1 (6.1–13.6) HPV-6/11 signal +/–: 2.2 (1.5–3.1) 1+: 4.6 (2.6–8.2) 2–4+: 3.9 (0.8–17.9)	Filter in-situ hybridization, 6/11, 16/18	Adjusted for age, number of sexual partners, age at first sexual intercourse, number of live births, interval since last Pap, years of education
Chang *et al.* (1990c) China	5 condylomata acuminata 21 SCC	11 normal	Normal, 0 Condylomata acuminata, 100 SCC, 57.1	[∞ (5.6–∞)] [∞ (2.4–∞)]	DNA in-situ hybridization, 6, 11, 16 and 18	Series of genital biopsies from pathology departments

Table 39 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
Yokota *et al.* (1990) Japan	31 ICC	666 with normal cervices from out-patient clinics and screening centers	*Cases* HPV-16, 39 HPV-18, 10 HPV-6/11, 3 *Controls* HPV-16, 2 HPV-18, 1 HPV-6/11, 1	HPV, [31.2 (13.7–71.1)] HPV-16, [34.4 (13.7–86.4)] HPV-18, [32.2 (5.2–193.6)]	Filter in-situ hybridization 6/11, 16, 18	Outpatients
Zhang (1990) China	116 ICC	36	ICC, 51 Control, 6	HPV, [17.6 (5.2–53.7)] HPV-16, [∞ (5.1–∞)]	Southern blot 16, 33, 31	Cross-sectional
Hildesheim *et al.* (1991) Latin-America	766 ICC	1532 hospital and community controls		HPV-16/18, 4.3 (3.0–6.0) HPV-16/18+HSV, 8.8 (5.9–13.0)	Filter in-situ hybridization 6/11, 16/18	Same study as Reeves *et al.* (1989). This analysis focuses on the interaction HSV/HPV.
Ohta *et al.* (1991) Japan	24 ICC 62 Pap IIIa 56 Pap III 29 Pap IIIb 8 CIS	124 (Pap I–II)	ICC, 65 Pap II, 50 Pap I–II, 6	HPV, [72.1 (14–361)]	ViraPap™ ViraType™	
Si *et al.* (1991) China	318 ICC 14 CIN 48 condylomata 34 cervicitis	24 normal biopsies	ICC, 60 CIN, 50 Condylomata, 28 Cervicitis, 20 Control, 8.3	HPV, [22 (5.0–95.4)] HPV-16, [16.1 (3.9–149.8)] HPV-18, [∞ (0.2–∞)]	Dot blot/Southern blot 16, 11	Cross-sectional
Wei *et al.* (1991) China	169 for HPV 34 CIS 10 ICC 8 CIN I 6 CIN II 5 CIN III	77 cervicitis 2 cervical polyps	ICC, 40 CIS, 71 CIN III, 40 CIN II, 33 CIN I, 25 Control, 36.7	[1.23 (0.3–4.8)] [4.44 (1.9–10.7)]	Dot blot 6b, 11, 16, 18	Survey of 6710 women. Biopsy of those with abnormalities. HPV in biopsies
Kadish *et al.* (1992) USA	63 LSIL 126 HSIL/ICC	122 controls	LSIL, 92.1 HSIL/ICC, 66.7 Control, 40	[17.3 (6.1–52.9)] [3.0 (1.7–5.2)]	Southern	
Kanetsky *et al.* (1992) New York, USA	4 ICC 1 CIS 1 CIN I	272 (226 tested for HPV)	Case, 33 Control, 2.7	HPV, 18.3 (1.9–121)	ViraPap™ 6/11, 16/18, 31/33/35	278 black/elderly women attending screening program. Also questionnaire for risk factors

Table 39 (contd)

Reference and study area	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV test	Comments/adjustments
Lörincz *et al.* (1992) USA	153 ICC	1566 normal cervices	Control, 6.4 Case, 89.5 HPV-16, 52.6 HPV-18, 26.3	HPV-16, 260.0 (216.9-311.9) HPV-18, -45, -56, 296.1(198.9–441.1)	Southern hybridization	Pooled analysis and HPV testing of subjects from 8 studies. No adjustments, crude odds ratios
Mandelblatt *et al.* (1992) New York, USA	6 (4 ICC, 2 CIN)	226	Case, 33.3 Control, 2.7	[12.2 (1.2–122.9)]	Dot blot	Women aged 65 or more
Saito *et al.* (1992) Japan	20 CIN I–III 22 ICC	599 hospital	CIN I–III, 45 ICC, 68.2 Control, 4.2	[180 (6.2–52.3)] [47.2 (16.3–141.7)]	Dot blot	
Thankamani *et al.* (1992b) India	102 ICC	12 hysterectomies	HPV-11, 36 Control, 0 HSV, 53	[10 000 (1.5–10 000)]	Hybridization 11, (HSV-2)	HPV-11 only. Also HSV-2
Marin *et al.* (1994) Slovenia	60 SCC 6 adenocarcinomas	42 normal	HPV-16, -18 Adenocarcinoma 33.3 33.3 SCC 43.3 7.0 Control 11.9 4.8	*HPV-16* [3.7 (0.4–36.0)] [5.7 (1.8–19.1)]	In-situ hybridization	

[ ] Calculated by the Working Group

SCC, squamous-cell carcinoma; ICC, invasive cervical carcinoma; CIN, cervical intraepithelial neoplasia; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; Pap, Papanicolaou smear; HSV, herpes simplex virus

Schneider *et al.* (1987b) studied HPV in Germany by filter in-situ hybridization methods with probes for HPV-6/11, -16 and -18 in 616 women with a history of hysterectomy. HPV was present in 33% of 120 women with cervical neoplasia and in 14% of 442 women with benign disease. [The OR was 2.2 (95% CI, 1.2–3.9) for any HPV and 2.8 (95% CI, 1.4–5.5) for HPV-16/18.]

Choo *et al.* (1988), in Taiwan, used dot blot and Southern blot hybridization to search for HPV-16, -18, -31 and -33 in 31 cases of invasive cervical carcinoma and 190 cytologically healthy women (viral DNA was studied in cervical swabs). The prevalences of HPV-16 were 51% among carcinoma cases and 2.6% among controls [OR, 39 (95% CI, 13–123)].

In Austria, Czerwenka *et al.* (1989) examined a series of cytology specimens with southern blot, dot blot and in-situ hybridization. Specimens classified as normal or Pap II were HPV positive by Southern blot in 27/166 (16.3%) whereas smears that were Pap III-IV were HPV positive in 55/77 (74%) [OR, 14.7 (95% CI, 7.3–29.9)].

In a study from Hong Kong, Donnan *et al.* (1989) assessed prevalence of HPV-16 by Southern blot in 30 invasive cervical cancer cases and 17 gynaecological controls. A total of 11 cases and one control were positive (OR, 9.3 (95% CI, 1.0–84.1)).

Reeves *et al.* (1989) conducted a population-based case–control study in Panama, Costa Rica, Colombia and Mexico including 759 cases and 1467 age-matched, randomly selected controls. HPV DNA was determined by filter in-situ hybridization with probes for HPV-16/18 and -6/11. Known and suspected risk factors for cervical cancer were assessed via direct interview. Prevalences for any HPV were 67.1% for cases and 35.4% for controls; those for HPV-16/18 were 62% for cases and 32% for controls; and those for HPV-6/11 were 17% for cases and 7% for controls. Relative risks for cancer, adjusted for age, number of sexual partners, age at first sexual intercourse, number of live births, interval since last Pap smear and years of education were: for any HPV, [3.7 (95% CI, 3.0–4.5)]; for HPV-16/18 signal +/–, 2.1 (95% CI, 1.6–2.8); signal 1+, 4.1 (95% CI, 3.2–5.4); signal 2–4+, 9.1 (95% CI, 6.1–13.6); for HPV-6/11 signal +/–, 2.2 (95% CI, 1.5–3.1); signal 1+, 4.6 (95% CI, 2.6–8.2); signal 2–4+, 3.9 (95% CI, 0.8–17.9). [Several publications based on the same study provided slightly different risk estimates (Reeves *et al.*, 1987; Brenes *et al.*, 1987; Reeves *et al.*, 1989; Herrero *et al.*, 1990; Hildesheim *et al.*, 1991; Herrero *et al.*, 1992)].

Yokota *et al.* (1990) studied HPV infection in women from Tokyo, Japan, attending out-patient gynaecology clinics and screening centres. Filter in-situ hybridization methods were employed with probes for HPV-6/11, -16 and -18. In 31 invasive cervical cancer cases, the prevalences were 3%, 39% and 10% and in 666 control women without cervical pathology were 1%, 2% and 1%. For invasive cervical cancer cases, the ORs were [31.2 (95% CI, 13.7–71.1)] for any type of HPV, [3.7 (95% CI, 0.4–31)] for HPV-6/11, [34.4 (13.7–86.4)] for HPV-16, and [32.2 (95% CI, 5.2–193.6)] for HPV-18.

Kadish *et al.* (1992) in the USA used cervicovaginal lavage and Southern hybridization to investigate 329 women attending a colposcopy clinic. The HPV DNA prevalence was 49/122 (40.2%) among controls and 142/189 (75.1%) of patients with SIL or cancer [OR, 4.5 (95% CI, 2.7–7.6)]. Among patients with LSIL, 58/63 (92.1%) were HPV positive [OR, 17.3 (95% CI, 6.1–52.9)]. Of the patients with HSIL or cancer, 84/126 (66.7%) were HPV positive [OR, 3.0 (95% CI, 1.7–5.2)].

Lörincz *et al.* (1992) reported on a pooled analysis of subjects included in eight studies that were investigated using Southern blot, including the aforementioned Lörincz *et al.* (1987). A total of 153 cases of invasive carcinoma were compared to 1566 controls with normal cervices. The prevalence of HPV in controls was 6.4%. The prevalence among cases was 89.5%, of which 52.6% was HPV-16 and 26.3% HPV-18. The type specific ORs were 260.0 (95% CI, 216.9–311.9) for HPV-16, 296.1 (95% CI, 198.9–441.1) for HPV-18, -45 or -56, and 31.1 (95% CI, 18.7–51.8) for HPV-31, -33, -35, -51 and -52.

Mandelblatt *et al.* (1992) investigated HPV DNA by dot blot in six cases of cervical neoplasia and 226 controls aged 65–96 years from New York, USA. HPV DNA was detected in two cases and six controls [OR, 17.6 (95% CI, 1.4–155.0)].

Saito *et al.* (1992) assessed HPV DNA by dot blot in 22 invasive cervical cancer cases and 599 cytologically normal women from Osaka, Japan. The prevalence of infection was 68.2% in cases and 4.2% in controls [odds ratio, 47.3 (95% CI, 16.3–142)].

Monsonego *et al.* (1993) studied a series of patients from Paris, France, with squamous genital cancer of the cervix (15), vagina (3), vulva (3) and anus (2). The study also included 48 specimens from the same locations without abnormal cytological features that served as controls. Blot hybridization was used to detect and type HPV DNA. The prevalence of all types of HPV among controls was 26/48 (54.2%). Among squamous genital cancer cases the HPV DNA prevalence was 19/23 (82.6%) [OR, 4.0 (95% CI, 1.1–16.5)]. The study also included the male partners of cases and controls. The HPV DNA prevalences in the lesions sampled were 6/16 (37%) in spouses of squamous cancer patients and 18/43 (42%) in spouses of negative controls.

Marin *et al.* (1994) investigated 66 invasive cancers and 42 hospital controls from Ljubljana, Slovenia, using in-situ hybridization. Among controls, the prevalence rate of HPV-16 was 11.9% and that of HPV-18 was 4.8%. The HPV prevalence in 60 squamous-cell carcinomas was 43.3% for HPV-16 and 7.0% for HPV-18. In six cases of adenocarcinoma the prevalence of HPV-16 was equal to the prevalence of HPV-18 (33.3%). The estimated odds ratios for HPV-16 were [3.7 (95% CI, 0.4–36.0)] for adenocarcinoma and [5.7 (95% CI, 1.8–19.1)] for squamous-cell carcinoma (see also Table 31).

Table 40 includes the case–control studies that used PCR amplification methods to assess exposure to HPV.

In Spain and Colombia, Muñoz *et al.* (1992) conducted a population-based case–control study including 436 incident cases of squamous-cell invasive cervical cancer and 387 population controls. Specimens tested with PCR were 229/436 (53%) of the cases and 228/387 (60%) of the controls. HPV detection was carried out using PCR methods based on the *L1* region consensus primers. Hybridization was performed sequentially with probes to HPV-6, -11, -16, -18, -31, -33, -35 under high-stringency conditions. Subsequently, the filters were screened with a generic probe containing a mixture of amplifiers of HPV-16 and -18 (Bauer *et al.*, 1991). The ORs for HPV DNA were 46.2 (95% CI, 18.5–115.1) in Spain and 15.6 (95% CI, 6.9–34.7) in Colombia. The attributable fraction derived from this study indicated that 67.5% of cases in Spain and 66.0% in Colombia could be attributed to HPV. Odds ratios were also calculated by type specific HPVs for Spain and Colombia combined, as follows: HPV-16 (29.7 (95% CI, 14.5–57.4)), HPV-18 (19.4 (95% CI, 4.0–93.7)), HPV-31, -33, -35 [21.4 (95% CI, 6.1–75.6)] and

Table 40. Case–control studies of invasive cervical cancer using HPV hybridization assays including amplification (PCR) methods

Reference and country	Cases (number and type)	Controls (number and type)	HPV prevalence (%)			Odds ratio (95 % CI)	HPV test/comments/adjustments
			Type	Cases	Controls		
Muñoz *et al.* (1992) Spain	250 incident cases	238 population-based controls	All	69.0	4.6	46.2 (18.5–115.1)	PCR. Adjusted for age, study area, number of sexual partners, education, age at first birth and history of a prior Pap smear
Cali, Colombia	186 incident cases	149 population-based controls	All	72.4	73.3	15.6 (6.9–34.7) Spain and Colombia, combined HPV-16, 29.7 (14.5–57.4) HPV-18, 19.4 (4.0–93.7) HPV-31, -33, -35, 21.4 (6.1–75.6) HPV unidentified, 79.6 (11.1–572.4)	
Eluf-Neto (1994) Brazil	199 hospital-based cases	225 hospital-based controls	Any	84	17	37.1 (19.6–70.4)	PCR. Odds ratios adjusted for age, socioeconomic status, number of Pap smears, parity, number of sexual partners, age at first sexual intercourse and duration of oral contraceptive use
			16	54	5	74.9 (32.5–173)	
			18	9	1	56.9 (11.7–276)	
			31/33	3	0		
			16/18/31/33	66	6	75.1 (34.2–165)	
			Undefined	19	10	13.8 (6.4–29.6)	
Peng *et al.* (1991) China	101 hospital-based cases	146 gynaecological clinic-based controls	16	31.7	1.4		PCR. Adjusted for age, income, residence, age at first marriage and cigarette smoking
			33	3.0	0		
			16/33	34.7	1.4	32.9 (7.7–141.1)	
ter Meulen *et al.* (1992) Tanzania	50 cervical carcinomas (hospital-based) 3 vaginal carcinomas	359 non-cancer gynaecological patients	Any	89	59	[1.6 (0.8–3.2)]	Southern blot and/or PCR
			16	38		[6.6 (2.9–15.4)]	
			18	32		[4.2 (1.8–9.9)]	
			45	6			
			Other	13			

Table 40 (contd)

Reference and country	Cases (number and type)	Controls (number and type)	HPV prevalence (%)			Odds ratio (95 % CI)	HPV test/comments/adjustments
			Type	Cases	Controls		
Das *et al.* (1992a) India	96 hospital-based cases	22 asymptomatic normal women	Any 16 18	98 64 3	18 18 0	[211.5 (30.1–2048)]	Southern blot and/or PCR
Shen *et al.* (1993) Taiwan, China	78 cervical cancers	55 uterine leiomyomas		66.7	5.5	HPV, [33.6 (9.5–184.2)] HPV + CMV, [∞ (13.6–∞)]	PCR. Results show a strong interaction between HPV and CMV.
Asato *et al.* (1994) Japan	52 cervical cancers	2873 Pap I–II		88.5	9.5	[72.5 (30.5–209.6)]	PCR, *L1* consensus primers
Griffin *et al.* (1990) United Kingdom	19 squamous-cell carcinomas	10 normal	Any	84.2	60	[3.6 (0.5–31.0)]	PCR. HPV tested in paraffin wax-embedded material
Arends *et al.* (1993) United Kingdom	47 cervical cancers	24 normal	16/18	79	0	[∞ (17.2–∞)]	PCR

[ ] Calculated by the Working Group
CMV, cytomegalovirus; PCR, polymerase chain reaction; Pap, Papanicolaou smear

unidentified HPV-types [79.6 (95% CI, 11.1–572.4)]. An additional analysis stratified by HPV status was performed. This analysis showed that, among HPV-negative cases, the risk factors identified were related to sexual behaviour. However, among HPV-positive cases and controls, the only significant differences were the use and the duration of use of oral contraceptives (Bosch *et al.*, 1992).

Of the subjects included in these studies, 823 cervical specimens were also tested by ViraPap™. In Spain, 250 cases and 238 controls were investigated. The HPV DNA prevalence was 41% among cases and 2.5% among controls (OR, 29.1 (95% CI, 10.3–81.9)). In Colombia, 186 cases and 149 controls were investigated. The HPV DNA prevalence was 30.4% for cases and 0.7% among controls (OR, 93.2 (95% CI, 12.6–687.3)). The intensity of the hybridization signal in the ViraPap™ system was quantified into five categories. The corresponding odds ratios were: very weak positive, 1.0; positive (2–3), 8.6 (95% CI, 1.7–42.2); strong positive (4–5), 10.3 (95% CI, 1.7–64.2). Southern hybridization was also used in 617 specimens. In Spain, 184 cases and 158 controls were tested. The HPV DNA prevalence among cases was 36.4% and among controls 5.7% (OR, 8.8 (95% CI, 4.1–19.0)). In Colombia, 132 cases and 143 controls were tested. The HPV DNA prevalence was 25.0% for cases and 4.2% among controls (OR, 9.6 (95% CI, 3.7–25.1)). The results of this study have also been partially reported elsewhere (Muñoz *et al.*, 1994).

Moreno *et al.* (1995) conducted a statistical analysis of the CIN III and cervical cancer studies in Spain and in Colombia. Risk factors were compared between the two types of cases and between cases and controls to ascertain factors that might favour the progression from CIN III to invasive cancer. Neither entity differed in exposure to HPV, HPV types or estimates of HPV viral load in any significant way.

In São Paulo, Brazil, Eluf-Neto *et al.* (1994) conducted a hospital-based case–control study including 199 histologically confirmed consecutive cases and 225 controls from a diversity of diagnoses. A PCR system was performed directly on crude cell suspensions by a combination of general primer-mediated and type-specific PCR (GP-PCR/T-PCR). The HPV attributable fraction calculated from this study was 86%. The ORs for type specific HPVs were 74.9 (95% CI, 32.5–173.0) for HPV-16, 56.9 (95% CI, 11.7–276.0) for HPV-18, 75.1 (95% CI, 34.2–165.0) for HPV-16, -18, -31 and -33 and 13.8 (95% CI, 6.4–9.6) for unidentified HPV types. After adjustment, a residual association was found for increasing parity (p for trend = 0.02). A non-statistically significant trend was observed for number of sexual partners and for duration of use of oral contraceptives (Eluf-Neto *et al.*, 1994).

Peng *et al.* (1991) conducted a case–control study in Sichuan, China, with 101 cases and 146 hospital controls. HPV-16 and -33 were detected by PCR. Prevalences were 31.7% in cases and 1.4% in controls for HPV-16, 3.0% in cases and 0% in controls for HPV-33 and 34.7% in cases and 1.4% in controls for HPV-16/33 with an odds ratio of 32.9 (95% CI, 7.7–141.1) adjusted for age, income, residence, age at first marriage and cigarette smoking. In this study, no association was found between cervical cancer and herpes simplex virus type 2 (OR, 1.3; 95% CI, 0.7–2.3).

ter Meulen *et al.* (1992) determined HPV by PCR in 50 biopsies of invasive cervical cancer and 359 cervical swabs of noncancer gynaecological patients in Tanzania. Any HPV was found in 59% of controls and 89% of cases. Type-specific prevalences in cases were 38% for HPV-16,

32% for HPV-18, 6% for HPV-45 and 13% for other HPV type. Crude relative risks were: [1.6 (95% CI, 0.8–3.2)] for any HPV, [6.6 (95% CI, 2.9–15.4)] for HPV-16 and [4.2 (95% CI, 1.8–9.9)] for HPV-18. In this study, HIV seroprevalence was also investigated and found to be strongly correlated to HPV prevalence. The controls were HIV-positive in 12.8% of instances. In contrast, among 270 cases of cervical carcinoma, only 3% were HIV-positive. Among controls, the stronger risk factors for HPV positivity was young age and HIV seropositivity. [The Working Group noted the high prevalence of HIV among controls, the young age of controls as compared to cases and the fact that HPV was detected in biopsies among cases and in cervical swabs among controls.]

Das *et al.* (1992a) reported a series of biopsies from 96 consecutive cases of cervical cancer from India and compared them with 22 biopsies from normal controls attending the same clinic. All specimens were investigated with Southern hybridization, and 91.7% of the cases were found to be HPV-positive. The eight cases that tested HPV negative were further investigated using PCR and six were found to be HPV-positive. HPV-16 accounted for 64% of the HPV types. Four of the 22 controls were HPV-positive using PCR (18%) [OR, 211.5 (95% CI, 30.1–2048)]. [The Working Group noted the lack of systematic investigation of specimens using PCR.]

Shen *et al.* (1993) conducted a case–control study in Taiwan with 78 invasive cervical cancer cases and 55 controls with uterine leiomyoma. HPV and cytomegalovirus (CMV) infections were determined by PCR. Prevalence of HPV was 66.7% in cases and 5.5% in controls. The odds ratio for any HPV was [33.6 (95% CI, 9.5–184.2)] and a strong interaction with CMV was found giving an infinite odds ratio for HPV plus CMV, based on 40 cases and no controls positive for both.

In a short communication, Asato *et al.* (1994) reported PCR-*L1* consensus primer-based HPV prevalence rates in 52 cervical cancer cases and 2873 women with Pap I-II cervices from Ryukyus, Japan. A total of 46 cases (88.5%) and 274 (9.5%) controls were positive [crude OR, 72.5 (95% CI, 30.5–209.6)].

Griffin *et al.* (1990) assessed the prevalence of HPV-6, -11, -16 and -18 using PCR and Southern blot in paraffin-embedded blocks from 19 cases of squamous-cell carcinoma and 10 normal cervices from Leeds, UK. HPV-6/11 was detected in 10 cases and five controls [OR, 1.1 (95% CI, 0.2–5.1)]; HPV-16/18 was detected in 16 cases and five controls [OR, 5.3 (95% CI, 0.9–31)].

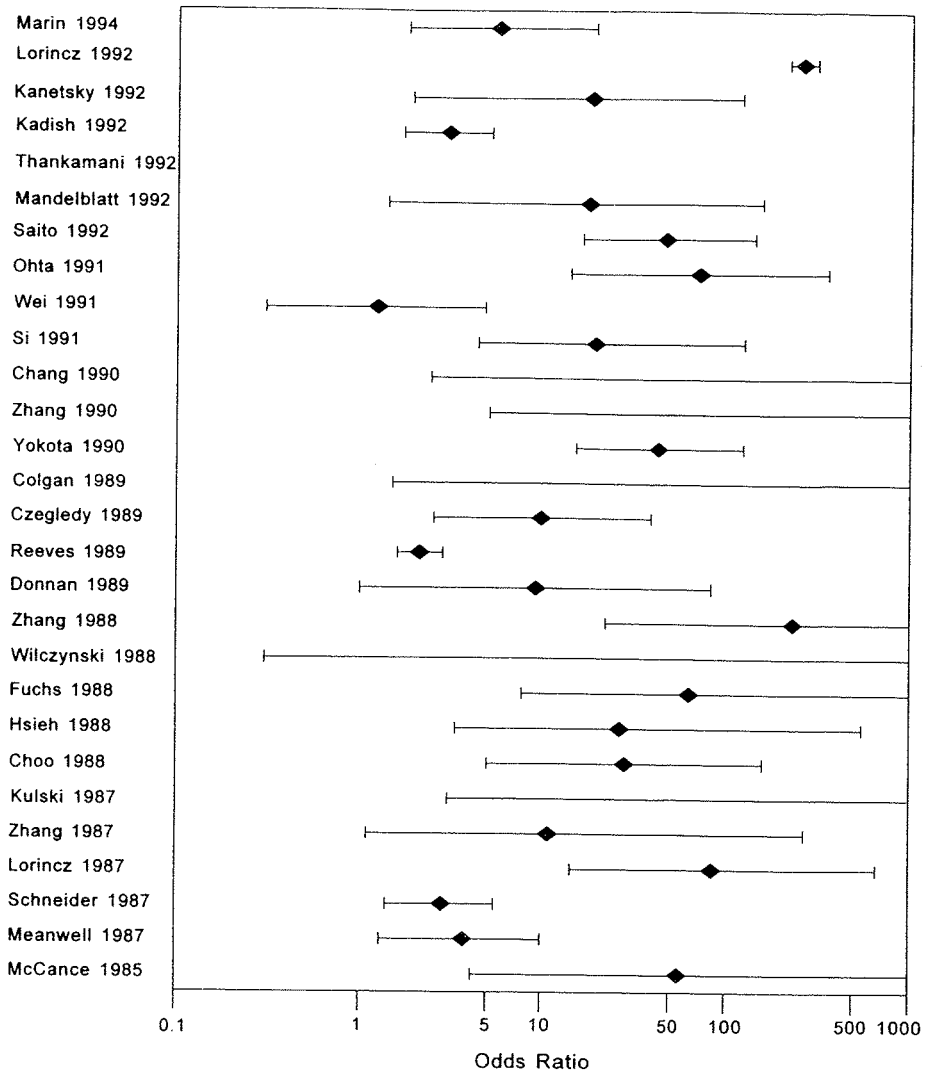
Arends *et al.* (1993) used a type-specific PCR-based assay for HPV-6, -11, -16, -18 and -33 on 47 cervical carcinomas and 24 samples of histologically normal cervices. The incidence of HPV-16 and -18 was 79% in carcinomas and 0% in the normal controls [OR, ∞ (95% CI, 17.2–∞)]. HPV-6 and -11 were not found in cancer cases or controls.

Figures 16 and 17 show the odds ratios and 95% confidence intervals for associations found in case–control studies between HPV-16 (or its nearest surrogate) and risk of invasive cervical cancer. Figures 16 and 17 show data obtained by methods other than PCR and by PCR, respectively.

Table 41 summarizes studies that used serological assays to detect antibodies against HPV proteins. The table includes mainly studies of anogenital cancer. Some published studies have also

explored HPV seropositivity in cases of CIN (see above and Table 37) and in skin cancer (Steger *et al.*, 1990). Much additional work is currently underway in this active area.

Figure 16. Odds ratios and 95% confidence intervals for associations found in case–control studies using non-PCR methods between HPV-16 (or its nearest surrogate) and invasive cervical cancers

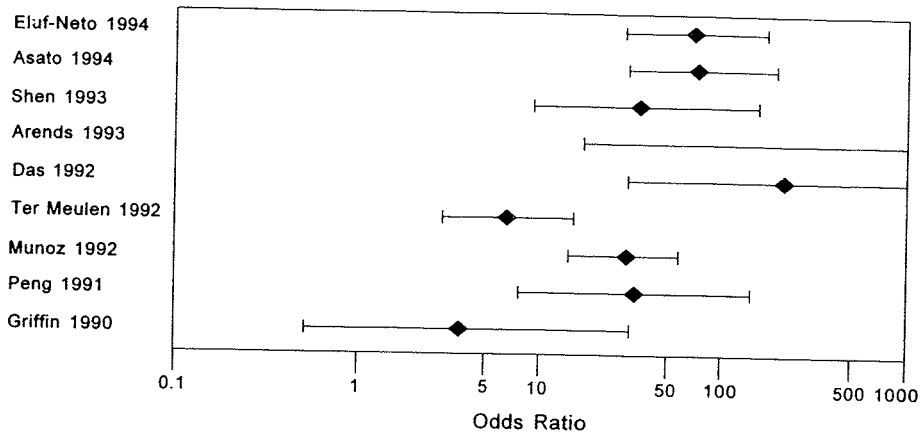


Data are taken from Table 39

Several antibody-detection systems have been used and the majority have been validated against cervical cytological/histological diagnoses and/or HPV DNA in cervical cells. The serological detection rate among HPV DNA-positive cases in the studies reported range between 50

and 70%. Moreover, some of the assays perform differently in cases of invasive cervical cancer (seropositivity rates are higher) than in cases of preinvasive lesions (see also section 1.3.4).

Figure 17. Odds ratios and 95% confidence intervals for associations found in case–control studies using PCR methods between HPV-16 (or its nearest surrogate) and invasive cervical cancers



Data are taken from Table 40.

This section focuses on the studies that have reported antibody seroprevalence rates in cases and in a group of non-cervical cancer controls. However, with few exceptions these comparisons rarely comply with the requisites of design and analysis of rigorous epidemiological investigations.

In a study in Australia, Baird (1983) collected sera from 60 cases of anogenital warts, 92 cases of CIN and 46 cases of cervical cancer. The sera was examined for IgG antibodies to a group-specific BPV-2 antigen using a solid-phase enzyme-linked immunosorbent assay, and compared with sera collected from 256 controls (48 children, 108 adults, 60 attendees of STD clinics and 40 non-cervical cancer patients). Prevalences were 10% in controls, 95% in cases of anogenital warts, 60% in CIN cases and 93% in cervical cancer patients. Estimated crude odds ratios were [171 (95% CI, 40.6–845.3)] for anogenital warts, [13.4 (95% CI, 5.8–31.5)] for CIN and [129 (95% CI, 30.3–643.8)] for cervical cancer.

In Germany, Jochmus–Kudielka et al. (1989) collected sera from 46 patients with HPV-associated lesions, 88 patients with cervical cancer and from 385 controls (49 laboratory personnel and 336 patients, both men and women, with no HPV-related infections) and tested them by the Western blot technique for the presence of antibodies to HPV-16 proteins E4 and E7 that had been expressed in *Escherichia coli* as fusion proteins. Prevalences of E4 and E7 were 18% and 4% respectively for control patients with no HPV-related infections, 12% and 2% for control laboratory personnel, 23% and 0% for patients with condyloma acuminata, 43% and 0% for CIN I–II patients, 32% and 16% for CIN III patients and 16% and 20% for cervical cancer patients. Compared to an age-matched selection of controls, the crude ORs for cervical cancer were 2.3 [(95% CI, 0.9–5.6)] for E4 and 18.0 [(95% CI, 3.9–115.7)] for E7. The authors

Table 41. Case–control studies of CIN, invasive cervical cancer and cancer at other sites using serological assays for HPV antigens

Reference and study	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV serology test	Comments/adjustments
Baird (1983) Australia	60 anogenital warts 92 CIN 46 cervical cancers	48 children 108 adults 60 STD clinic 40 non-cervical cancers	Anogenital wart, 95 CIN, 60 Cervical cancer, 93 Control, 10	[171 (40.6–845.3)] [13.4 (5.8–31.5)] [129 (30.3–643.8)]	Solid-phase enzyme-linked immunosorbent assay for an IgG antibody to a group-specific BPV-2 antigens	Odds ratios calculated using STD clinic patients and non-cervical cancer patient as controls
Jochmus-Kudielka *et al.* (1989) Germany	44 HPV-associated lesions 88 cervical cancers	336 patients with no HPV-related infections 49 laboratory personnel	*E4/E7* Control, 18/4 Laboratory personnel, 12/2 Condyloma, 23/0 CIN I-II, 43/0 CIN III, 32/16 Cervical cancer, 16/20	Compared to age-matched controls: Cervical cancer for E4, 2.3 [(0.9–5.6)] ($p = 0.53$) Cervical cancer for E7, 18.0[(3.9–115.7)]	Western blot HPV-16 ORFs E4 and E7 proteins	Anti-E7 antibodies may represent a marker for cervical development. Anti-E4 antibody may be correlated with virus replication.
Mann *et al.* (1990) Panama	186 invasive cervical cancers	172 age-matched controls	*Cases/controls* IgA to E7, 21/9 IgG to E7, 25/6 IgA to 245, 15/11 IgG to 245, 19/11	 5.3 (2.4–11.6) 2.7 (1.3–5.3) 1.7 (1.2–3.3) 1.0 (0.4–1.6)	ELISA IgG and IgA antibodies to HPV-16 (E7 peptide and to peptide 245, epitope in E2)	Odds ratios adjusted for cigarette smoking, education, pregnancies, time since last Pap and age at first sexual intercourse. Conclusions: Antibodies to E7 are markers for invasive cervical cancer. Seropositivity correlated poorly with clinical stage, survival, or the presence of HPV-DNA in tumour.
Steger *et al.* (1990) Germany	54 malignant melanomas 60 basal-cell carcinomas 44 Hodgkin's diseases 11 squamous-cell skin carcinomas 32 cervical cancers	445 randomly selected controls	Malignant melanoma, 28 Basal-cell carcinoma, 40 Hodgkin's disease, 48 Squamous-cell skin carcinoma, 73 Cervical cancer, 37 Control, 20	[1.6 (0.8–3.1)] [2.7(1.5–4.9)] [3.7(1.9–7.3)] [10.8(2.5–52.6)] [2.4(1.1–5.5)]	HPV-8-specific IgG antibodies to L1 (Western blot analysis with the entire HPV-8 L1 as antigen)	
Bleul *et al.* (1991) Tanzania and Germany	Invasive squamous-cell cervical carcinomas Tanzania: 116 Germany: 94	Age-matched controls with other gynaecological problems Tanzania: 116 Germany: 94	*Tanzania* Control, 0; case, 8.6 *Germany* Control, 0; case, 3.2	 [∞ (237–∞)] [∞ (0.4–∞)]	Anti HPV-18 E6 and E7 antibodies ELISA	Analysis with a synthetic 28-mer peptide comprising E7/1 region

Table 41 (contd)

Reference and study	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV serology test	Comments/adjustments
Köchel *et al.* (1991) Germany	46 cervical cancers	31 non-genital cancer patients 41 healthy women	*HPV-16/18 E6 & E7* Case, 63 Cancer control, 6.5 Healthy control, 9.8 *HPV-16/18 L1 & L2* Case, 54.3 Cancer control, 41.9 Healthy control, 43.9	As compared to healthy controls: *HPV-16/18 E6 & E7* [15.8(4.3–63.3)] *HPV-16/18 L1 & L2* [1.5 (0.6–3.9)]	Antibodies to HPV-6b, HPV-16 and HPV-18 L1, L2, E4 and E7 Western blot	
Kanda *et al.* (1992) Japan	12 CIN 108 cervical cancers	140 healthy women	*Anti-HPV-16 E1/E4 cases/controls* CIN, 0/0 Cancer, 10.2/0 *Anti-HPV-16 E7 cases/controls* CIN, 0/0 Cancer, 20.4/0	One-tailed *p* values [< 0.0001] [< 0.0001]	ELISA Western blot HPV-16 E1/E4 and E7	Prevalences available for other cancers: ovarian (n = 28), endometrial (n = 6), choriocarcinoma (n = 3), vulvar (n = 2), VIN (n = 3)
Lehtinen (1992) Finland	Newly diagnosed cervical carcinomas: 42 squamous-cell carcinomas 19 adenocarcinomas	61 age-matched controls	*Squamous-cell carcinomas cases/controls* HPV-6 E2, 17/5 HPV-16 E2, 43/13 *Adenocarcinoma cases/controls* HPV-6 E2, 11/11 HPV-16 E2, 42/16 *Any cases/controls* HPV-6 E2, 15/7 HPV-16 E2, 43/15	IgA HPV-16 E2 peptide 245 Squamous-cell, 13 (2.4–249) Adenocarcinoma, 9 (1.8–194) Any, 9.5 (2.8–57.2) No difference with HPV-6 No differences with IgG	ELISA HPV-16 E2 IgA and IgG antibody levels	Unadjusted odds ratios
Mandelson *et al.* (1992) USA	69 invasive cervical cancers	81 controls	*HPV-16 E7* Level Cases/controls 0 66/71 + 1 12/17 > +1 22/12	+ 1, 0.8 (0.4–1.8) > +1, 3.8 (1.7–8.5) Others (HPV-6, -16/18 L1 & L2 & other Es) were not associated)	IgG antibodies to HPV-6 (L1, L2) HPV-16/18 (E2, E4, E6, E7, L1 & L2) Western blot assay	Adjusted for age, cigarette smoking, age at first sexual intercourse, lifetime number of sexual partners, number of prior births, number of prior Pap smears in previous decade

Table 41 (contd)

Reference and study	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV serology test	Comments/adjustments
Müller *et al.* (1992) Colombia and Spain	100 HPV-16 invasive 15 HPV-other invasive 62 HPV-negative invasive cancers 49 HPV-16 CIN III	177 controls of invasive cases 49 controls of CIN cases	*Any E6/any E7* Invasive control, 4/15 CIN-control, 6/20 HPV-16-invasive, 16/42 HPV-other-invasive, 0/27 HPV-negative-invasive, 2/23 HPV-16 CIN, 4/18 HPV-positive-invasive	*Any E7* [4.0 (2.2–7.4)] [2.0 (0.5–7.6)] [1.6 (0.7–3.5)] [0.9 (0.3–2.7)] [3.7 (2.0–6.7)]	ELISA with 4 HPV-16 E6-E7 peptides	Results for E6 can be also computed but the prevalences are much smaller than E7. Cases were selected because of HPV DNA status and controls.
Sasagawa *et al.* (1992) Japan	30 invasive cervical cancers 26 CIN III	38 healthy women	*E6/E7* CIN, 19/8 Invasive cancer, 27/33 Control, 0/0	*One-tailed p-values E6/E7* [< 0.01/0.16] [< 0.001/0.0001]	HPV-16 E6 & E7 ELISA	
Ghosh *et al.* (1993) United Kingdom	92 cervical cancers 90 CIN	84 healthy women	*L1/E4/E6/E7* Control, 4/24/5/10 CIN I/condyloma, 18/36/14/14 CIN II-III, 10/39/13/6 Cervical cancer, 5/43/35/25	Age-sex-matched (69 cases and 69 controls) *Cervical cancer* E4, [2.5 (1.1–5.5)] E6, [16.7 (3.5–108.3)] E7, [3.9 (1.2–13.0)]	E4, E6, E7, L1 Western blot analysis of recombinant HPV proteins	
Jha *et al.* (1993) United Kingdom	219 population-based cervical cancers	387 age-matched women	*Cases/controls* HPV-16 E7, 14/7 HPV-18 E7, 7/5 HPV-16/18 E7, 20/12	 1.9 (1.1–3.5) 1.8 (0.8–3.9) 1.9 (1.2–3.2)	HPV-16 E7 and /or HPV-18 E7 ELISA	Adjusted for age, number of sexual partners, age at first sexual intercourse, years of oral contraceptive use, number of normal Pap smears in the past four years
Heino *et al.* (1993) Sweden	64 anal epidermoid carcinomas	79 healthy blood donors	*Prevalence IgA E2:9* Case, 89; control, 24	*IgA E2:9*, [25.7 (9.3–74.3)] L1, L3, E7 not significantly associated	ELISA IgA and IgG antibodies to 5 HPV-16 peptide antigens	
Paez *et al.* (1993) Japan	51 squamous-cell carcinomas 4 adenosquamous-cell 2 adenocarcinomas	200 healthy blood donors	*E7/L2* Squamous-cell, 20/29 Adenosquamous-cell, 0/75 Adenocarcinoma, 0/0 All cancer, 18/32 Control, 5/9	*E7*, [4.0 (1.4–11.3)] *L2*, [4.7 (2.1–10.4)]	Antibodies to HPV-16 E7 and L2 proteins Western blot assay	

Table 41 (contd)

Reference and study	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95 % CI)	HPV serology test	Comments/adjustments
Stacey *et al.* (1993) Australia	Cervical cancers: 10 E7 seropositive 10 E7 seronegative	10 healthy controls	*Prevalence of bE7 RIPA* E7 seropositive, 100 E7 seronegative, 50 Any, 75 Control, 0	[$p = 0.0001$]	HPV-16 E7 protein by recombinant baculovirus (bE7 RIPA)	Authors conclude that the bE7 RIPA assay provides a more sensitive method for the detection of E7 antibodies in human sera than does the currently widely used Western blotting method.
Viscidi *et al.* (1993) Colombia and Spain	97 invasive cancers 49 CIN	121 controls of invasive cases 48 controls of CIN cases	*E6 or E7 cases/controls* CIN: 8 / 2 Invasive: 72 / 6	*E6 or E7 cases/controls* [4.2 (0.4–102..0)] [42.2 (16.4–113.7)]	HPV-16 E6 and E7 TT-RIPA	
Sun *et al.* (1994a) Colombia and Spain	97 population-based invasive cervical cancers	121 population-based controls	*ELISA* / *RIPA* E6 15/5 56/2 E7 41/17 43/4 E6 or E7 46/21 72/6	E6 ELISA, 3.5 (2.1–5.8) E6 RIPA, 74.7 (25.2–219.5) E7 ELISA, 3.3 (2.7–4.0) E7 RIPA, 17.7 (11.1–29.7) E6 or E7 ELISA, 3.3 (2.8–3.9) E6 or E7 RIPA, 42.2 (28.3–62.7)	Radioimmunoprecipitation (in vitro-translated HPV-16 E6 & E7) versus ELISA (E6 and E7 synthetic peptides)	Comparison of peptide-ELISA versus TT-RIPA. Odds ratios not adjusted
Sun *et al.* (1994b) Brazil	194 hospital-based invasive cervical cancers	217 hospital-based controls	E6: 54/6 E7: 30/5 E6 or E7: 63/10 E6 and E7: 21/0.5 E6 or E7 high antibody titres: 41/0.5	35 (15–83) 10 (4–25) 28 (13–61) 87 (10–736) 239 (29–1946)	Radioimmunoprecipitation HPV-16 E6 and E7	Odds ratios adjusted for age, socioeconomic status, number of Paps, parity, NOSP, AFSI, duration and use of oral contraceptives sensitivity and specificity for invasive cancer: E6 or E7: 63 and 90; E6 or E7 (high titres): 41 and 99.7

[ ] Calculated by the Working Group

CIN, carcinoma *in situ*; TT-RIPA, radioimmunoprecipitation of the translated protein; NOSP, number of sexual partners; AFSI, age at first sexual intercourse; VIN, vulvar intraepithelial neoplasia; ORF, open reading frame; STD, sexually transmitted disease; BPV, bovine papillomavirus; ELISA, enzyme-linked immunosorbent assay; Pap, Papanicolaou smear

conclude that the presence of anti-E7 antibodies may represent a marker for cervical cancer development and that the presence of anti-E4 antibodies may be correlated with virus replication.

In Panama, Mann *et al.* (1990), using an enzyme-linked immunosorbent assay, tested sera from 186 cases of invasive cervical cancer and from 172 age-matched controls for IgG and IgA antibodies to HPV-16 E7 peptide and to peptide 245, representing an epitope in E2. Prevalences (%) in cases and controls were 21 and 9 for IgA to E7, 25 and 6 for IgG to E7, 15 and 11 for IgA to peptide 245 and 19 and 11 for IgG to peptide 245, respectively. The ORs for cervical cancer, adjusted for cigarette smoking, education, number of pregnancies, age at first sexual intercourse and time since last Pap, were 5.3 (95% CI, 2.4–11.6) for IgG to E7, 2.7 (95% CI, 1.3–5.3) for IgA to E7, 1.7 (95% CI, 1.2–3.3) for IgG to peptide 245 and 1.0 (95% CI, 0.4–1.6) for IgA to peptide 245. Seropositivity correlated poorly with clinical stage, survival or the presence of HPV–DNA in tumour. The authors concluded that these data suggest that antibodies to E7 are markers for invasive cervical cancer.

In Germany, Steger *et al.* (1990), using Western blot analysis with the entire L1 as antigen to detect HPV-8-specific IgG antibodies, tested serum from 445 randomly selected controls and from 201 patients with various cancers (54 with malignant melanoma, 60 with basal-cell carcinomas, 44 with Hodgkin's disease, 11 with squamous-cell skin carcinoma and 32 with cervical cancer). Prevalences of HPV-8 L1 antibodies were 20% in the control group, 28% in patients with malignant melanoma, 40% in patients with basal-cell carcinomas, 48% in patients with Hodgkin's disease, 73% in patients with squamous-cell skin carcinoma and 37% in patients with cervical cancer. The corresponding estimated odds ratios were [1.6 (95% CI, 0.8–3.1)] for malignant melanoma, [2.7 (95% CI, 1.5–4.9)] for basal-cell carcinomas, [3.7 (95% CI, 1.9–7.3)] for Hodgkin's disease, [10.8 (95% CI, 2.5–52.6)] for squamous-cell skin cancer and [2.4 (95% CI, 1.1–5.5)] for cervical cancer.

Suchánková *et al.* (1991) tested a total of 140 sera from Czech women with either normal cervical diagnoses (n = 85), CIN (n = 21) or cervical cancer (n = 34) compared to 10 children's sera as controls. They assayed IgG antibody by ELISA against an HPV-16 E7 synthetic peptide (16/E7-2) and by Western blot against a genitally engineered HPV-16 E7 fusion protein. The two assay results were highly significantly correlated. Twelve of 34 cancer patients (35.2%) tested positive in one or both assays compared to six of 106 (5.7%) CIN and normal patients. None of the 10 children's sera was reactive.

In a study in USA, Mandelson *et al.* (1992) measured IgG antibodies to HPV-6-encoded L1 and L2 and to HPV-16- and HPV-18-encoded E2, E4, E6, E7, L1 and L2 bacterial fusion proteins in a Western immunoblot assay among 69 cases with invasive cervical cancer and 81 control women. The intensities of the Western blot bands were graded as 0 (negative), +1, +2 or +3. Prevalences (%) for anti HPV-16 E7 among cases and controls were 66 and 71 for grade 0, 12 and 17 for grade +1 and 22 and 12 for grade > +1. The OR (adjusted for age, cigarette smoking, age at first sexual intercourse, lifetime number of sexual partners, number of prior births, number of prior Pap smears in previous decade) for HPV-16 E7 was 3.8 (95% CI, 1.7–8.5) for grade > +1. Odds ratios for the other fusion proteins were not statistically significant. The authors conclude that these findings strongly support an association between antibodies to HPV-16 E7 and invasive cervical cancer.

Müller *et al.* (1992) collected sera from 177 cases of invasive cervical cancer and their 177 controls, and sera from 49 cases of CIN III and their 49 controls, all participating in a case–control study of cervical cancer and CIN III in Spain and Colombia, and examined them for antibodies to four HPV-16 E6 and E7 peptides. Cases of invasive cervical cancer were categorized, according to their PCR-based HPV DNA status, into HPV-16 DNA positive (n = 100), HPV DNA positive for other types (n = 15), and HPV DNA negative (n = 62). All CIN cases selected were positive for HPV-16 DNA. Prevalences (%) of antibodies to any of the four E6 peptides and to any of the four E7 peptides for each group were four and 15, respectively, for controls of invasive cases, 6 and 20 for controls of CIN cases, 16 and 42 for cases of HPV-16 positive invasive cancer, 0 and 27 for cases of invasive cancer positive for other HPV types, 2 and 23 for cases of invasive cancer HPV negative, and 4 and 18 for cases of HPV-16 positive CIN. Estimated crude ORs using positivity to any of the E7-related peptides were [3.7 (95% CI, 2.0–6.7)] for the HPV positive invasive group, [4.0 (95% CI, 2.2–7.4)] for the HPV-16 invasive group, [2.0 (95% CI, 0.5–7.6)] for the other HPV types group, [1.6 (95% CI, 0.7–3.5)] for the HPV-negative group and [0.9 (95% CI, 0.3–2.7)] for the HPV-16 CIN group. Thus, HPV-16 E6/E7 seropositivity was most associated with invasive cancer containing HPV-16.

In the United Kingdom, Ghosh *et al.* (1993) collected sera from 90 cases of CIN and 92 cases of cervical cancer and examined them in comparison with those of 84 healthy controls for antibodies to HPV early proteins E4, E6, E7 and the major capsid protein L1 using Western blot analysis of recombinant HPV proteins. Prevalences (%) for L1/E4/E6/E7 were 4/24/5/10 for controls, 18/36/14/14 for CIN I and condylomata, 10/39/13/6 for CIN II–III and 5/43/35/25 for cervical cancer. In an analysis of 69 cases and 69 controls matched by age, the estimated odds ratios for cervical cancer were [2.5 (95% CI, 1.1–5.5)] for E4, [16.7 (95% CI, 3.5–108.3)] for E6 and [3.9 (95% CI, 1.2–13.0)] for E7.

Jha *et al.* (1993) collected sera from 219 cases of cervical cancer and their 387 controls participating in a population-based case–control study in the United Kingdom and examined them for antibodies to HPV-16 E7 and/or HPV-18 E7 by ELISA. Prevalences (%) for cases and controls were 14 and 7 for HPV-16 E7, 7 and 5 for HPV-18 E7 and 20 and 12 for HPV-16/18 E7. The ORs adjusted for age, number of sexual partners, age at first sexual intercourse, years of oral contraceptive use and number of Pap smears in the past four years were 1.9 (95% CI, 1.1–3.5) for HPV-16 E7, 1.8 (95% CI, 0.8–3.9) for HPV-18 E7 and 1.9 (95% CI, 1.2–3.2) for HPV-16/18 E7. [The Working Group noted that blood collection followed case–control diagnosis by several years.]

Krchnák *et al.* (1993) studied seroreactivity to four HPV-18 E7 peptides in 65 invasive cancer patients and 65 controls matched on age and place of residence in the Czech Republic. Seroreactivity was extremely low in cases (< 5% to any peptide) and totally absent in controls.

Onda *et al.* (1993) in Japan tested seroreactivity to HPV-15 E4 and E7 ELISA antigens as well as HPV DNA type among 51 women with invasive cervical cancer compared to 22 women with CIN (10 with carcinoma *in situ*). The ELISA antigens were bacterially expressed. *L1* consensus primers were used for PCR. Ten out of 17 (58.8%) HPV-16 DNA-positive cancer cases were positive for either anti-E4 or anti-E7, compared to 24 women (12.5%) with cancer

containing another HPV type and 0/10 women with HPV-negative carcinoma. The only other seropositive patient had HPV-negative carcinoma *in situ*.

Viscidi *et al.* (1993) collected sera from 97 cases of invasive cervical cancer and their 121 controls, and sera from 49 cases of CIN and their 48 controls, participating in a case–control study of cervical cancer and CIN in Spain and Cali, Colombia, and examined them for antibodies to HPV-16 E6 and E7 proteins by radioimmunoprecipitation of the in-vitro translated proteins (TT-RIPA). Prevalences (%) for invasive cases and controls were 56 and 2 for E6, 43 and 4 for E7, 72 and 6 for E6 or E7 and 26 and 0 for E6 and E7. Prevalences (%) for CIN cases and controls were 2 and 0 for E6, 6 and 2 for E7, 8 and 2 for E6 or E7 and 0 and 0 for E6 and E7. The estimated crude OR for E6 or E7 was [42.2 (95% CI, 16.4–113.7)] for invasive cervical cancer and [4.2 (95% CI, 0.4–102.0)] for CIN.

Dillner *et al.* (1994) conducted a seroepidemiological study of cervical cancer and HPV in northern Sweden. Sera from 94 cases of incident cervical cancer were compared to 188 age- and sex-matched controls. Twelve antigens from HPV-6, -11, -16 or -18 were tested as a panel for IgG and IgA reactivity. Significantly increased odds ratios of about 3 were found for multiple antigens with the highest OR (9.2 (95% CI, 4.4–19.4)) associated with IgG to an HPV-18 E2 antigen.

Nindl *et al.* (1994) analysed sera obtained from 137 Mexican cervical cancer patients using several different assays for HPV-16 proteins E6 and E7. Specifically, ELISA using as antigen either synthetic peptides or the complex E7 protein was compared to RIPA using viral protein made by in-vitro transcription/translation. As controls, sera from 163 healthy Mexican women and 35 healthy German men were tested. For all assays, cancer cases had higher seroreactivity (17–47%) than controls (who were rarely seropositive).

Sun *et al.* (1994a) extended the work of Viscidi *et al.* (1993) by testing sera further from 97 cases of invasive cervical cancer and 121 controls participating in a population-based case–control study of cervical cancer in Spain and Cali, Colombia. They examined them for antibodies to HPV-16 E6 and E7 by both the radioimmunoprecipitation assay with in-vitro-translated HPV-16 E6 and E7 proteins (TT-RIPA), and an enzyme-linked immunosorbent assay (ELISA). Prevalences (%) for cases and controls using peptide-ELISA were 15 and 5 for E6, 41 and 17 for E7, 46 and 21 for E6 or E7and 8 and 2 for E6 and E7. Prevalences (%) for cases and controls using TT-RIPA were 56 and 2 for E6, 43 and 4 for E7, 72 and 6 for E6 or E7 and 27 and 0 for E6 and E7. The unadjusted ORs using peptide-ELISA were 3.5 (95% CI, 2.1–5.8) for E6, 3.3 (95% CI, 2.7–4.0) for E7, 3.3 (95% CI, 2.8–3.9) for E6 or E7 and 3.5 (95% CI, 1.4–8.9) for E6 and E7. In comparison, the unadjusted odds ratios using TT-RIPA were much larger: 74.7 (95% CI, 25.2–219.5) for E6, 17.7 (95% CI, 11.1–29.7) for E7, 42.2 (95% CI, 28.3–62.7) for E6 or E7 and [∞ (95% CI, 10.6–∞)] for E6 and E7.

Masked sera from 194 cases of invasive cervical cancer and 217 controls participating in a hospital-based case–control study of cervical cancer in Brazil were examined for antibodies to HPV-16 E6 and E7 by radioimmunoprecipitation assay (Sun *et al.*, 1994b). Prevalences (%) for cases and controls were 54 and 6 for E6, 30 and 5 for E7, 63 and 10 for E6 or E7, 21 and 0.5 for E6 and E7 and 41 and 0.5 for high antibody titres to E6 or E7. The ORs adjusted for age, socioeconomic status, number of Papanicolaou smears, parity, number of sexual partners, age at first sexual intercourse and duration of oral contraceptive use were 35 (95% CI, 15–83) for E6,

10 (95% CI, 4–25) for E7, 28 (95% CI, 13–61) for E6 or E7, 87 (95% CI, 10–736) for E6 and E7 and 83 (95% CI, 10–689) for high antibody titres (cpm > 6000) for E7 and 239 (95% CI, 29–1946) for high antibody titres (cpm > 6000) for E6 or E7. Antibody prevalences (%) for HPV-DNA 16 and HPV DNA-negative invasive cases were 61/41 for E6, 43/14 for E7, 73/41 for E6 or E7, and 31/14 for E6 and E7. The sensitivity and specificity for invasive cancer were 63% and 90% for E6 or E7 and 41% and 99.7% for high antibody titres (cpm > 6000) to E6 or E7.

Dillner *et al.* (1995) extended their previous seroepidemiological study of 94 Swedish cervical cancer cases and 184 matched controls by testing the sera for IgG and IgA to several promising new antigens. Seropositivity in the tests was, for most, significantly associated with risk of cervical cancer, with odds ratios ranging up to 15. The three serological assays showing the strongest associations with cervical cancer were E6 : 10 IgA (OR, 15 (95% CI, 6.0–49)), HPV-16 virus-like particle IgG (OR, 9.3 (95% CI, 3.8–27.5)) and E1: 19 IgG (OR, 7.8 (95% CI, 4.0–16)). Even higher odds ratios were observed when combinations of assays were used to discriminate cases from controls.

Nonnenmacher *et al.* (1995) tested the serological response to HPV-16 virus-like particles among 89 women with HPV-16 DNA-positive cervical cancer and 49 with CIN III compared to 162 controls in Colombia and Spain. Seropositivity was strongly associated with risk of cervical cancer [OR, 9.5 (95% CI, 4.9–19.3)] and CIN [OR, 22.6 (95% CI, 9.7–56.8)] in both countries. In contrast to E6 and E7 RIPA data from the same population (Müller *et al.*, 1992), seropositivity to virus-like particles was significantly higher among CIN patients than among cancer patients.

From the studies in Table 41 the following conclusions seems to emerge:

(i) The association of HPV derived antibodies with cervical cancer

Differences in HPV antibodies between cases of cervical cancer and controls have consistently been detected by serological assays. Studies based on HPV-derived antibodies are generally consistent with the findings of the studies based on HPV DNA detection, but with some exceptions, the magnitude of the risk estimates is lower than that obtained by recent HPV DNA-based comparisons.

(ii) Titre of antibody

One study indicated that high antibody titres to E6 and E7 was a stronger discrimination between cases and controls than antibody positivity (Sun *et al.*, 1994b).

(iii) Clinical stage of cervical neoplasia

Although comparability of studies cannot be formally established, antibodies to HPV-derived proteins are more often found in cases of invasive cervical cancer than in cases of preinvasive lesions. One study suggested that seroprevalence of antibodies to E6 protein were directly related to clinical stage (Sun *et al.*, 1994b). The finding is not replicated by other studies (Heino *et al.*, 1993).

2.4.2 Other cancers

(a) Anogenital cancers

There are few case–control studies of the association between HPV and cancers of the genital tract other than cervix (for review see Daling & Sherman, 1992; Holly & Palefsky,

1993). In this section, studies in which history of genital warts was analysed as a surrogate measurement of HPV infection are described.

(i) Cancer of the penis

Table 42 summarizes four studies. HPV-16 was the predominant HPV type (> 60%) in all the reports. One study using PCR reported HPV prevalences of 49% (Maden *et al.*, 1993) for penile cancer and another, using in-situ hybridization, found over 90% HPV positivity for penile intraepithelial neoplasia (PIN) II and III (Demeter *et al.*, 1993).

Maden *et al.* (1993) reported an odds ratio of 5.9 (95% CI, 2.1–17.6) for history of genital warts. A further study tested normal foreskin from adults and found strong associations between both overall HPV and HPV-16 and penile carcinoma (Varma *et al.*, 1991).

Brinton *et al.* (1991) conducted a case–control study of penile cancer in China involving 141 cases and 150 community controls. On examination, 13 cases and one control had genital warts at the time of the study [OR, 15.1 (95% CI, 2.2–647)].

(ii) Cancer of the anus

Six case–control studies have reported on HPV and anal cancer (Table 43). Control material included, variously, colon adenocarcinoma and anal tissue from the control patients. The HPV DNA prevalence observed in epidermoid anal cancer cases ranged from 21.3% (Beckmann *et al.*, 1989) to 80% (Ogunbiyi *et al.*, 1994a). The prevalence of HPV DNA in anal adenocarcinoma was zero in one study (Beckmann *et al.*, 1989). The prevalence among controls ranged from 0% to 14% and the odds ratio estimates were consistently greater than 20.

(iii) Cancers of the vulva

Three case–control studies have reported on cases of cancer of the vulva (see Table 44). In the USA, Brinton *et al.* (1990) compared the history of genital warts in a series of 209 cases of vulvar cancer and 348 community controls. Cases reported warts in 14.8% of instances and controls in 1.4%. The odds ratio was 15.2 (95% CI, 5.5–42.1). A possible interaction with smoking was suggested. Smokers with a history of warts had 35 times the risk of vulvar cancer over controls.

In Denmark, Hørding *et al.* (1993) tested 62 women with vulvar cancer and 110 specimens of vulvar tissue from women who died in accidents. Using PCR techniques, HPV (HPV-6/11, -16, -18, -33) DNA was detected in 30.6% of the cases and in none of the controls. HPV prevalence was more common if the cancer tissue had VIN III lesions in the adjacent region.

In Australia, Kulski *et al.* (1989) used filter in-situ hybridization to test for HPV DNA specimens from the cervix and the vulva of women referred because of clinical or morphological abnormalities or past HPV infection. Of the samples, 74% of the cervical scrapes and 68% of the vulvar scrapes were HPV positive. None of 35 women without signs of cervical abnormality tested positive for HPV DNA. [The difference in HPV prevalence between cases and controls may be overestimated due to the selection criteria of cases and controls.]

Table 42. Case–control studies of penile neoplasms

Reference and country	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Brinton *et al.* (1991) China	141 cases of penile cancer	150 community controls	Case, 9 Control, 1 HPV prevalence assessed by genital warts	Genital warts: [15.1 (2.2–647)]	History of genital warts	
Varma *et al.* (1991) USA	23 cases (30 specimens) of penile carcinoma	20 foreskins from adults	*Cases* HPV, 87 HPV-16, 78 *Controls*, 0	[∞ (17.7–∞)] [∞ (11.3–∞)]	PCR In-situ hybridization	Cases and controls were tested on paraffin embedded specimens from archival material. Tests included HPV-6/11 and -16 probes. Prevalence reported includes the combined results of the PCR and in situ tests and refers to the 23 patients.
Demeter *et al.* (1993) USA	44 PIN	88 men with condyloma acuminatum (not tested for HPV)	PIN I, 62.5 (58% HPV-6/11) PIN II, 90 (60% HPV-16, -31) PIN III, 92.3 (92.3% HPV-16)		RNA–RNA in-situ hybridization Southern blot	Controls not tested for HPV. Concordance on HPV between in situ hybridization and Southern blot was 74%. Concordance on type among positive cases was 100%. No evidence of viral integration
Maden *et al.* (1993) USA	110 penile cancers (67 tested for HPV)	355 men from general population matched by age at date of diagnosis	*Cases* HPV, 49 HPV-16, 70 *Controls*, not tested HPV prevalence assessed by genital warts	History of genital warts: 5.9 (2.1–17.6)	PCR History of genital warts	Cases tested on paraffin-embedded specimens. Subjects interviewed were 50.2% of the cases and 70.3% of the controls.

[ ] Calculated by the Working Group
PIN, penile intraepithelial neoplasia; PCR, polymerase chain reaction

Table 43. Case–control studies of anorectal cancer

Reference and country	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Daling *et al.* (1987) USA	148 anal cancers	166 colon cancers	*History of genital warts* Squamous-cell homosexual men, 47.1 heterosexual men, 28.6 women, 28.3 Transitional-cell, 0 Control, 1–2	26.9 (2.8-257.1)	History	No HPV tests performed
Palmer *et al.* (1989) United Kingdom	45 anal cancers	28 sex-matched patients undergoing rectal surgery	HPV, 61 HPV-16, 56 HPV-18, 5 Control, 0	[∞ (8.8–∞)] [∞ (7.4–∞)] [∞ (0.1–∞)]	Southern blot, dot blot In-situ hybridization	HPV-16 was predominantly integrated into cellular DNA
Beckmann *et al.* (1989) USA	23 anorectal carcinomas *in situ* 47 invasive carcinomas 42 transitional-cell carcinomas 14 adenocarcinomas	110 colon adenocarcinomas (fixed tissue)	*In situ*, 56.5 Invasive, 21.3 Transitional, 2.4 Adenocarcinoma, 0 Control, 0	[∞ (27–∞)] [∞ (6.2–∞)] [∞ (0.1–∞)]	In-situ hybridization	Type distribution was similar between in situ and invasive carcinomas. Of 23 HPV-positive cases, 8 were HPV-6, 10 were HPV-16, 1 was HPV-18, and 4 were HPV-X. Control tissue was a strip of anal epithelium extending from anorectal junction to the anal margin.
Scholefield *et al.* (1992) United Kingdom	152 women with CIN III investigated for AIN/anal cancer (n = 29)	50 women without CIN (2 with CIN I) (AIN, n = 0)	Case, 51 Control, 14	[6.5 (2.1-21.1)]	PCR	
Shroyer *et al.* (1992) USA	5 anorectal cancers	22 colon adenocarcinomas	Case, 100 Control, 0	[∞ (12.1–∞)]	PCR (100%) In-situ hybridization (80%)	
Ogunbiyi *et al.* (1994a) United Kingdom	40 invasive vulvar cancers investigated for HPV anal infection and anal cancers from biopsy material taken using a proctoscope in conjunction with a colposcope	80 hospital	Vulvar cancer, 75 Anal SIL, 76.9 Anal cancer, 80 Control, 13.7	Anal neoplasms: 22.0 (5.4–98.6)	PCR	Study of concurrent lesions in the vulva and the anal region. Controls were selected if they had no history of anogenital HPV infection or neoplasia. Cases were not excluded if history of HPV infection. Only HPV-16 investigated

[ ] Calculated by the Working Group

AIN, anal intraepithelial neoplasia (grades I, II, III); SIL, squamous intraepithelial lesion; PCR, polymerase chain reaction; CIN, cervical intraepithelial neoplasia; X, HPV type unknown

Table 44. Case–control studies of vulvar and ovarian cancers

Reference and country	Cases (Number and type)	Controls (Number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Kaufman *et al.* (1987) USA	12 ovarian cancers	4 normal ovaries	Case, 83 Control, 0	HPV-6b, [∞ (1.1–∞)]	In-situ hybridization	Signal intensity specifically located in the nucleus using HPV-6b probe. Signal with HPV-11 very weak
Kulski *et al.* (1989) Australia	128 CIN or HPV signs	35 women free of cervical abnormalities	Cervical scrape, 74 Vulvar scrape, 68 Cervical + vulvar, 57 HPV-16/18, 61 HPV-6/11, 14 HPV-31, -33, 3 Control, 0	[∞ (23.6–∞)] [∞ (11.1–∞)]	DNA filter in-situ hybridization	Study of the coexistence of HPV infections in the vulva and the cervix. Cases included patients selected because of HPV clinical and/or morphological signs. HPV types investigated were 6/11, 16/18, 31/33.
Brinton *et al.* (1990) USA	209 vulvar cancers	348 community controls	*History of genital warts* Case, 14.8 Control, 1.4	15.2 (5.5-42.1)	History of warts	No HPV detection was performed. Interaction between genital warts and smoking is suggested.
Sherman *et al.* (1991) USA	53 vulvar cancers 180 VIN	466 population controls	Vulvar cancer, 41.7 VIN, 38.7 Control, 4.5	17.3 (6.3-47.2) 15.8 (8.4-29.8)	History of genital warts (condyloma)	
Hording *et al.* (1993) Denmark	79 vulvar cancers (62 tested)	110 normal vulvar tissues from accident casualties	Case, 30.6 (61% with associated VIN III and 13% without VIN III) HPV-16, 68.4 HPV-18, 15.8 HPV-33, 15.8 Control, 0	[∞ (40–∞)]	PCR	HPV-positive cases were younger and more frequently had multicentric neoplasms. HPV-6/11, -16, -18 or -33 were investigated. No cases of HPV-6/11 were found.

[ ] Calculated by the Working Group
VIN, vulvar intraepithelial neoplasia; PCR, polymerase chain reaction

(b) Other cancers

(i) Cancer of the mouth

Three studies have compared HPV DNA prevalence in cases of oral cancer and tissues from individuals without oral cancer (Table 45). In three of them the prevalence of HPV among cases was significantly higher than among controls.

In Taiwan, Chang *et al.* (1989) compared tissue specimens from 17 cases of oral cancer with specimens from normal gingival tissue from 17 patients using Southern blot techniques. Among cases, 13/17 (76.4%) were HPV-16 positive and, among controls, 1/16 (5.9%) was positive for an unknown type. The estimated OR was [52.0 (95% CI, 4.4–1454.0)]. Three oral papillomas were also studied and all were HPV-16 positive; the HPV-16 sequences detected in the oral cancers and papillomas were episomal. Twelve of the 17 cases were also betel chewers and/or smokers.

Brandsma and Abramson (1989) investigated 21 cases of cancer of the mouth (10) and tongue (11) using Southern blot and compared HPV detection with site-matched specimens from 18 controls. Two cases were HPV positive compared with none of the controls (see also Table 46).

Maden *et al.* (1992) investigated 131 male cases of oral cancer and 136 male population controls matched by age and date of diagnosis from Washington State, USA. Random digit dialling was used to identify eligible controls. Exfoliated cells from the mouth were used as primary material for HPV testing and PCR methods were used. The study included an extensive questionnaire on exposure to the key risk factors for oral cancer and the risk estimates were adjusted for age, smoking, alcohol consumption and lifetime number of sexual partners. The prevalence of HPV-6 DNA was 19% among cases and 9% among controls. The crude OR was 2.9 (95% CI, 1.1–7.3) and the adjusted OR was 2.8 (95% CI, 1.1–7.3). HPV-16 DNA was found in 6% of the cases and in 1% of the controls. The crude OR was 6.2 (95% CI, 0.7–52.2). In this study no association was found between oral cancer and history of the most common sexually transmitted diseases including herpes simplex virus (HSV)-1 or HSV-2. However, a non-significant increase in risk was associated with the presence of HSV-2 antibodies (adjusted OR, 1.8 (95% CI, 0.7–4.6)).

In Germany, Ostwald *et al.* (1994) used a PCR-based method to compare HPV DNA prevalence between 26 cases of oral carcinoma and 97 healthy volunteers. Cell scrapes from the buccal mucosa were collected from both cases and controls. From cases, additional specimens were taken from the normal mucosa distant from the cancer location. Among cases, 61% showed evidence of HPV DNA against 1% among controls [odds ratio, 153.6 (95% CI, 17.7–3449.4)] and 18 out of the 26 cases were smokers.

Table 46 summarizes studies that investigated cases of cancers from different sites in the upper digestive and respiratory tracts in relation to the presence of HPV DNA. Controls for these cases were normally obtained from normal patients undergoing scrapes or biopsies from matched sites for reasons other than cancer.

Table 45. Case–control studies of cancer of the oral cavity

Reference and country	Cases (number and type)	Controls (number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Chang *et al.* (1989) Taiwan	17 oral carcinomas 3 oral papillomas	17 normal gingival tissues	*HPV-16* Carcinoma, 76.4 Papilloma, 100 *Any HPV* Control, 5.9	[52.0 (4.4–1454.0)]	Southern blot	HPV-6/11, -16 and -18 investigated. Interaction between betel chewing and HPV is suggested.
Abdelsayed (1991) USA	18 dysplasias or carcinomas in non-alcohol/non-tobacco users	18 dysplasias or carcinomas in alcohol and/or tobacco users	Case, 0 Control, 11		In-situ hybridization	HPV-6/11, -16/18 and -31/33/35 investigated. One control was positive for HPV-6/11 and the other for HPV-16/18.
Maden *et al.* (1992) USA	131 oral cancers	136 population controls matched on age and date of diagnosis	*Case* HPV-6, 19 HPV-16, 6 *Control* HPV-6, 9 HPV-16, 1	2.8 (1.1–7.3) 6.2 (0.7–52.2)	PCR	Odds ratio for HPV-6 adjusted for age, smoking, alcohol consumption and lifetime number of partners
Müller *et al.* (1994) Germany	26 oral carcinomas	97 normal oral mucosae from volunteers	Case, 61 (HPV-16 and -18 predominant) Control, 1	[153.6 (17.7–3449.4)]	PCR and Southern blot	HPV prevalence in the normal mucosa of cases decreased with distance from the cancer site.

[ ] Calculated by the Working Group
PCR, polymerase chain reaction

Table 46. Case–control studies of cancer of the upper digestive and respiratory tracts

Reference and country	Cases (Number and type)	Controls (Number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Brandsma & Abramson (1989) USA	101 carcinomas Nose (2) Mouth (10) Tongue (11) Tonsil (7) Pharynx (8) Larynx (60) Oesophagus (3)	116 hospital controls matched by site	Nose, 0 Mouth, 0 Tongue, 18.2 Tonsil, 28.5 Pharynx, 12.5 Larynx, 5.0 Oesophagus, 0 Control, 1.7	All cases [4.90 (0.93–34.3)]	Southern blot	
Bryan *et al.* (1990) United Kingdom	13 pharynx and larynx carcinomas	14 with normal nasopharynx mucosa	Case, 84.6 HPV-11, 30.8 HPV-6/11, 53.8 Control, 64.3	[3.1 (0.37–30.26)]	PCR	Specimens tested for HPV-6 and -11 types
Niedobitek *et al.* (1990) Germany	28 carcinomas of the tonsil	30 chronic tonsilitis	Case, 21.4 Control, 0	[∞ (1.4–∞)]	In situ hybridization	
Williamson *et al.* (1991) South Africa	14 oesophageal carcinomas	41 hospital controls	Case, 43 Control, 15	[4.38 (0.92–21.5)]	PCR	Biopsies from normal mucosa of the cases showed 66.6% HPV positivity rate.
Benamouzig *et al.* (1992) France	12 oesophageal cancers	24 hospital controls	Case, 25 Control, 4.2 (dot blot results)	[7.67 (0.57–220.8)]	In-situ hybridization Dot blot	Dot blot hybridization gave higher positivity rates than in-situ hybridization. Normal mucosa of the cases showed HPV DNA in 41.6%.
Snijders *et al.* (1992b) The Netherlands	10 carcinomas of the tonsil	7 tonsilitis patients	Case, 100 HPV-16, 40 HPV-33, 40 HPV-16/33, 10 Control, 0	[∞ (7.5–∞)]	PCR Southern blot RNA–PCR In-situ hybridization	E7 transcripts of HPV-16 were exclusively located within carcinoma cells. Stroma was negative.
Tyan *et al.* (1993) Taiwan	30 carcinomas of the nasopharynx 44 other carcinomas of the head and neck	11 normal tissues from nasopharynx and oral cavity	Nasopharynx, 46.7 Other head and neck cancer, 29.5 Control, 9.1	[8.4 (1.0–406.3)] [4.1 (0.5–195.8)]	PCR and DNA sequencing	Coinfection of HPV with EBV occurred in 46.6% of nasopharingeal carcinomas and in 11.4% of the other head and neck cancers. HPV-16 accounted for 96% of the HPV DNA positive specimens. Only HPV-16 DNA was tested.

Table 46 (contd)

Country (Reference)	Cases (Number and type)	Controls (Number and type)	HPV prevalence (%)	Odds ratio (95% CI)	HPV test	Comments/adjustments
Watanabe *et al.* (1993) Japan	12 carcinomas of the tonsil and pharynx	28 tonsilitis	Case, 20 Control, 0 (PCR results)	[∞ (4.4–∞)]	Dot-filter Southern blot PCR	Study comparing primarily the results of the three detection methods

[ ] Calculated by the Working Group
PCR, polymerase chain reaction; EBV, Epstein-Barr virus

(ii) Cancer of the tonsil

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 [$p < 0.001$].

Snijders *et al.* (1992b) used PCR and Southern hybridization techniques to test 10 cases of carcinoma of the tonsil and seven cases of tonsillitis as controls. All cases tested positive for HPV against none of the controls [OR, ∞ (95% CI, 7.5–∞)]. The presence of HPV DNA in cancer cells was further tested with in-situ hybridization. In all instances, the stroma was HPV-negative and HPV DNA was detected in the cancer cells.

In Japan, Watanabe *et al.* (1993) tested 12 cases of carcinoma of the tonsil and pharynx and used 28 specimens from cases of acute tonsillitis as controls. Three methods for HPV testing were used, dot-filter, Southern hybridization and PCR. Using the PCR results, the HPV DNA prevalence was 20.0% among cases and zero in controls [OR, ∞ (95% CI, 4.4–∞)].

(iii) Cancer of the pharynx, larynx and oesophagus

Table 46 summarizes the results of studies that included short series of cases of cancers of the pharynx, larynx, oesophagus and a miscellaneous group of other cancer sites of the head and neck. HPV DNA was detected using a variety of hybridization methods. Brandsma and Abramson (1989) tested 101 cases of carcinomas of the upper respiratory and digestive tract against a site matched group of 116 of hospital controls. For all cancer sites combined, the OR was [4.9 (95% CI, 0.93–34.4)]. In most investigations, the HPV prevalence ratio among cases was higher than 1. However none of the studies showed statistically significant differences. [The number of specimens examined in each study was small (< 50). Lack of power may explain the lack of significance of the differences observed.]

(iv) Colon cancer

In the USA, three reports from the same research group explored the presence of HPV markers in specimens of colon cancer using different techniques. Kirgan *et al.* (1990a,b) compared 43 cases of colorectal cancer (30 invasive and 13 *in situ*), 30 adenomas of the colon and 30 specimens of normal colon. The methods used initially to detect HPV were immuno-histochemistry on sections from paraffin-embedded blocks followed by in-situ hybridization of the specimens that tested positive. HPV antigen prevalence was 97% among cancers, 60% among adenomas and 23% among normal controls [$p < 0.001$ for comparisons of HPV antigen between each group of cases and controls]. HPV DNA was found by in-situ hybridization in 27% of adenomas, 43% of the carcinoma specimens tested and in none of the controls. [Differences in HPV DNA prevalence were significant only for the group of carcinoma *in situ* (69%) and controls (0%) ($p = 0.004$)]. In a third study, *L1*-PCR and Southern blot were used in specimens from 38 carcinomas, 21 adenomas and 24 normal mucosa. Prevalence rates were 32% in carcinomas, 38% in adenomas and 8% in normal mucosa [OR for carcinoma, 5.7 (95% CI, 1.0–41.4); OR for adenoma, 6.8 (95% CI, 1.1–55.0)] (McGregor *et al.*, 1993). [These studies from one laboratory are not consistent with the case series reported in section 2.2.3.]

(v) Cancer of the ovary

One study (see Table 44) (Kaufman *et al.*, 1987) compared HPV DNA prevalence in 12 ovarian cancers and from four ovaries of women with benign disease of the ovary or uterus. Ten of the cases of ovarian cancer (83%) were HPV positive against none in the control groups [$p < 0.001$]. HPV-6b was the only type identified.

(vi) Cancer of the urinary bladder

In Japan, Anwar *et al.* (1992b) compared 48 specimens from bladder cancer cases with 21 specimens from normal bladder. PCR methods were used in combination with dot and Southern blot hybridization. Prevalences of HPV DNA were 81% in cases and 33% in controls [OR, 8.67 (95% CI, 2.38–33.27)]. HPV-16, -18 and -33 accounted for 62% among cases and 14 % among controls [OR, 10.0 (95% CI, 2.3–50.0)]. [These data are not consistent with the case series reported in section 2.2.3.]

(vii) Cancer of the conjunctiva

In the USA, McDonnell *et al.* (1986) reported the prevalence of HPV capsid antigen using immunoperoxidase staining of 50 conjunctival papillomas and 61 dysplastic conjunctival lesions (24 mild to moderate dysplasia, 37 severe dysplasia to invasive cancer). Positive staining was seen in 23 papillomas (46%) and in five dysplastic lesions (8.2%) but in none (of 20) control conjunctival sarcoidosis biopsy specimens [OR, ∞ (95% CI, 3.6–∞) and OR, ∞ (95% CI, 0.3–∞), respectively].

(viii) Lung cancer

In France, Bejui-Thivolet *et al.* (1990b) reported on the prevalence of HPV DNA in 10 cases of squamous-cell metaplasia and 33 cases of squamous-cell carcinoma of the bronchus. In-situ hybridization with butinylated probes types 6, 11, 16 and 18 were used on paraffin-embedded tissue. HPV-6 DNA was identified in one bronchial metaplasia. HPV DNA was found in six carcinomas (18%). HPV-18 was found in three cases, HPV-16 in one case, HPV-11 in one case and HPV-6 in one case. Ten specimens from normal mucosa and alveolar tissues were used as controls and were all negative for HPV DNA [$p = 0.18$].

2.4.3 Cofactors

Given the high prevalence of anogenital HPV infections and the relative rarity of anogenital cancers, it is worth considering those additional carcinogenic cofactors that might cause a small proportion of infected persons to progress to malignancies. The discussion of HPV 'cofactors' refers, accordingly, to exposures that accelerate or otherwise increase the rate of transition to malignancy following HPV infection, including factors that increase the likelihood that infection will persist.

Possible HPV cofactors for cervical cancer will be mentioned briefly here for completeness, but a complete summary is not attempted. The magnitude of the association between HPV and cervical neoplasia is such that even if there is confounding or effect modification from other factors, this could not explain the large relative risks seen in the epidemiological studies. The separate topic of the identification of HPV-independent factors that might cause the small

proportion of apparently HPV-negative cervical cancers will not be addressed and nor will HPV as a cofactor for other cancers be described, because of the scarcity of relevant data.

To study HPV cofactors for cervical cancer requires a study group known to be exposed to HPV. Thus, the review of studies of cofactors is restricted to recent projects with reliable HPV DNA typing data and odds ratios for other factors reported within a well-defined HPV-positive stratum (Bosch *et al.*, 1992; Koutsky & Kiviat, 1993; Muñoz *et al.*, 1993; Schiffman *et al.*, 1993; Eluf-Neto *et al.*, 1994; Muñoz *et al.*, 1994; de Sanjosé *et al.*, 1994; Strickler *et al.*, 1995).

The variable 'earlier age at first sexual intercourse' has been observed to be a risk factor among HPV-positive women for both invasive cancer (Bosch *et al.*, 1992) and CIN III (Muñoz *et al.*, 1993). This sexual behavioural variable is likely a proxy for age at first exposure to HPV infection and could be reflecting 'latency' (here meaning the long time from HPV exposure to cancer diagnosis) or a 'vulnerable period' in which infection at younger ages is more carcinogenic for some reason.

High parity (e.g. more than five live births) has been consistently observed to elevate the risk among HPV-positive women of both cervical cancer (Bosch *et al.*, 1992; Eluf-Neto *et al.*, 1994) and CIN III (Muñoz *et al.*, 1993; Schiffman *et al.*, 1993). However, interpretation is complicated by the finding of similar elevations of risk associated with multiparity among apparently HPV-negative women. In epidemiological terms, parity would therefore be considered to be an 'independent' risk factor if the HPV-negative women were truly negative (a questionable assumption). Hormonal, traumatic, immunological and nutritional hypotheses have been advanced to explain the risk associated with multiparity, but there are insufficient data to decide among them.

Also suggestive of hormonal influences, the use of oral contraceptives has been found to be a possible HPV cofactor in several studies of invasive cervical cancer (Bosch *et al.*, 1992; Eluf-Neto *et al.*, 1994) and CIN III (Negrini *et al.*, 1990; Muñoz *et al.*, 1993; Schiffman *et al.*, 1993). Because of the concordant findings regarding parity and oral contraceptive use, hormonal influences can be considered the most promising candidates in the search for HPV cofactors.

Two studies (Koutsky *et al.*, 1992; de Sanjosé *et al.*, 1994) have reported that seropositivity to *Chlamydia trachomatis* is associated with an elevated risk of CIN III among HPV-positive women. Another study (Strickler *et al.*, 1995) found HTLV-1 seropositivity to be a possible viral cofactor. However, no putative viral cofactor has been associated consistently with risk in the few studies presenting relevant data, with the obvious exception of HIV-associated immunosuppression leading to an increased risk of HPV infection and CIN (and anal neoplasia). A possible etiological role of HSV-2 in cervical carcinogenesis, suggested by a previous generation of seroepidemiological case–control studies, has not been consistently observed in more recent studies that take HPV infection properly into account (Hildesheim *et al.*, 1991; Bosch *et al.*, 1992; Jha *et al.*, 1993; Koutsky & Kiviat, 1993; de Sanjosé *et al.*, 1994).

Cigarette smoking has also not been observed to be a strong HPV cofactor for either cervical cancer, despite strong *a priori* suspicions (Bosch *et al.*, 1992; Eluf-Neto *et al.*, 1994) and consistently elevated risks in case–control studies (Winkelstein, 1990), or high-grade CIN (Koutsky *et al.*, 1992; Muñoz *et al.*, 1993; Schiffman *et al.*, 1993). However, no larger epidemiological study of smoking, HPV and invasive cervical cancer has yet been performed in a geographic region where intensive smoking among women is prevalent.

In addition to a possible genotoxic role, it is also possible that smoking plays a role in immunosuppression (Barton *et al.*, 1988) permitting an incipient HPV infection to become persistent.

At least one group has presented case–control data suggesting that relative nutritional deficiencies of folate or other micronutrients (e.g. vitamin C, carotenoids) could be an HPV cofactor (Butterworth *et al.*, 1992). The case–control and scant clinical trial evidence for nutritional cofactors are, overall, still weak.

Immunological variables are likely to be among the most important HPV cofactors, based on the HIV immunosuppression data and animal models. Ongoing case–control and cohort studies among HPV-infected women are considering, in particular, the roles of HLA genotypes, seroreactivity, and in-vitro measurements of cell-mediated immunity (e.g. lymphocyte IL-2 production following HPV antigen challenge).

Finally, it is interesting to consider that most HPV cofactors identified in epidemiological studies have tended to distinguish high-grade CIN and invasive cervical cancer on the one hand from low-grade CIN and HPV infection on the other. Few cofactors, if any, have been observed to distinguish invasive cancer from high-grade CIN, except for the substantially older age of invasive cases (Moreno *et al.*,1995). This observation, if confirmed, might suggest that the risk of progression of HPV infection/low-grade CIN to prevalent, chronic CIN III is more influenced (and modifiable) by external factors than the subsequent risk of invasion, which could be stochastic and primarily dependent on time spent with CIN III.

2.5 Special populations

2.5.1 Skin cancer in patients with epidermodysplasia verruciformis (EV)

Epidermodysplasia verruciformis (EV) is a very rare, inherited condition which was first described in 1922 (Lewandowsky & Lutz, 1922). During the subsequent 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 on sun-exposed sites. Basal-cell carcinomas, although described, appear to be rare, and there are no reports of increased risk of malignant melanoma in EV patients. There are no published SMRs available for skin cancer in this population, but a squamous-cell carcinoma:basal-cell carcinoma ratio of 16:1 was reported in one study of 66 EV patients. A postal questionnaire sent to Japanese doctors found that most of these EV patients had developed virus warts by the age of 10 years, whilst squamous-cell carcinoma developed between the ages of 30–50 years. The average lag time between onset of EV-type skin wart infection and squamous-cell carcinoma was 24.5 years (Tanigaki *et al.*, 1986).

Other virus-associated diseases, including hepatitis B infection (van Voorst Vader *et al.*, 1986; see also IARC, 1994b), genital carcinoma and Burkitt's lymphoma have been described in EV patients (Lutzner & Blanchet-Bardon, 1985). 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). The genetic basis of this disease is not known.

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 sun-exposed sites (Tanigaki *et al.*, 1986). Furthermore, squamous-cell carcinomas appear to develop more frequently in Caucasian EV patients living in sub-tropical and tropical climates than 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 living in Europe (Orth *et al.*, 1979), 58% in Japan (Tanigaki *et al.*, 1986) and 100% of patients living in South America (Rueda & Rodriguez, 1976).

HPV types in warts and skin cancers in EV patients

(i) Skin warts

EV patients develop a variety of skin warts. Common and plane warts (verruca vulgaris (VV) and verruca planar (VP)) are also seen in the general population, but there are also EV-specific lesions, namely red plaque-like lesions (RP) and scaly, pityriasis versicolor-like lesions (PV) (Orth *et al.*, 1979).

Table 47 summarizes studies of HPV typing of EV-associated skin warts. All studies used hybridization methods without amplification. Reports are based on a limited number of specimens, often from single patients, and only one study includes information on control material (Jacyk *et al.*, 1993a). Some studies do not specify the type of skin wart examined and many do not specify the number of samples examined.

VP and VV lesions appear to be associated predominantly with HPV types 3 and 10. However, the large number of HPV types found in EV lesions where the clinical type of wart has not been specified (HPV-12, -15, -21, -22, -23, -25, -46 and -47; reviewed in de Villiers, 1989) suggests that this apparent association should be interpreted with caution. Kanda *et al.* (1989) found no relationship between HPV type and clinical lesion. HPV-5, -8, -9, -14, -17, -20 and -38 have been found in PV-like virus warts in EV-patients. Multiple HPV types were found in PV-like lesions.

(ii) Squamous-cell carcinoma

Table 48 summarizes data available on HPV types found in EV-associated skin cancer, including single case reports and case series. Reports are based on very limited numbers of tumours and no study includes information on control material. All studies were performed using hybridization methods without amplification.

HPV-5, -8, -14, -17 and -20 have been identified in EV-associated invasive squamous-cell carcinoma, although only in a small number of samples. HPV-16 was isolated from a case of Bowen's disease of the thumb in one patient (Ostrow *et al.*, 1987b). HPV-5, -8 and -20 DNA has been found in squamous-cell carcinoma as oligomers and monomers, some in concatemeric form (approximately 100 copies/cell) (Pfister *et al.*, 1983b; Deau *et al.*, 1991; Yutsudo *et al.*, 1994). HPV-20 transcripts in squamous-cell carcinomas were demonstrated in one study (Yutsudo *et al.*, 1994).

Both wild-type HPV-5 genomes and deleted forms have been found in primary and metastatic tumour (Ostrow *et al.*, 1982; Yabe *et al.*, 1989). In addition, sequence variants of the

Table 47. HPV detected by Southern blot in skin warts in EV patients

Reference	Study area	No. of lesions (No. of patients)	Clinical description	Overall HPV positivity (%)	HPV type-specific positivity					
					1–3 and 10	5	8	17	20	Others[a]
Orth *et al.* (1979)	Europe	Multiple lesions (14)	VP	NA	+					HPV-2
			RP	NA	+	+				
			PV	NA		+				
Ostrow *et al.* (1982)	USA	Multiple lesions (2)	NA	NA		+				
Pfister *et al.* (1983b)	Turkish patient	Multiple lesions (1)	NA	NA		+	+			4 others not identified
Kremsdorf *et al.* (1984)	France	Multiple lesions (8)	VV/VP	NA					+	HPV-14a, -14b, -15 and -21
			PV	NA				+		HPV-19, -21, -23 and -24
Lutzner *et al.* (1984)	France	Multiple lesions (11)	NA	NA	+	+	+		+	HPV-2, -14, -22, -9 and -9-related
van Voorst Vader *et al.* (1986)	Netherlands	Multiple lesions (1)	NA	NA		+	+	+		HPV-19 and -24
Ostrow *et al.* (1987b)	USA	6 (1)	VV/VP	83	4/6					One type not identified. HPV-16 in Bowen's disease
Kanda *et al.* (1989)	Japan	Multiple lesions (12)	VP, VV	NA	+					HPV-14 and -38
			PV	NA		+		+	+	HPV-12, -14 and -38 (multiple HPV types in some lesions)
			RP	NA						
			RP	NA					+	HPV-14 and -21
Jacyk & de Villiers (1993)	South Africa	Multiple lesions (20)	VP	NA	+	+				
			PV	NA		+		+		
Jacyk *et al.* (1993a)	South Africa	Multiple lesions (5)	Seborrheic keratoses	NA		+				No HPV found in 10 keratoses from non-EV patients
			PV	NA		+		+		
			VP	NA	+	+				
Jacyk *et al.* (1993b)	South Africa	Multiple lesions (1)	PV	NA						HPV-9
			VV	NA						HPV-4 and -9
Yutsudo *et al.* (1994)	Japan	Multiple lesions (1)	PV	NA				+	+	HPV-38
		Multiple lesions (1)	VV, VP	NA	+					

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

[a]Of those types tested

Table 48. HPV detected by Southern blot in skin cancer in EV patients

Reference	Study area	No. of tumours (No. of patients)	Overall HPV positivity (%)	HPV type-specific positivity number or (%)				Comments
				5	8	17	20	
Ostrow *et al.* (1982)	USA	2 (2)		(2/2)				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 (1)		(1/1)				100 copies/cell. Oligomeric DNA, some persisting in concatemeric form
Lutzner *et al.* (1984)	France	7 (5)		57	29			HPV-14 found in one SCC. The seven skin cancers include three cases of Bowen's disease and four cases of SCC.
Orth (1986)	International	28* (14)	96	75	17			HPV-14b (1 tumour) (approximately 100–300 copies/cell). *Whether all of these are new cases that have not been reported previously is unclear.
van Voorst Vader *et al.* (1986)	Netherlands	1 (1)		(1/1)				HPV-17 and -24 found in peri-lesional tissue
Ostrow *et al.* (1987b)	USA	1 (1)						HPV-16 found in Bowen's disease of thumb
Kanda *et al.* (1989)	Japan	6 (NA)	33			17	17	
Yabe *et al.* (1989)	Japan	1 (1)		1/1				HPV-5 found in benign lesions. Deleted forms of HPV-5 found in both primary and metastatic tumour from same patient
Yutsudo *et al.* (1994)	Japan	1 (1)					(1/1)	100 copies/cell. Episomal DNA as oligomers and monomers. HPV transcripts in SCC suggests infection.

EV, epidermodysplasia verruciformis; NA, not available; SCC, squamous-cell carcinoma; PV, pityriasis versicolor-like lesion

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.

2.5.2 *Studies of cancer incidence in transplant patients*

Tumours that may have a viral etiology may be those that occur at high frequency in transplant recipients. The most recent cohort study comparing cancer incidence in transplant recipients with that in the general population (Birkeland *et al.*, 1995), confirms the findings of many smaller studies (see, for example, Matas *et al.*, 1975; Kinlen *et al.*, 1979). In the study of Birkeland *et al.* (1995), 5692 transplant recipients (1964–82) were followed from 1968–86 using data from the Nordic cancer registries. The transplant recipients were found to have a twofold to fivefold increased risk of many common tumours, including those of the colon, rectum, larynx and lung. A very high, 10–30-fold increase was seen for non-Hodgkin's lymphoma, skin cancer and urogenital and anogenital carcinomas.

(a) *HPV infection, CIN and invasive cervical and anogenital carcinoma in transplant recipients*

A number of studies have been conducted to estimate the prevalence of cervical lesions and/or HPV infection among groups of women who are immunosuppressed following renal transplantation. Some have also included control groups of immunocompetent women. The results of these studies are summarized in Table 49.

(i) *HPV infection and CIN*

The prevalence of cervical HPV infection in transplant recipients has been estimated at between 20 and 45%, while condylomata have been reported in 8–30% of women (Table 49).

In one study (Alloub *et al.*, 1989) in the United Kingdom, there was no significant difference between the prevalence of HPV DNA in 49 renal transplant patients attending for routine follow-up and in 69 control women from a gynaecology ward who had no history of CIN and had had a negative smear within the last two years (45% and 38%, respectively). HPV-16/18 DNA was, however, more common in the transplant recipients than controls (27% and 6%, respectively; $p < 0.005$) but there was no significant difference in the prevalence of HPV-6/11 (24% and 32%, respectively).

Two other studies have reported significantly increased rates of HPV infection in transplant recipients. In the USA, Halpert *et al.* (1986) found cytological evidence of HPV in 18 of 81 women (22%) who had received a renal transplant more than one year previously, and in only two of 81 (2.5%; $p < 0.01$) hospitalized immunocompetent women, matched to the transplant patients by age, race and age at first coitus. Fairley *et al.* (1994a) used PCR to detect HPV in 15 of 69 (22% women who had received a renal transplant in Australia more than six months previously, compared with 18 of 89 (20%) of women on dialysis therapy and one of 22 (4.5%) 'normal' women with mild renal impairment. In this latter study, five (7%) transplant recipients, 4 (4%) women on dialysis and no 'normal women' had CIN.

Schneider *et al.* (1983) reviewed the histological reports and slides from a group of 132 women who received renal transplants in Virginia, USA, between 1962 and 1979. Eleven women (8%) developed koilocytotic atypia, which is considered diagnostic of condyloma, a

Table 49. Prevalence of cervical HPV infection, CIN and invasive carcinoma of the cervix in renal allograft recipients

Reference	Area	Detection method	Number and % with HPV or lesion				Relative risk and 95% CI	Comments
			Transplant patients		Controls			
			No.	%	No.	%		
HPV Infection								
Schneider *et al.* (1983)	USA	Cytology (koilocytotic atypia)	11/132	8	–			
Halpert *et al.* (1986)	USA	Cytology	18/81	22	2/81	2.5		$p < 0.01$
MacLean *et al.* (1986)	New Zealand	Cytology	5/24	21	–			
Alloub *et al.* (1989)	United Kingdom	DNA hybridization	22/49	45	26/69	38		
		HPV-6/11	12/49	24	22/69	32		
		HPV-16/18	13/49	27	4/69	6		$p < 0.005$
Gentile *et al.* (1991)	Italy	Cytology/histology	12/39	31	–			
Gitsch *et al.* (1992)	Germany	Histology (condyloma)	7/23	30	–			
Fairley *et al.* (1994a)	Australia	PCR (*L1* consensus primers)	15/69	22	1/22	4.5		$p = 0.05$
Invasive carcinoma								
Schneider *et al.* (1983)	USA		1/132	0.8	–			
MacLean *et al.* (1986)	New Zealand		0/24	0	–			Mean time since transplant 61 months
Fairley *et al.* (1994b)	Australia & New Zealand		12 cases	NA	–		SIR, 3.3 (1.7–5.8)	Mean follow-up 5.8 years
Birkeland *et al.* (1995)	Denmark, Finland, Norway & Sweden		28 cases	NA	–		SIR, 8.6 (5.7–13)	Mean follow-up 4.8 years

Table 49 (contd)

Reference	Area	Detection method	Number and % with HPV or lesion				Relative risk and 95% CI	Comments
			Transplant patients		Controls			
			No.	%	No.	%		
CIN								
Porreco *et al.* (1975)	USA		3/131	2.3	–		SIR [14 (2.8–40)]	Mean follow-up 3.6 years
Cordiner *et al.* (1980)	United Kingdom	Cytology/histology	5/26	19	–			After a mean of 3.8 years immunosuppression
Ingoldby *et al.* (1980)	United Kingdom		0/50	0	–			Three years follow-up, 1–2 cases expected
Schneider *et al.* (1983)	USA		6/132	4.5	–			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)]	Mean time since transplant, 47 months
Alloub *et al.* (1989)	United Kingdom	Histology	24/49	49	7/69	10	[8.5 (3.0–25)]	
Gentile *et al.* (1991)	Italy	Cytology/histology	1/39	2.6	–			Mean time since transplant, 77 months
Gitsch *et al.* (1992)	Austria	Histology	2/23	8.7	–			
David *et al.* (1993)	Germany		5/58	8.6	–			
Fairley *et al.* (1994a)	Australia	Cytology	5/69	7.2	0/22	0		

[ ] Calculated by the Working Group
CIN, cervical intraepithelial neoplasia; SIR, standardized incidence ratio

mean of 22 months after transplantation. Of these eleven women, six also developed CIN a mean of 38 months after transplantation.

Gitsch *et al.* (1992) found cervical condyloma in seven of 23 renal transplant recipients (30%), six (86%) of whom were positive for HPV by in-situ hybridization (two each for HPV-6/11, -16/18 and -31/33). In comparison, eight of 14 (56%; $p > 0.1$) immunocompetent women with cervical condyloma, matched to the transplant patients by age and parity, were HPV positive (three each for HPV-6/11 and -16/18 and two for HPV-31/33).

CIN has been detected in up to 50% of women following renal transplantation (Table 49) and a number of case–control and cohort studies have suggested a higher incidence of CIN among transplant recipients than in the general population. These studies have not, in general, reported data for HPV infection.

Porreco *et al.* (1975) identified 131 women four months following renal transplantation in Colorado, USA. During an average of 3.6 years follow-up, three women developed intra-epithelial carcinoma of the cervix, compared with 0.22 expected on the basis of rates in Colorado, giving a standardized incidence ratio (SIR) of 14 [95% CI, 2.8–40].

Ingoldby *et al.* (1980) followed 50 women who had received renal transplants in the United Kingdom. There were no new cases of CIN during an average of three years of follow-up while one or two cases would have been expected.

In the study of Halpert *et al.* (1986) described above, 12% of renal transplant recipients had CIN compared with 2.5% of the matched control group [crude OR, 5.6 (95% CI, 1.1–38)]. In the study of Alloub *et al.* (1989) described above, 49% of transplant recipients had CIN, compared with 10% of the control group [OR, 8.5 (95% CI, 3.0–25)]. Blessing *et al.* (1990) used in-situ hybridization to test for HPV-4–6, -8, -11, -16 and -18 in 22 samples from seven women with cervical, vaginal and vulvar intraepithelial lesions. HPV-6 was identified in one sample, HPV-16 in 12 samples (from six patients) and HPV-18 in four samples (from three patients).

(ii) Invasive carcinoma

Two cohort studies have followed large groups of patients following renal transplantation. Fairley *et* al. (1994b) followed a cohort of 15 820 patients (8785 men and 7035 women) identified through the Australia and New Zealand Dialysis and Transplant Registry. A total of 8215 patients had received renal transplants and 7605 were on dialysis therapy between 1976 and 1992. The cohort was followed for cancer incidence until March 1992 and the expected numbers of cancers were calculated from national rates. Twelve women who had received renal transplants developed cervical cancer giving an SIR of 3.3 (95% CI, 1.7–5.8) compared with two women on dialysis therapy (SIR, 0.74 (95% CI, 0.1–1.2)).

Birkeland *et al.* (1995) identified 2369 women who had received renal transplants in Denmark, Finland, Norway and Sweden from 1964 to 1982. They were followed until 1986 and expected numbers of cancers were calculated based on the national rates. Twenty eight women developed cervical cancer, giving an SIR of 8.6 (95% CI, 5.7–13).

(iii) Other anogenital cancers

Few case series define and, therefore, reliably document the incidence of anogenital carcinoma in transplant recipients, but it does appear to be higher than that seen in the general population. In one series, Penn (1986) found that patients with anogenital carcinoma accounted

for 2.8% (65/2150) of tumours in transplant recipients compared with 0.5% of tumours in the general population.

In two cohort studies (Fairley *et al.*, 1994b; Birkeland *et* al., 1995), the incidence of anogenital cancer in renal transplant recipients has been reported to be between 2.1 and 56 times that expected from rates in the general population (Table 50). In one study (Fairley *et al.*, 1994b), this excess was seen only in patients who had received renal transplants, and not in those on dialysis therapy; SIR for transplant and dialysis patients were as follows: vulvar cancer, 56 (95% CI, 36–83) and 4.2 (95% CI, 0.4–12), respectively; penile cancer, 24 (95% CI, 6.4–60) and no case observed but 0.23 expected; anal cancer, 40 (95% CI, 11–102) and no case observed but 0.13 expected.

Table 50. Incidence from cohort studies of anogenital carcinomas among renal transplant recipients

Reference	Country	Population (follow-up)	Cancer site	Sex	SIR	95% CI	Comments
Birkeland *et al.* (1995)	Denmark, Finland, Norway and Sweden	2369 women 3323 men (1964–86)	Vulva/vagina Rectum Rectum	F M F	31 4.5 2.6	15–55 2.3–7.9 0.7–6.6	11 observed 12 observed 4 observed
Fairley *et al.* (1994b)	Australia and New Zealand	7035 women 8785 men (1976–92)	Vulva Anus Penis	F M + F M	56 40 24	36–83 11–102 6.4–60	24 observed 4 observed 4 observed

The prevalence of anal HPV-16 infection has been determined in only one series of transplant patients using a PCR technique on anal biopsy material (Ogunbiyi *et al.*, 1994b). HPV DNA was found in 36 of 76 biopsies from transplant patients (47%) compared with 18 of 145 biopsies from controls (12%; $p < 0.05$). Anogenital intraepithelial neoplasia was found in 26/133 patients compared with 1/145 control subjects ($p < 0.05$). Anogenital carcinoma was found in only one transplant patient and in no control in this series. HPV-6/11, -16/18 and -5 were found in one further giant anal condyloma using immunocytochemistry, in-situ hybridization, Southern blot and PCR (Soler *et al.*, 1992). Only one report exists for HPV typing in anogenital carcinoma. This study, of a single case of a metastatic perianal squamous-cell carcinoma in a transplant recipient, documents integration of HPV-11 in both primary and metastatic tumour (Manias *et al.*, 1989).

(b) HPV DNA in transplant-associated skin lesions

(i) Skin warts

Individual case reports document the presence of multiple HPV types, including EV-associated types, in skin warts of transplant recipients. These include HPV-2 (Euvrard *et al.*, 1991; Purdie *et al.*, 1993), HPV-5/8 (Lutzner *et al.*, 1983; Purdie *et al.*, 1993), HPV-27 (Ostrow *et al.*, 1989b) and HPV-49 (Favre *et al.*, 1989).

Table 51 summarizes the results of larger studies of transplant-associated virus warts (> 5 lesions). In studies using in-situ hybridization or Southern blot, the overall detection rate of HPV DNA where multiple probes were employed ranged from 20% to 82%, with most studies

Table 51. Prevalence of HPV DNA in skin warts of transplant recipients

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
					1–4/10	5/8	6/11 16/18	Other HPV 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
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 virus 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 polymorphism at PvuII and PstI sites.
Blessing *et al.* (1990)	United Kingdom	In-situ hybridization (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
Euvrard *et al.* (1993)	France	In-situ hybridization (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, in-situ hybridization 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)	44 (36 patients)	19 (43)	26/44	4/44	5/44	41 (1)	Multiple types found in some lesions
Pélisson *et al.* (1994)	France	In-situ hybridization (1a, 2a, 5, 6a, 11a, 16 and 18)	8 (transplant) 7 (non-transplant controls) 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

Table 51 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type- specific positivity				Comments
					1–4/10	5/8	6/11 16/18	Other HPV types (no. of lesions)	
Shamanin *et al.* (1994a)	United Kingdom	PCR and direct sequencing (1–4, 10, 5/8, 6/11, 16/18 and others)	50	28 (60)	2/50	0/50	1/50	27 (6), 28 (2), 57 (1), 12 (1), 15 (1), 17 (2), 25 (1), 29 (4), 49 (1), un-characterized (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

NA, not available; PCR, polymerase chain reaction; EV, epidermodysplasia verruciformis
[a]Of those types tested

identifying HPV DNA in over 60% of lesions. Three studies employed PCR amplification and, in these, the detection rate was between 55 and 61%. The failure to detect HPV DNA in a number of skin warts, even in studies employing PCR, suggests that the results should be interpreted with caution. Since control skin warts from immunocompetent patients were not employed in many studies, it is not known whether this reflects the limitations of the methods employed or a potential reservoir of currently unknown, and therefore undetectable, HPV types in transplant recipients.

Common skin-associated HPV types 1, 2, 3, 4 and 10 were by far the most common types to be identified in skin warts in studies where probes for these HPV types were employed. When the data from these studies, which used different methodologies, were combined, HPV-1, -2, -3, -4 or -10 were found in 80/237 (34%) of transplant samples and in 6/16 (38%) of control samples [$p = 0.95$]. Mucosal HPV types 6/11 and 16/18 were found in 23/199 (11%) of transplant samples and in 0/16 (0%) of controls where probes detecting these HPV types were employed [$p = 0.32$]. HPV-5/8 was found in 22/314 (7%) of transplant samples and in 0/16 (0%) of controls [$p = 0.65$]. Similarly, one recent study found EV-related HPV types (not further characterized) in 14/50 (28%) of skin warts and common skin-associated HPV types in only 2/50 (4%) of skin warts [$p = 0.05$] (Shamanin *et al.*, 1994a).

(ii) Verrucous keratoses (precancerous lesions)

Table 52 summarizes HPV DNA prevalence in case series (> 5 lesions) of verrucous keratoses. Only three case–control studies have been carried out and the numbers examined were small.

HPV DNA detection rates in transplant-associated verrucous keratoses in studies employing Southern blot or in-situ hybridization without amplification were 0–40%, with most studies detecting HPV DNA in approximately 20–30%. In studies employing PCR, detection rates were 0–73% but in three of these, in which multiple probes were employed, HPV DNA detection rates were between 24 and 73% (Shamanin *et al.*, 1994a; Stark *et al.*, 1994; Tieben *et al.*, 1994).

Combining data from studies using probes designed to detect type-specific HPV, albeit using different methodologies showed that, overall, common skin-associated HPV types 1–4 and 10 were found in 27/219 (12%) of transplant samples compared to 4/23 (17%) of control samples [$p = 0.68$]. HPV types 5/8 were found in 16/292 (5.4%) of transplant samples and 2/36 (5.6%) of control [$p < 0.5$], and mucosal HPV types 6/11 and 16/18 were found in 23/231 (10%) of transplant samples and 0/36 (0%) of control samples [$p = 0.05$].

(iii) Squamous-cell carcinoma

Table 53 summarizes HPV DNA prevalence in case series of transplant-associated squamous-cell carcinoma. Only two case–control studies have been performed and the numbers examined are small.

HPV DNA detection rates for studies employing Southern blot and in-situ hybridization without amplification in transplant squamous-cell carcinomas ranged from 5–100%. Studies employing PCR identified HPV DNA in 0–81% of lesions. In one case–control study employing PCR and multiple probes, HPV DNA was found in 2/9 (22%) of control squamous-cell carcinomas compared with 10/30 (33%) of transplant-associated squamous-cell carcinomas (Stark *et al.*, 1994).

Table 52. Prevalence of HPV DNA in verrucous keratoses of transplant recipients

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV-type specific positivity				Comments
					1–4/10	5/8	6/11 16/18	Others	
Rüdlinger *et al.* (1986)	United Kingdom	In-situ hybridization (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	In-situ hybridization (4, 5/8)	19	5/19 (26)	2/19	3/19	–	–	Frozen tissue. No control samples examined
Euvrard *et al.* (1991)	France	In-situ hybridization (1, 2, 5, 16/18)	7	0/7 (0)	0/7	0/7	0/7	–	Frozen tissue
Viac *et al.* (1992)	France	In-situ hybridization (multiple probes)	11	4/11 (36)	2/11	0/11	0/11	Uncharacterized HPV types (2/11)	Frozen tissue
Euvrard *et al.* (1993)	France	In-situ hybridization (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, in-situ hybridization 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
Trendfield *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
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	In-situ hybridization (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

Table 52 (contd)

Reference	Study area	Detection method (types included)	No. of cases	Overall HPV positivity (%)[a]	HPV type-specific detected				Comments
					1–4/10	5/8	6/11 16/18	Others	
Shamanin *et al.* (1994a)	United Kingdom	Southern blot and PCR (1–4, 10, 5/8, 6/11, 16/18 and others)	29	29/40 (73)	2/40	0/40	0/40	HPV-9, -15, -17, -20, -27, -29 and -49 found in 10/40 lesions. Uncharacterized HPV types found in 7/40 further lesions	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 HPV types (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	0/10	0/10	HPV type 36 (1/10). Uncharacterized HPV type (1/10)	Frozen tissue. No control samples

PCR, polymerase chain reaction; NA, not available
[a]Of those tested

Table 53. Prevalence of HPV DNA in squamous-cell carcinoma of transplant recipients

Reference	Study area	No. of cases	HPV detection method (types included)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
					1–4/10	5/8	6/11 16/18	Other types	
Barr *et al.* (1989)	United Kingdom	25	Dot blot (1, 2, 4, 5/8)	16/25 (64)	1/25	15/25	–	–	Frozen tissue from five patients
Magee *et al.* (1989)	Texas, USA	8	In-situ hybridization (1–4, 16/18, 6/11)	8/8 (100)	0/8	–	8/8	–	–
Blessing *et al.* (1990)	United Kingdom	11	In-situ hybridization (4, 5/8)	2/11 (18)	2/11	0/11	–	–	Frozen tissue
Dyall-Smith *et al.* (1991)	United Kingdom	188	PCR amplification (1–4, 5, 7, 9, 11, 16/18, 19, 25)	0/188 (0)	0/188	0/188	0/188	–	Frozen tissue. No control SCC studied
Viac *et al.* (1992)	France	8	In-situ hybridization (multiple probes)	2/8 (25)	1/8	0/8	1/8	–	–
Euvrard *et al.* (1993)	France	46	In-situ hybridization (1, 2, 5, 16/18)	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	10	Dot blot and Southern blot (1–4, 10, 5/8, 6/11, 16/18)	6/10 (60)	2/10	2/10	0/10	Unknown HPV types (4/10)	–
Smith *et al.* (1993)	Australia	20	PCR amplification (probes not specified)	0/20 (0)	–	–	–	–	–
Soler *et al.* (1993)	France	26	Southern blot, PCR and in-situ hybridization (1–4, 5/8, 6/11, 16/18)	21/26 (81)	0/26	6/26	20/26	–	Frozen tissue. Multiple HPV types found in single lesions
Trenfield *et al.* (1993)	Australia	40	Southern blot (multiple probes)	2/40 (5)	1/40	0/40	0/40	HPV-36 found in 1 SCC	Frozen tissue
Pélisson *et al.* (1994)	France	13	In-situ hybridization (1a, 2a, 5, 6a/11a, 16/18)	8/13 (62)	3/13	1/13	7/13	–	Frozen tissue. No control SCC studied
McGregor *et al.* (1994)	United Kingdom	14 transplant 22 non-transplant	PCR amplification (5/8, 6/11, 16/18)	0/14 (0) (transplant) 0/22 (0) (non-transplant)	– –	0/14 0/22	0/14 0/22	–	Paraffin-embedded material

Table 53 (contd)

Reference	Study area	No. of cases	HPV detection method (types included)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
					1–4/10	5/8	6/11 16/18	Other types	
Shamanin *et al.* (1994a)	United Kingdom	23	Southern blot and PCR (1, 2, 3, 5, 7, 10, 37, 40)	17/23 (74)	0/23	0/23	0/23	HPV-27, -29 and -47 found in 5/23 SCC. Unknown types were found in a further 8/23 SCC.	Frozen tissue. No control samples examined
Stark *et al.* (1994)	United Kingdom	30 transplant patients 9 controls	Southern blot and PCR (1–4, 5/8, 6/11, 16/18)	10/30 (33) (transplant) 2/9 (22) (control)	3/30 1/9	0/30 1/9	2/30 0/9	Unknown HPV types identified in 6/30 SCC	Frozen samples
Tieben *et al.* (1994)	Netherlands	24	PCR and direct sequencing (multiple probes)	5/24 (21)	1/24	1/24	0/24	HPV-14 found in 2/24 SCC. Unknown types found in 2/24 SCC	Frozen tissue
Berkhout *et al.* (1995)	Netherlands	53	PCR (degenerate nested primer, direct sequencing)	43/53 (81)	0/53	0/53	0/53	HPV-24 (1) HPV-19 (1) HPV-20 (3) HPV-25 (2) HPV-15 (9) HPV-38 (1) HPV-23 (3) Undefined (15)	Multiple HPV types found in some lesions

PCR, polymerase chain reaction; SCC, squamous-cell carcinoma
[a]Of those types tested

Combining the data from these studies, as above, showed common skin-associated HPV types 1–4 and 10 were found in 34/452 (7%) of transplant and 1/9 (11%) of control samples. HPV-5/8 was found in 27/458 (6%) of transplant and 1/31 (3%) of non-transplant samples. Mucosal HPV types 6/11 and 16/18 were found in 53/430 (12%) of transplant and 0/31 (0%) of controls. In three studies in which multiple probes were employed, a number of other HPV types, including HPV-36, -27, -29, -47 and -14, were identified in some squamous-cell carcinomas (Shamanin *et al.*, 1994a; Stark *et al.*, 1994; Tieben *et al.*, 1994). A study using nested primers specifically designed to detect EV HPV types found a broad spectrum of these types in 81% (43) of squamous-cell carcinomas, including HPV-15, -19, -20, -23, -24, -25 and -38. The significance of these findings and their role in transformation remains unclear (Berkhout *et al.*, 1995).

(iv) Basal-cell carcinoma

Table 54 summarizes HPV DNA prevalence in a small number of case series and one case–control study of basal-cell carcinoma. Detection rates were between 0% and 100% but the numbers of tumours were very small. Combining the data from these studies, as above, showed common skin-associated HPV types 1–4 and 10 were found in 4/20 (20%) of transplant basal-cell carcinomas. HPV types 5/8 were not identified in any of 31 transplant basal-cell carcinomas or of 15 control basal-cell carcinomas. HPV types 16/18 were found in 3/31 (10%) of transplant basal-cell carcinomas in one study (Pélisson *et al.*, 1994) and in none of 15 control basal-cell carcinomas in another study (McGregor *et al.*, 1994).

(c) HPV infection and cancer at other sites

There are few reports of HPV infection in association with cancer at other sites in transplant recipients. Querci della Rovere *et al.* (1988) documented HPV-11 infection in a case of bladder cancer in a renal-transplant recipient. In two series of bladder cancer patients, HPV infection (HPV-16/18) was only found in one patient in each series (of 10 and 22 patients, respectively) who had received a renal transplant (Kitamura *et al.*, 1988; Maloney *et al.*, 1994).

HPV was identified in one malignant melanoma in an immunosuppressed patient but not in 35 other malignant melanoma specimens from immunocompetent patients (Scheurlen *et al.*, 1986b). Other case reports documented HPV-16 in an oropharyngeal carcinoma following cardiac transplantation (Demetrick *et al.*, 1990), HPV-16 in a carcinoma of the tongue (Lookingbill *et al.*, 1987) and HPV-2 in a spinocerebellar tumour following renal transplantation (Sassolas *et al.*, 1991). Koilocytosis and hyperkeratosis suggestive of HPV infection were reported in three cases of head and neck cancer following renal, cardiac and bone-marrow transplantation (Bradford *et al.*, 1990).

2.5.3 Studies in HIV-infected persons

Infection with human immunodeficiency virus (HIV) leads to a profound alteration of the immune function that differs from that of most other immunosuppressive conditions in being increasingly severe over a period of a few to many years. There are several possible mechanisms by which HIV could affect the natural history of HPV and related neoplasia, see Figure 18. HIV-induced immunosuppression might reactivate latent HPV infection and lead to higher HPV

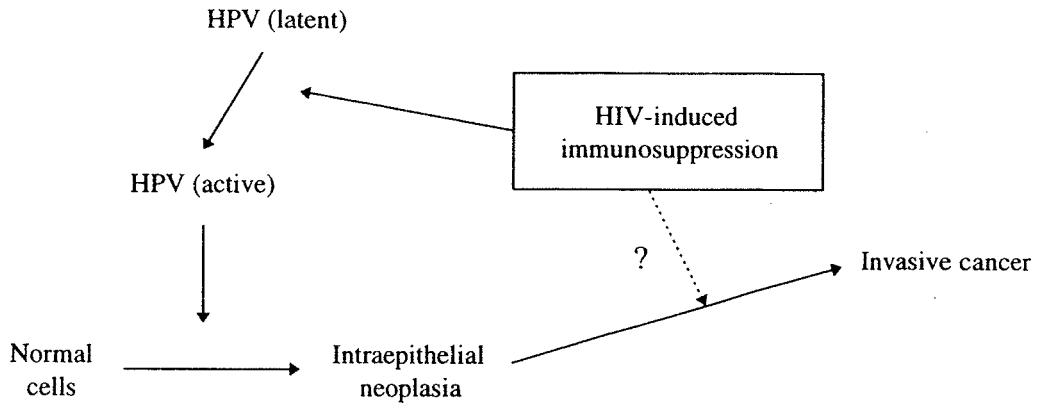
Table 54. Prevalence of HPV DNA in basal-cell carcinoma of transplant recipients

Reference	Study area	No. of cases	HPV detection method (including types)	Overall HPV positivity (%)[a]	HPV type-specific positivity				Comments
					1–4/10	5/8	6/11, 16/18	Other types	
Rüdlinger *et al.* (1986)	United Kingdom	1	Southern blot (1–4, 5/8, 6/11, 16)	0/1 (0)	0/1	0/1	0/1	–	–
Obalek *et al.* (1988)	–	2	Southern blot (1, 4, 5, 10, 11, 16/38)	2/2 (100)	2/2	0/2	0/2	–	–
Euvrard *et al.* (1993)	France	2	In-situ hybridization (mixed probe)	0/2 (0)	0/2	0/2	0/2	–	–
Trenfield *et al.* (1993)	Australia	11	Southern blot (1–4, 5/8, 11, 16/18)	1/11 (9)	1/11	0/11	0/11	–	–
McGregor *et al.* (1994)	United Kingdom	11 (transplant) 15 (non-transplant)	PCR amplification (5/8, 6/11, 16/18)	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	4	In-situ hybridization (1, 2, 5, 6/11, 16/18)	3/4 (75)	1/4	0/4	3/4	–	Frozen tissue. No control BCC samples
Shamanin *et al.* (1994a)	United Kingdom	5	PCR and direct sequencing (degenerate primers designed to detect a range of cutaneous HPV types)	3/5 (60)	0/5	0/5	0/5	X	Frozen tissue
Tieben *et al.* (1994)	Netherlands	4	PCR (four consensus primers designed to detect cutaneous HPV types)	0/4 (0)	0/4	0/4	0/4	0/4	Frozen tissue

PCR, polymerase chain reaction; BCC, basal-cell carcinoma; X, uncharacterized HPV types
[a]Of those types tested

replication, and this would be important if a dose–response situation exists for the carcinogenic potential of HPV-infection. It is also possible that severe immunosuppression might influence directly the risk of progression from a premalignant to a malignant stage. Finally, it is theoretically possible that HIV could have a direct oncogenic potential but there is little evidence to support such a model of association.

Figure 18. Possible interactions between HIV-induced immunosuppresion and HPV infection in carcinogenesis



Research attempting to disentangle the potential relationship between HIV and HPV-associated malignancy has so far been primarily based on small cross-sectional and case–control studies of populations at particular risk of HIV-infection, with precancerous lesions rather than invasive neoplasm as the outcome of interest. The short existence of the HIV epidemic, the initial male predominance in many societies and the young populations at risk have, in particular, limited the possibilities for studying large numbers of HIV-infected women, especially those with cancer. The ability to control for confounding is particularly relevant while studying the influence of HIV on HPV-related malignancy. Not only are both HIV and most known high-risk types of HPV sexually transmitted, but HIV-infected persons have a lifestyle that for other reasons may increase their risk of certain cancers. The small sample size in many of the published studies among HIV-infected subjects limits their possibility to adequately control for behavioural covariates and risk factors associated with HIV infection.

(a) Studies of the uterine cervix

The influence of HIV on HPV expression and HPV-associated malignancy of the uterine cervix has been dealt with in a limited number of review papers (Palefsky, 1991; Rabkin & Blattner, 1991; Sillman & Sedlis, 1991; Northfelt & Palefsky, 1992; Braun, 1994; Stratton & Ciacco, 1994).

(i) Precancerous lesions

Table 55 summarizes the studies on precancerous lesions. In the late 1980s, the first case reports and case series were published suggesting an association between HIV-induced immuno-

Table 55. Prevalence of HPV in precancerous lesions of the uterine cervix in HIV-infected persons

Reference and study area	HIV positive, number and type	HIV negative, number and type	HPV prevalence		Cervical abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Byrne *et al.* (1989) United Kingdom	19 recruited from HIV+ STD-clinic attendees		95		3 CIN III 1 CIN II 1 atypia 1 SPI 1 HPV		Observed at colposcopy	Pap smear and biopsy	Prevalence of HPV in lower genital tract
Schrager *et al.* (1989) USA	35 IVDU and partners of IVDU	23 IVDU and partners of IVDU	HIV+, 26 HIV–, 4	7.6 (0.9–65)	*Squamous atypia* HIV+, 31 HIV–, 4	10 (1.2–85)	Cytological or histopathological findings	Pap smear	HIV-infected: fewer used barrier contraceptives, more had had STD.
Feingold *et al.* (1990) USA	35 IVDU and partners of IVDU	32 IVDU and partners of IVDU	HIV+, 49 HIV–, 25	2.8 (1.0–8.0)	*SIL* HIV+, 40 HIV–, 9	6.4 (1.6–25)	Southern blot (cervicovaginal lavage)	Pap smear	More non-whites among HIV-positive women
Schäfer *et al.* (1991) Germany	111 gynaecological in-patients or HIV infection	76 IVDU	HIV+, 48 HIV–, 20	[3.7 (1.9–7.3)]	*Dysplasia or neoplasia* HIV+, 41 HIV–, 9	[7.0 (2.9–17)]	Cytology	Pap smear	Crude odds-ratio
Vermund *et al.* (1991) USA	51	45	HIV+, 53 symptomatic, 70 asymptomatic, 22 HIV–, 22	[3.9 (1.6–9.6)]	*SIL* Symptomatic HIV+, 42 Asymptomatic HIV+, 17 HIV–, 13	[8.1 (2.9–22)] [1.0 (0.3–3.7)] 4.6 (0.8–28)	Southern blot (11, 16, 18) (lavage)	Pap smear	Extension of study by Feingold *et al.* (1990)
Kreiss *et al.* (1992) Nairobi, Kenya	147 prostitutes	51 prostitutes	HIV+, 37 HIV–, 24	1.7 (0.8–3.6)*	*CIN* *Overall* HIV+, 26 HIV–, 24 *HIV+* HPV+, 47 HPV–, 9 *HIV–* HPV+, 57 HPV–, 7	0.9 (0.2–3.5)* 9.4 (1.7–52) 17 (1.4–217)	Dot blot/Southern blot (6, 11, 16, 18, 31, 33, 35)	Cytology	*Adjusted for age and years of prostitution. Women with CIN and HPV: 6/11, 4 cases 16/18, 4 cases unknown, 5 cases

Table 55 (contd)

Reference and study area	HIV positive, number and type	HIV negative, number and type	HPV prevalence		Cervical abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Laga *et al.* (1992) Kinshasa, Zaire	47 prostitutes	48 prostitutes	HIV+, 38 HIV–, 8 *HIV+/CIN+*, 73 *HIV+/CIN–*, 30	6.8 (1.9–26.8) 6.2 [1.3–29]	*CIN* HIV+, 27 HIV–, 3	15 (1.8–95)	ViraType™ Southern blot (low stringency) (6,11,16,18,31,33, 35)	Cytology	13 Pap smears inadequate for interpretation
ter Meulen *et al.* (1992) Tanzania	46 gynaecological in-patients	313 gynaecological in-patients	Any type: HIV+, 78 HIV–, 56 *Type 16/18* HIV+, 30 HIV–, 14	*HPV (total)** 2.52 (p = 0.02) *HPV-16/18* 2.42 (p = 0.02)	HIV+, 2.4 HIV–, 2.8		PCR (consensus primer and type-specific, 16, 18)	Pap smear	*Adjusted for age
Conti *et al.* (1993) Italy	273 former IVDU	161 former IVDU			HIV+, 42 HIV–, 8	4.2 (2.1–8.4)* *HPV–/HIV+* 1.2 [p > 0.05] *HPV+/HIV–* 11 (2.8–42) *HPV+/HIV+* 64 (19–214)	Cytological diagnosis	Cytology confirmed by biopsy	Cross-sectional study, potential selection bias (inflated) *Adjusted for age and number of sexual partners *CIN II–III/HIV+*: $CD4^+$ count< 500/mm^3 versus $CD4^+$ ≥ 500/mm^3, 5.4 (2.6–11)
Maggwa *et al.* (1993) Nairobi, Kenya	205 attendees of family-planning clinic	3853 attendees of family-planning clinic			HIV+, 4.9 HIV–, 1.9	2.8 (1.3–5.9)		Cytology	Adjusted for age, sexual behaviour and demographic variables
Ho *et al.* (1994) New York, USA	97 IVDU, HIV–related diseases, or partners of IVDU	110 IVDU, HIV–related diseases, or partners of IVDU	*All HPV types* HIV–, 23 HIV+, 50 $CD4^+$% > 20, 45 $CD4^+$% ≤ 20, 61 *Types 16,18,31,33, 35* HIV–, 6.4 HIV+, 14	 3.3 (1.8–6.1) 2.8 (1.3–6.0) 5.3 (2.2–13) 3.5 (1.3–9.2)			Southern blot hybridization		*Strong HPV signal* HIV–, 1.0 HIV+ $CD4^+$% > 20, 2.6 (0.62–11) $CD4^+$% < 20, 5.9 (1.4–25) Includes some persons previously studied by Vermund *et al.* (1991).

Table 55 (contd)

Reference and study area	HIV positive, number and type	HIV negative, number and type	HPV prevalence		Cervical abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Klein *et al.* (1994) New York, USA	114 IVDU, or HIV-positive partners	139 IVDU, or HIV-positive partners			HIV–, 10 HIV+, 22 $CD4^+\% > 20$, 17 $CD4^+\% \leq 20$, 35 *Multivariate analysis* HPV infection High-risk HPV Strong HPV signal Low $CD4^+$	 2.5 (1.2–5.1) 1.8 (0.7–4.6) 4.8 (2.0–12) 6.8 (2.9–15.7) 12 (4.1–34) 10.8 (3.5–33.7) 3.1 (1.0–9.5)	Southern blot hybridization	Cytology	No demographic or behavioural variables associated with SIL. Included persons studied by Vermund *et al.* (1990) and Ho *et al.* (1994)
Seck *et al.* (1994) Dakar, Senegal	HIV-1, 18 HIV-2, 17 from infectious disease clinic	58 women in infectious disease clinic	*HIV-1* STH, 19 PCR, 75 *HIV-2* STH, 47 PCR, 73 *HIV–* STH, 4 PCR, 2	 6.4 (0.6–80) 11.5 (2.7–56) 24.1 (3.5–257) 10.5 (24–52)	*HIV-1 versus HIV* normal dysplasia *HIV-2 versus HIV* normal dysplasia	 1 23.3 (2.9–205) 1 9.3 (1.1–79)	Southern transfer hybridization (6,11,16,18,31,33, 35) PCR (consensus primer)	Cytology	
Williams *et al.* (1994) San Francisco, USA	55 IVDU	59 IVDU	*Dot blot* HIV+, 19 HIV–, 5 *PCR* HIV+, 57 HIV–, 13	 4.2 (1.0–25) 8.9 (3.2–27)	9/11 SIL in HIV+	6.1 (1.2–61)	ViraType™ and PCR	Cytology	Recruited from larger cohort (see also Table 56)
Wright *et al.* (1994a) New York, USA	398 attendees of AIDS clinics, STD-clinics, methadone clinics	357 same	HIV+, 61 HIV–, 36 $p < 0.01$		*CIN I* HIV–, 4 HIV+, 13 *CIN II–III* HIV–, 1 HIV+, 7	 [4.3 (2.3–8.1)] [15 (3.6–64)]	PCR *L1* consensus primers	Cytology + histology (biopsy)	Independent variables in multiple regression model for CIN risk: HPV-DNA, HIV-positivity, $CD4^+ < 200/mm^3$ and > 34 years of age

[ ] Calculated by the Working Group

CIN, cervical intraepithelial neoplasia; SIL, squamous intraepithelial lesion; IVDU, intravenous drug users; STH, Southern transfer hybridization; PCR, polymerase chain reaction; STD, sexually transmitted disease; SPI, subclinical papillomavirus infection; Pap, Papanicolaou; $CD4^+$, $CD4^-$ cells

suppression and cervical intraepithelial neoplasia (Bradbeer, 1987). Henry *et al.* (1989) reported mild to moderate dysplasia with atypical condyloma in all of their first four consecutively identified HIV-positive women at a medical centre in Minnesota, USA.

Byrne *et al.* (1989) re-examined 19 of 36 women diagnosed with HIV-positivity after routine testing became available at St Mary's Hospital in London. Seven (37%) cervical smears were reported to be abnormal, out of which four (21%) were histologically verified as intraepithelial neoplasia (Table 55). Disease at more than one site was detected in half of the patients, and, overall, 18 (95%) showed evidence of clinical/subclinical HPV infection of the lower genital tract (including vagina, vulva, perineum). The disease would have remained undetected in more than half of the group had colposcopy not been undertaken. Nine of the women were intravenous drug abusers.

In an analysis of cervicovaginal smears with the cytopathologist blinded to the subject's viral status, a significantly higher percentage of cytological squamous atypia was documented in HIV-positive (11/35; 31%) compared to HIV-negative women (1/23; 4%) (Schrager *et al.*, 1989). Furthermore, cytological or histopathological findings suggestive of HPV infection were observed in 26% of HIV-positive women compared to 4% of HIV-negative women. However, the controls in this study were not comparable to HIV-positive cases in terms of sexual behaviour, history of sexually transmitted diseases and frequency of use of barrier methods of contraception.

Vermund *et al.* (1991) extended a study by Feingold *et al.* (1990) on HPV-associated disease in intravenous-drug-using women or women with heterosexual contact with male drug users in the USA. In this study of 96 women, non-white subjects were disproportionately represented among HIV-infected women but other behavioural and sociodemographic characteristics were similar. Symptomatic HIV-positive women were more likely to be HPV positive by Southern blot hybridization (70%) than asymptomatic (22%) or HIV-seronegative women (22%). Among symptomatic HIV-positive women, a strong association between HPV and squamous intraepithelial lesions was documented [OR, 8.1 (95% CI, 2.9–22)] whereas the association was non-significant for the other two groups. These and other studies conducted in the late 1980s and early 1990s suggested that more severe HIV-induced immunosuppression might exacerbate HPV-mediated cervical cytological abnormalities (Maiman *et al.*, 1991; Schäfer *et al.*, 1991; Conti *et al.*, 1993).

Kreiss *et al.* (1992) performed a nested case–control study of 147 HIV-positive and 51 HIV-negative women within a large cohort of prostitutes established in Nairobi but were unable to document significant differences with respect to the prevalence of HPV-DNA in the two groups (adjusted OR, 1.7 (95% CI, 0.8–3.6)). Papanicolaou smears were only available on the most recently enroled 63 women in the study. Based on cytological examination of this subset, CIN was unrelated to HIV seropositivity. Furthermore, among women with cervical HPV DNA, HIV infection was not associated with an increased prevalence of CIN (47% in HIV-positive versus 57% in HIV-negative women). [A strength of this study is that the populations studied were relatively homogeneous with respect to sexual behaviour and condom use.]

In contrast, in a somewhat smaller but otherwise similarly designed nested case–control study in Kinshasa, Zaire, Laga *et al.* (1992) found a significantly higher prevalence of HPV DNA in HIV-positive cases (18/47; 38%) compared to controls (4/48; 8%) (OR, 6.8 (95% CI,

1.9–26.8)). HPV was detected both by ViraType™ and Southern blotting. Pap smears were obtained on all women but 13 were inadequate for interpretation. Eleven (27%) HIV-positive women had CIN compared with one (13%) of the HIV-negative women (OR, 15 (95% CI, 1.8–95)). Eight (73%) of the 11 HIV-positive women who had CIN also had HPV DNA detected, compared to nine (30%) of 30 with no CIN ($p = 0.02$, Fisher's exact test). Cases and controls in this study did not differ on important demographic or sexual behavioural characteristics but clinical acquired immunodeficiency syndrome (AIDS) was observed more frequently (7% of HIV-positive cases) than in the study population reported by Kreiss *et al.* (1992) (0.7%).

In a cross-sectional study of 359 gynaecological in-patients without cancer from Tanzania (ter Meulen *et al.*, 1992), 1/42 (2.4%) of HIV-positive women compared with 8/285 (2.8%) of HIV-negative women had an abnormal Pap-smear. None of the HIV-positive women were suspected of being severely immunosuppressed, owing to the lack of severe HIV-related symptoms. Nevertheless, HIV-positive women were 3.3 times more likely to be positive for HPV-16/18 (detected by PCR) than HIV-negative women (OR, 3.3; $p = 0.02$) after adjusting for differences in sexual behaviour and history of sexually transmitted diseases. [No analysis of the association between HPV and smear abnormality by HIV status was presented by the authors.]

In their large study of 4058 women attending two peri-urban family-planning clinics in Nairobi, Kenya, Maggwa *et al.* (1993) documented CIN on the Pap smears of 10 of 205 (4.9%) HIV-positive women, compared to 72 of 3853 (1.9%) HIV-seronegative women (OR, 2.7 (95% CI, 1.3–5.5)). This association remained after controlling for sexual behaviour and other risk factors. [HPV testing was not performed in this study.]

In a study of 673 Spanish prostitutes and 1182 non-prostitutes attending a family-planning clinic, the odds ratio for cervical intraepithelial neoplasia in HIV-positive prostitutes was 14.2 (95% CI, 4.8–42.4) compared to non-prostitute women [HIV status unknown] whereas there was no increased risk of CIN among HIV-negative prostitutes (OR, 1.2 (95% CI, 0.5–2.8)). Within the group of prostitutes, the odds ratio for CIN in HIV-positive compared to HIV-negative women was 12.7 (95% CI, 3.9–40.9) (de Sanjosé *et al.*, 1993).

In a study of 93 women (58 HIV negative, 18 HIV-1 positive, 17 HIV-2 positive) from Senegal (Seck *et al.*, 1994), detection of HPV-16, -18, -31, -33, -35 was significantly associated with HIV-1 and HIV-2 infection (HPV-16/18 versus HPV-negative: OR, 56 (95% CI, 26–121); HPV-31/33/35 versus HPV-negative: OR, 8.4 (95% CI, 2.2–32.4)). HIV infection (HIV-1 or HIV-2) was associated with evidence of dysplasia following adjustment for age and sexual behaviour variables (OR, 5.5 (95% CI, 1.0–30)). Among HIV-positive women with HPV DNA detected by PCR, dysplasia was demonstrated by cytological analyses in 2/6 (33%) with mild HIV-disease, and 7/10 (70%) with severe HIV-disease (OR, 4.7 (95% CI, 0.4–73)).

In a study of 33 HIV-1 infected women in the USA, Vernon *et al.* (1994) found frequent co-localization of HIV-1 and HPV in CIN lesions. HIV and HPV were each detected by PCR in 17 (52%) cervical biopsy samples. HIV and HPV were detected together in 10 (50%) of the 20 samples showing CIN, but in none of 13 samples showing normal histology or inflammatory atypia ($p = 0.002$, 1-tailed test).

Whereas most studies published so far have either used HIV-positivity *per se* or degree of severity of HIV-associated disease as a surrogate marker for level of immune status, more recent studies increasingly include an evaluation by level of $CD4^+$ cell count.

In a cross-sectional study of 434 female former intravenous drug abusers in Italy, Conti *et al.* (1993) found histological evidence of CIN in 115 of 273 (42%) HIV-positive women and in 13 of 161 (8%) HIV-negative women (OR, 4.2 (95% CI, 2.1–8.4), after adjustment for age and number of sexual partners). The prevalence of CIN increased with the stage of HIV infection with an OR of 5.4 (95% CI, 2.6–11) for women with a $CD4^+$ cell count < 500/mm^3, compared with those with a count ≥ 500/mm^3. There was an interaction between cytologically diagnosed HPV infection and HIV status with odds ratios for CIN of 1.2 (non-significant) in HPV-negative/HIV-positive women, 11 (95% CI, 2.8–42) in HPV-positive/HIV-negative women and 64 (95% CI, 19–214) in HPV-positive/HIV-positive women, when compared with HPV-negative/HIV-negative women. [The selection of study subjects may have inflated the prevalence of CIN.]

Ho *et al.* (1994) found in their analysis of 207 primarily intravenous drug using women that young age (OR, 2.5 (95% CI, 1.3–4.8)) and HIV-positivity (OR, 3.0 (95% CI, 1.5–5.7)) were the only independent demographic and behavioural factors to be associated with HPV DNA positivity as measured by Southern blot. The association with HIV was only changed marginally between the univariate and the multivariate analyses indicating limited confounding influence. Prevalence of HPV increased with decreasing $CD4^+$ cell level from 23% among immunocompetent HIV-negative subjects to 45% in mild-to-moderate immunosuppressive conditions (HIV-positivity and $CD4^+$% > 20) and to 61% in severe immunosuppression ($CD4^+$% < 20). HPV-16, -18, -31, -33 and -35 were not particularly strongly associated with HIV-positivity. A general increase in the number of detectable viral copies of HPV with increasing immunosuppression was indirectly supported by the finding of a significant association between strong Southern blot hybridization signal strength and increasing HIV-induced immunosuppression (see Table 55). Among 29 study subjects who did not have any sexual exposure in the previous year, 1/16 of HIV-seronegative women were HPV-positive (6.3%), compared with 8/13 of HIV-positive women (61.5%). This is in line with the hypothesis that HIV-induced immunosuppressed individuals may be prone to persistent HPV infection.

In a study in the USA of 253 women at risk of HIV infection because of intravenous drug abuse or through a partner who used intravenous drugs, Klein *et al.* (1994) identified SIL in 22% of HIV-positive women compared with 10% of HIV-negative women (OR, 2.5 (95% CI, 1.2–5.1)). In multivariate analyses, the presence of SIL was independently related to the presence of high-risk HPV types (12 (95% CI, 4.1–34)) and severe HIV-related immunosuppression (3.1 (95% CI, 1.0–9.5)).

Wright *et al.* (1994a) performed a cross-sectional study of 398 HIV-positive and 357 HIV-negative women recruited from HIV/AIDS clinics, STD clinics or methadone clinics in the USA. HIV-positive women were more likely to have a history of prostitution, intravenous drug usage, genital warts and genital herpes than HIV-negative women. Eighty (20%) of the HIV-positive women had CIN confirmed by biopsy (CIN I: 52 (13%), CIN II–III: 28 (7%) compared to 15 (4.2%) of HIV-negative women (CIN I: 13 (4%), CIN II–III: 2 (1%)). No invasive cancers were found. HPV DNA detected by PCR (*L1* consensus primer) was observed in 213 (61%) HIV-positive women compared to 114 (36%) HIV-negative women ($p < 0.01$). In multiple regression analysis, HPV DNA positivity, HIV positivity, $CD4^+$ cell count< 200/mm^3, and age > 34 years were all found to be independently associated with CIN (all stages).

The influence of immunosuppression was further evaluated in a cross-sectional study by Williams *et al.* (1994) based on 114 intravenous drug users in San Francisco, USA. A close association between HIV, HPV and abnormal cervical cytology was observed, as shown in Table 56. In a multivariate model of risk factors for cervical epithelial abnormalities that excluded those showing only atypia with inflammation, both cervical HPV detected by dot blot (OR, 32 (95% CI, 2.9–354)) and positive HIV serostatus with $CD4^+$ cell count below 250/mm^3 (OR, 127 (95% CI, 7.5–2133)) were independent predictors.

Table 56. Relation between human immunodeficiency virus serostatus, presence of cervical human papillomavirus, and cervical cytology

HPV/HIV	Cervical cytology		Odds ratio	95% CI	*p*
	Abnormal	Normal			
Dot blot					
HPV–/HIV–	0	47			
HPV–/HIV+	5	31	7.3	0.7–354	0.08
HPV+/HIV–	1	2	16	0.2–1254	0.2
HPV+/HIV+	4	4	38	2.7–1888	0.001
PCR					
HPV–/HIV–	0	41			
HPV–/HIV+	3	17	6.8	0.5–367	0.1
HPV+/HIV–	1	6	5.8	0.07–471	0.3
HPV+/HIV+	6	18	13	1.4–610	0.009

Adapted from Williams *et al.* (1994)

(ii) Progression of disease and treatment

Maiman *et al.* (1993) followed 44 HIV-positive and 125 HIV-negative women in New York for up to 43 months (mean 15 months). More HIV-positive women (39%) developed biopsy-proven recurrent CIN after treatment than HIV-negative women (9%; $p < 0.01$). CIN severity and lesion size were, however, similar in the two groups. Recurrent disease was associated with the degree of immunosuppression, occurring in 18% of women with a $CD4^+$ cell count > 500/mm^3 and in 45% of those with a $CD4^+$ cell count < 500/mm^3 ($p < 0.05$).

In Germany, Petry *et al.* (1994) carried out a prospective study of immunosuppressed women, who were either HIV-infected (n = 48) or transplant recipients (n = 52). The aim of the study was to evaluate progression from cervical HPV-positivity to CIN or from CIN I to CIN II–III. Women with cervical lesions were matched (1 : 2) with immunocompetent, HIV-negative controls and colposcopy, cytology and HPV DNA typing (ViraType™) performed at each visit. Progression was more common in the combined groups of immunosuppressed women (6/11, 55%) than in controls (2/21, 10%; $p = 0.01$, Wilcoxon test). All patients with a $CD4^+$ cell count of less than 400/mm^3 or who had been immunosuppressed for more than three years suffered from progressive lesions. The cure rate among controls was 18/20 regardless of whether

conization or laser-vaporization was used, but it was much lower (4/10) among immunocompromised patients. [Data for HIV-positive women and renal allograft recipients were not presented separately.]

It is possible, in theory, that CIN preceded HIV acquisition in these women, and that it may even have promoted HIV infection, as is the mechanism established for other sexually transmitted diseases with ulcerous lesions. Neither cross-sectional nor case–control studies will give us the necessary answer. However, the increased prevalence of CIN reported in late-stage HIV infection in other studies, together with the more frequent and more rapid recurrence of CIN lesions after treatment in HIV-positive individuals suggests that HIV infection precedes CIN rather than the reverse.

(iii) Invasive cervical cancer

Invasive cervical cancer (ICC) has, since January 1993, been included as an AIDS-defining illness in HIV-positive women (Centers for Disease Control, 1992), primarily because of the plausibility of an association. There are at present few data to substantiate an increased risk of ICC among HIV-infected women. Increased occurrence of ICC has not been observed in the USA among women at high risk for AIDS (Rabkin *et al.*, 1993; Wright *et al.*, 1994a). In a large linkage study between AIDS and cancer registries in seven health departments in the USA, which has been reported as an abstract, Coté *et al.* (1993) found ICC in AIDS patients to be only increased marginally over background levels. The lack of an increased risk of ICC may be explained partly by the late introduction of HIV in the female population. Possibly, HIV-infected women die before CIN progresses to ICC. Active-screening programmes among HIV-infected women may also reduce the likelihood of progression to ICC. HIV-infected women have a higher rate of sexually transmitted diseases than women in general, and are therefore more likely to be in close contact with the health-care system, both before and after their HIV infection.

Based on hospital records from Lusaka, Zambia, no evidence of an influence of the HIV-epidemic on ICC rates was documented (Rabkin & Blattner, 1991) despite nearly 10% of pregnant women and 18% of normal blood donors being HIV-infected by 1985 (Melbye *et al.*, 1986). [Life expectancy for an HIV-positive person in Africa is particularly low.]

(b) Studies of the anorectal region

General reviews covering aspects of anal cancer and HPV in HIV-infected individuals are sparse (Palefsky, 1991; Rabkin & Blattner, 1991). The most comprehensive and detailed review on the subject so far has been presented by Palefsky (1994).

The assessment of anorectal epithelial cytology poses special problems because of variability in the quality of the cellular presentation and faecal contamination. Furthermore, biopsy materials have, in the studies undertaken so far, only exceptionally been obtained to evaluate further the cytological results. A significant association between cytology and histopathology was observed in one study (Palefsky *et al.*, 1990), whereas Surawicz *et al.* (1993) reported that evaluation of 90 homosexual men referred for internal lesions from a cross-sectional community-based study by biopsy recorded a threefold higher prevalence of dysplasia than detected with cytology.

(i) Precancerous lesions

Table 57 summarizes the studies on precancerous lesions. In a prospective study of 61 homosexual men, cytological evidence of dysplasia with concomitant features of HPV infection was observed at least once in 24 men, and HPV without dysplasia on at least one occasion in a further 26 men (Frazer *et al.*, 1986). Twenty of the men were HIV positive and among these men a reduced $CD4^+/CD8^+$ ratio was associated with the presence of dysplasia.

HPV-6/11, -16/18 or -31, -33, -35 was found in anal swabs from 41 (39%) of 105 homosexual men from Washington DC and New York, USA (Caussy *et al.*, 1990a). This figure was 53% in HIV-infected subjects compared to 29% in HIV-negative subjects ($p = 0.01$). In HIV-infected subjects, a low $CD4^+$ cell count was independently associated with anal HPV detection whereas the number of partners and frequency of receptive anal intercourse was unimportant. Abnormal cytology was seen in 9/37 (24%) HIV-infected men compared to 4/55 (7%) HIV-negative men ($p = 0.03$) and was strongly associated with the detection of any HPV genotype by dot blot. None of 15 subjects with HPV detected only by PCR had anal epithelial abnormality.

Kiviat *et al.* (1990) reported 13/49 (27%) HIV-infected bisexual and homosexual men compared to 3/47 (6%) HIV-negative men to have detectable anal HPV by dot blot hybridization (OR, 10 (95% CI, 1.9–57)). [No data on anal cytology/histology were available.]

Anal HPV DNA was detected overall in 15% of 120 Danish homosexual men but in 61.1% of 33 men who were HIV-positive (Melbye *et al.*, 1990). As shown in Figure 19, HPV detection was closely associated with immunosuppression. Anal cytology was abnormal in 19.5% and correlated with HPV (OR, 6.1 (95% CI, 2.1–18)). Type-specific associations were found with HPV -31, -33 and -35 (OR, 8.5 (95% CI, 1.9–39)) and HPV-16/18 (OR, 3.1 (95% CI, 0.8–12)) but not HPV-6/11 (OR, 1.0 (0.11–9.7)). Overall, HPV was detected in 39% of subjects with abnormal cytology. HPV was found in all four subjects with an abnormal anal cytology and a $CD4^+/CD8^+$ ratio below 0.4, but in only three of 14 subjects (21.4%) with abnormal anal cytology and a ratio ≥ 1.3.

In their study of 97 HIV-positive homosexual men with CDC (Centers for Disease Control) group IV disease in San Francisco, USA, Palefsky *et al.* (1990) found HPV DNA (ViraPap™/-Viratype™) in 54% while 39% had abnormal anal cytology (for details see Table 57). Anal intraepithelial neoplasia (AIN) was diagnosed in 15 specimens (15%). Abnormal cytology was significantly associated with anal HPV (OR, 4.6; $p = 0.003$) and, among those infected with two or more HPV types, 10/12 had abnormal anal cytology (OR, 39). $CD4^+$ cell counts obtained from medical records were inversely associated with cytological abnormality but did not contribute significantly to a multiple regression model that also included HPV.

Based on a sample of 112 Australian homosexual men presenting consecutively for routine screening for STDs and HIV, 19% showed evidence of mild to moderate dysplastic changes (AIN I or AIN II) (Law *et al.*, 1991). HPV DNA (6/11, 16/18) was detected by dot blot hybridization in 40% of anal smear samples (6/11 in 18%; 16/18 in 11%; both groups in 12%). There was a significant association between the detection of HPV-16/18 DNA and anal dysplasia but not between HPV infection or anal dysplasia and HIV-positivity, immune status, sexual practices or other STDs.

Table 57. Studies of precancerous lesions of the anorectal region in HIV-infected persons

Reference and study area	Number of HIV+ cases	Number of HIV− controls	HPV prevalence		Anorectal abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Frazer *et al.* (1986) Australia	20 homosexual men	41 homosexual men	HIV+, 80* HIV−, 53*	[3.5, 1.0–12]	HIV+, 45* HIV−, 15*	[4.8 (1.4–16)]	Cytological reading	Cytology	*Based on least abnormal smear obtained from each subject
Caussy *et al.* (1990a) USA	43 homosexual men	62 homosexual men	HIV+, 53 HIV−, 29	$p = 0.01$	HIV+, 24 HIV−, 7	$p = 0.03$	ViraType™ and PCR (6/11, 16, 18, 33)	Cytology (ASIL)	
Kiviat *et al.* (1990) USA	49 homo/-bisexual men	47 homo/-bisexual men	HIV+, 27 HIV−, 6	10 (1.9–57)			ViraPap™		
Melbye *et al.* (1990) Denmark	33 homosexual men	87 homosexual men	HIV+, 61.1 $CD4^+$%, > 40, 10 40–31, 7.5 30–21, 13 20–11, 34 ≤ 10, 36			ASIL + HPV $CD4^+/CD8^+$ ratio ≥ 1.0, 5.9 [0.9–39] < 1.0, 30.0 (3.1–290)	ViraType™ (6/11, 16/18, 31,33,35)	Cytology (ASIL)	
Palefsky *et al.* (1990) San Francisco, USA	97 homosexual men with CDC group IV disease		All types, 54 HPV-6/11*, 23 HPV-16/-18*, 29 HPV-31, -33, -35*, 20		HIV+, 39 4 condylomas 19 atypias 11 AIN I 4 AIN II		ViraType™ (6/11, 16/18, 31, 33, 35)	Cytology and histology	*Alone or in combination with the other types
Law *et al.* (1991a) Australia	45 consecutive homosexual men for STD screening	67 consecutive homosexual men for STD screening	All men (HIV+, HIV−) HPV-6/11, 18 HPV-16/18, 11 Both, 12		All men (HIV+, HIV−) AIN I–II, 19 AIN I, 17 men AIN II, 4 men		Dot blot hybridization	Cytology	No correlation between HPV or dysplasia and HIV
Bernard *et al.* (1992) France	54 homosexual and IVDU men	54 male partners to women with cervical HPV or dysplasia	*HIV+* All types, 67 HPV-6/11, 17 HPV-16/18, -31/33/35, 83 *HIV−* All types, 54 HPV-6/11, 62 HPV-16/18, -31/33/35, 38	All types [1.7 (0.8–3.8)]	*HIV+* AIN (PIN) I, 9 AIN II–III, 24 *HIV−* AIN I, 20 AIN II–III, 6		In-situ hybridization		Link between CMV and high-risk HPV observed irrespective of HIV status

Table 57 (contd)

Reference and study area	Number of HIV+ cases	Number of HIV– controls	HPV prevalence		Anorectal abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Critchlow *et al.* (1992) USA	26 consecutive homosexual men for HIV testing	119 consecutive homosexual men for HIV testing	HIV+, 31 HIV–, 8	5.8 (1.1–30) adjusted for STD history, age, anorectal symptoms			Dot filter hybridization		HIV positivity did not influence type of HPV. HPV prevalence up with severity of HIV disease
Palefsky *et al.* (1992) San Francisco, USA	37 with stage IV HIV disease		Increased from 60 to 89		ASIL, 27–65 AIN, 8–32 AIN II–III, 0–16		ViraPap™/Type™	Cytology and anoscopy biopsy	Prospective study over an average of 17 months
Kiviat *et al.* (1993) USA	285 homosexual men seeking HIV testing	204 homosexual men seeking HIV testing	*Southern blot* HIV+, 55 HIV–, 23 *PCR* HIV+, 92 HIV–, 78	4.0 (2.7–6.2) 3.1 (1.6–5.8)	HIV+, 26 HIV–, 8 HIV+ only *Atypia* CD4⁺ < 200, 28 201–500, 25 501–800, 25 > 800, 30 *ASIL* CD4⁺ < 200, 36 201–500, 35 501–800, 25 > 800, 8	5.6 (3.0–10.5) HIV+ versus HIV– *Atypia* 4.2 (1.6–11) 3.3 (1.6–6.5) 2.7 (1.4–5.4) 2.6 (1.2–5.4 *ASIL* 9.9 (3.7–27) 8.7 (4.1–18) 5.1 (2.3–11) 1.3 (0.4–4.2)	Southern transfer hybridization and PCR (6/11, 16/18, 31/33/35)	Cytology Bethesda recommendation	*OR for ASIL by level of HPV DNA:* CD4⁺ count ≤ 500/mm³ versus > 500/mm³ *STH+/PCR+* 2.6 (1.2–5.7) *STH–/PCR+* 6.3 (0.8–72)
Brown *et al.* (1994) Indiana, USA	12 (10 men) from STD and gynaecology clinics	41 (27 men) from STD and gynaecology clinics	High risk HPVs: HIV+, 58 HIV–, 17	[6.8 (1.7–28)]			Hybrid Capture™		
Palefsky *et al.* (1994) San Francisco, USA	37 homosexual men from San Francisco General Hospital Cohort Study	28 homosexual men from San Francisco General Hospital Cohort Study	HIV+, 51 HIV–, 36	[4.6 (0.9–23)]	HIV+, 28 HIV–, 8		ViraPap™/Type™	Cytology	HPV and ASIL correlated with HIV+ and CD4⁺ count < 200/mm³ and current smoking

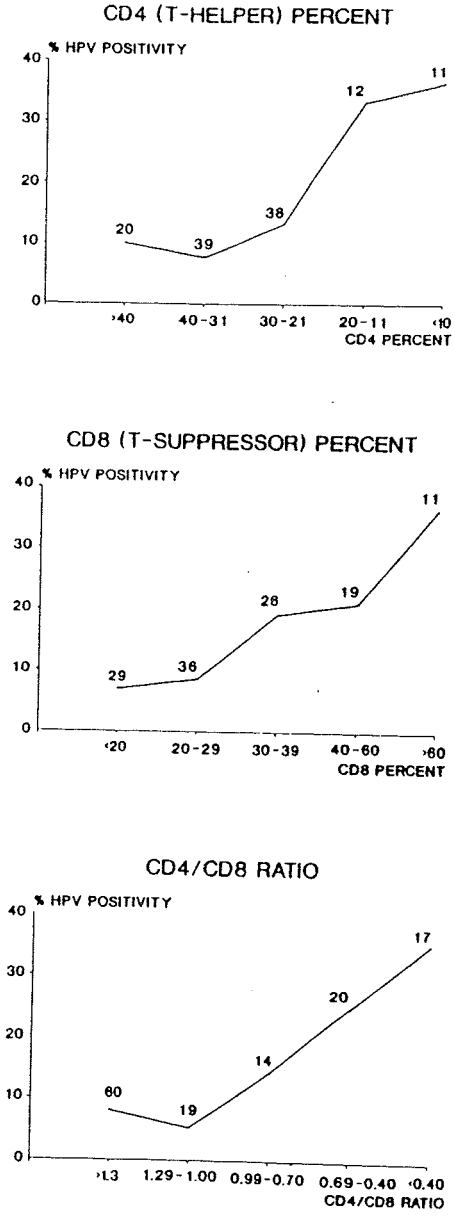
Table 57 (contd)

Reference and study area	Number of HIV+ cases	Number of HIV− controls	HPV prevalence		Anorectal abnormality		HPV test (types)	Pathology reading	Comments
			%	Odds ratio (95% CI)	%	Odds ratio (95% CI)			
Williams *et al.* (1994) San Francisco, USA	55 IVDU women	59 IVDU women	*Dot blot* HIV+, 32 HIV−, 14 *PCR* HIV+, 77 HIV−, 56		HIV+, 79	3.4 (0.9–16) *Dot blot* HPV−/HIV−, 1.0 HPV−/HIV+, 2.4 (0.4–16) HPV+/HIV−, 2.5 (0.04–38) HPV+/HIV+, 9.2 (1.6–64) (no association with PCR)	ViraPap™/Type™ and PCR	Cytology	Recruited from a larger cohort. No association between ASIL and CD4⁺ count

[ ] Calculated by the Working Group

PCR, polymerase chain reaction; CDC, Centers for Disease Control; STD, sexually transmitted diseases; IVDU, intravenous drug users; CMV, cytomegalovirus; ASIL, anal squamous intraepithelial lesions; DFH, dot-filter hybridization; AIN, anal intraepithelial neoplasia; PIN, penile intraepithelial neoplasia; STH, Southern transfer hybridization; CD4⁺, CD4⁺ cells; OR, odds ratio

Figure 19. Percentage of anal HPV DNA (ViraPap™/ViraType™) detected by level of $CD4^+/CD8^+$ markers in homosexual men



From Melbye *et al.* (1990)

Bernard *et al.* (1992) studied an equal number (54) of HIV-positive and HIV-negative men all presenting with anogenital lesions such as flat condyloma or condyloma acuminatum. Whereas most HIV-positive subjects were homosexual or intravenous drug users, the negative men were partners to women with genital HPV or dysplasia. High-risk HPV types (16/18, 31, 33, 35) were more common (83%) in HIV-positive persons. Low-risk types (6/11) were more

common in HIV-negative subjects (62%). High-risk types of HPV were seen in 94% (15/16) of men with AIN(PIN) II–III compared to 6% for low-risk HPVs, whereas 50% and 37.5%, respectively, were reported in AIN(PIN) I. A significant association between high-risk HPVs and the detection of cytomegalovirus in the same lesion was observed irrespective of HIV status.

Critchlow *et al.* (1992) reported a significant association between HIV-infection and anal HPV DNA as measured by dot filter hybridization after adjustment for STD history, age and current anorectal disease (OR, 5.8 (95% CI, 1.1–30)). HIV-infection did not influence type of HPV detected but severity of HIV-related disease was positively related to HPV prevalence.

In a larger and more-recent study by the same group (Kiviat *et al.*, 1993), a random sample of 285 HIV-positive and 204 HIV-negative homosexual men seeking HIV testing in Seattle, USA, was surveyed. HPV DNA was detected by Southern blot hybridization in 55% and 23% of HIV-positive and HIV-negative men, respectively (OR, 4.0 (95% CI, 2.7–6.2)) and by PCR in 92% and 78%, respectively (OR, 3.1 (95% CI, 1.6–5.8)). Each specific group of HPV DNA types surveyed was more common in HIV-infected men (Table 58). Detection of HPV by both Southern blot hybridization and PCR (assumed to indicate a high level of HPV) was significantly associated with anal intraepithelial lesions. However, after adjustment for level of detectable HPV DNA, severely immunosuppressed HIV-positive men ($CD4^+$ cell count $< 500/mm^3$) were at higher risk of anal intraepithelial lesions than men with a $CD4^+$ cell count of more than $500/mm^3$ (OR, 2.9 (95% CI, 1.4–6.2)). [This finding indicates a possible independent role of immunosuppression in addition to HPV.]

Sixty-six of 299 (22%) HIV-positive and 24 of 213 (11%) HIV-negative men from the above-mentioned study were referred for biopsies of internal anorectal lesions (Surawicz *et al.*, 1993). Of 78 men with HPV-associated internal lesions, only 36% had dysplasia diagnosed by cytology, while 92% had dysplasia evaluated by histology (27% high grade). Their findings of correlation of anal abnormalities with histological diagnosis are presented in Table 59. HIV-status did not influence the prevalence of high-grade lesions (27% and 25% in HIV-positive and HIV-negative men, respectively). Both high-risk and low-risk HPV types were common in many of the biopsy specimens but high-risk types 16/18/45 were more common among HIV-positive men (48%) than HIV-negative men (21%; $p = 0.08$).

In a study of 62 subjects with condylomata acuminata in the USA (Brown *et al.*, 1994), seven of 12 (58%) HIV-positive subjects (10 men) were positive for high-risk HPVs (HPV-16, -18, -31, -33, 35, -45, -51, -52 and -56) by Hybrid Capture™ compared with seven of 41 (17%) immunocompetent subjects [crude OR, 6.8 (95% CI, 1.7–28)]. All subjects were positive for low-risk HPV types (6, 11, 42–44), except one of the HIV-positive patients who was only positive for high-risk HPVs.

In a study of 37 HIV-positive and 28 HIV-negative homosexual men, Palefsky *et al.* (1994) found both anal intraepithelial lesions and the presence of HPV to be closely associated with HIV positivity in men with $CD4^+$ cell count below $200/mm^3$. Furthermore, a multivariate analysis indicated a possible influence of current smoking.

Breese *et al.* (1995) conducted a cross-sectional and follow-up study of 93 HIV-positive and 116 HIV-negative homosexual/bisexual men in the USA. Subjects were tested for anogenital HPV types 6/11, 16/18 and 31/33/35 (ViraPap™/ViraType™) at baseline and, for a subset

Table 58. Prevalence of anal HPV DNA in HIV-positive and HIV-negative homosexual men as detected by dot-filter hybridization, low- and high-stringency Southern transfer hybridization, and PCR

	HIV+ (%)	HIV− (%)	Odds ratio	95% CI
Dot blot	(n = 304)	(n = 211)		
Any HPV	52	18	5.1	3.3–7.9
Southern	(n = 285)	(n = 204)		
Any HPV	55	23	4.0	2.7–6.2
HPV-16, -18	21	7	5.0	2.6–9.6
HPV-31, -33, -35	15	3	8.7	3.5–26
HPV-6, -11	21	7	5.0	2.6–9.6
Unclassified HPV	16	8	3.7	1.7–6.3
Multiple HPV	15	3	8.5	3.4–25.2
PCR	(n = 241)	(n = 152)		
Any HPV	92	78	3.1	1.6–5.8
HPV-16, -18	53	38	3.6	1.8–7.2
HPV-31, -33, -35	43	15	7.4	3.4–16
HPV-6, -11	47	39	3.1	1.6–6.2
Unclassified HPV	19	22	2.2	1.0–4.9
Multiple HPV	44	23	4.9	2.4–10

From Kiviat *et al.* (1993)
PCR, polymerase chain reaction

Table 59. Correlation of anal abnormalities with histological diagnosis

Anoscopic abnormalities	Negative	Low-grade (AIN I)	High-grade (AIN II–III)	Total
Discrete warts	3	26	8	37[a]
Circumferential ring of warts	2	14	7	23
Flat white epithelium	1	11	6	18
Normal or non-HPV-associated findings	7	0	1	8[a]
Total	13	51	22	86[a]

From Surawicz *et al.* (1993); AIN, anal intraepithelial neoplasia
[a]Biopsies from 2 HIV-seronegative men in each of these categories were unsatisfactory

of men, six months later. Overall 20 (17%) HIV-negative and 57 (61%) HIV-positive men were positive for HPV ($p < 0.001$). HPV-16/18 DNA was detected most frequently, accounting for 51% and 65% of infections in HIV negative and HIV positive men, respectively. HPV was more common in men with AIDS related complex (ARC)/AIDS (74%) than in asymptomatic HIV-positive men (53%, $p = 0.08$); the prevalence of infection increased significantly with declining

$CD4^+$ cell count ($p < 0.05$). Persistent HPV infection after an initial positive test was also more common in men with ARC/AIDS (95%) than asymptomatic HIV-positive men (62%) or HIV-negative men (61%).

Several studies among women are under way, but so far the only one published in this area is by Williams *et al.* (1994). In their study of 114 intravenous drug users, these investigators found anal HPV to be twice as common as cervical HPV and associated with HIV-positivity by both dot blot (OR, 2.5 (95% CI, 0.9–7)) and PCR (OR, 2.6 (95% CI, 1.0–6.8)). Overall, anal intraepithelial lesions were seen in 14% (15/109) of the women of which 11 were HIV-infected (OR, 3.4 (95% CI, 0.9–16)). ASIL was closely associated with simultaneous high-level (dot blot-positive) HPV DNA and HIV positivity (OR, 9.2 (95% CI, 1.6–64)), but no association was found with level of $CD4^+$ cell count.

(ii) Progression of disease

In San Francisco, USA, Palefsky *et al.* (1992) followed 37 homosexual men with stage IV HIV-disease prospectively for an average of 17 months and found the prevalence of anal epithelial abnormalities to increase from 27% to 65% over this period. The percentage of men with AIN increased from 8 to 32% and high grade AIN from 0 to 16%. HPV DNA as detected by ViraPap™/ViraType™ technique increased from 60 to 89%. Among subjects who had no cytological abnormality at the start of the study, 11 of 12 (92%) who were HPV-positive and 5 of 13 (38%) who were HPV-negative developed anal disease during follow-up ($p = 0.02$).

(iii) Invasive anal cancer

Reports from Denmark, Sweden and the USA have shown significant increases in the incidence of epidermoid anal cancer, not only during the AIDS era, but over the last 30 years (Goldman *et al.*, 1989; Frisch *et al.*, 1993; Melbye *et al.*, 1994a). The increase has been more pronounced in women than in men and in urban areas than in rural areas. Furthermore, black people are at higher risk than whites and never-married men at higher risk than ever-married men. Interestingly, the increased risk of anal cancer in never-married men has been documented as far back as the 1940s and 1950s (Frisch *et al.*, 1993). These trends show that important behavioural and environmental changes were taking place before the beginning of the AIDS epidemic. However, data from the Surveillance, Epidemiology and End Results Programme (SEER) in the USA have shown a remarkable increase in incidence among men in the San Francisco Bay area in the last decade (1973–75: 0.5 per 10^5; 1988–89: 1.2 per 10^6; $p < 0.001$). Furthermore, the relative risk of anal cancer among never-married men compared to ever married men increased from 6.7 (95% CI, 4.7–9.5) in 1979–84 to 10 (95% CI, 7.5–14) in 1985–89 (Melbye *et al.*, 1994a).

Melbye *et al.* (1994b) used a linkage between AIDS (50 050 reports) and cancer (859 398 reports) registries in seven health departments in the USA to investigate the association between HIV infection and epidermoid anal cancer. Compared to general population rates, the relative risk of anal cancer at and after AIDS diagnosis (11 cases) was 84 (95% CI, 46–152) among homosexual men and 38 (95% CI, 9.4–151) among non-homosexual men (two cases). The relative risk of anal cancer was 14 (95% CI, 6.6–29) in the period two to five years before AIDS and 27 (95% CI, 16–47) during the two years before AIDS diagnosis (p for trend = 0.004) (Table 60).

Table 60. Relative risk (observed/expected ratio) of epidermoid anal and anorectal cancer among AIDS patients compared with population controls matched for age, sex, and race

Time from AIDS diagnosis	No. of cases		Relative risk (95% CI)
	Observed	Expected	
2–5 years before	7	0.502	14 (6.6–29)
0.25–2 years before	13	0.475	27 (16–47)
0.25 years before or after	9	0.113	80 (41.4–153)
0.25–0.75 years after	3	0.072	42 (13.4–129)
> 0.75–2.25 years after	4	0.082	49 (18.3–130)

From Melbye *et al.* (1994b)