Chapter 1
Cervical cancer and screening

Cervical cancer incidence and mortality worldwide

This section describes geographical patterns in cervical cancer incidence, survival and mortality, and the association of disease risk with classical demographic variables. Time trends in incidence and mortality, and the influence of screening programmes in determining them, are covered in Chapter 5.

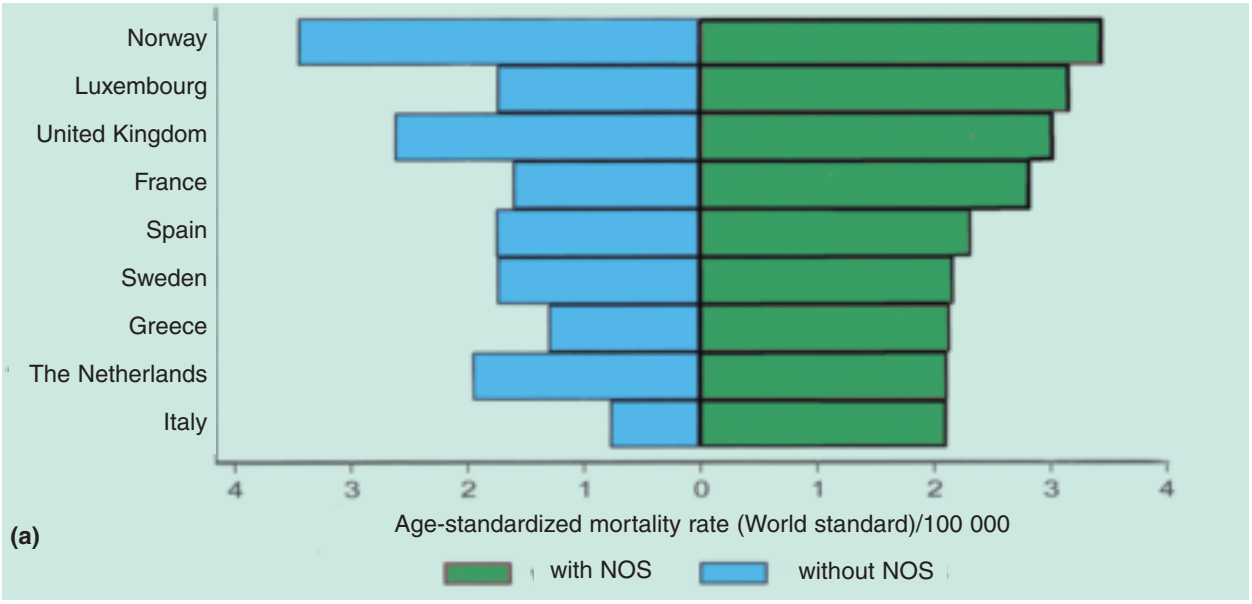
In examining differences in risk of disease between populations and over time, it is best to use, whenever possible, data on cancer incidence. However, mortality data are, in general, more widely available and cover longer periods of time. The use of mortality data as a substitute for incidence data is based on the assumption that the ratio of incident cases to deaths—as expressed by survival—is more or less the same in the populations being considered (including, for study of trends, over time). The section below on survival gives an indication of the validity of this assumption.

In some studies, mortality, in terms of numbers of deaths or probability of death, may actually be the focus of interest, for example in comparing overall cancer burden or the combined result of all cancer control interventions (including early diagnosis and therapy). In this context, variables that take into account age at death (person-years of life lost) and the level of disability between diagnosis and death (quality-adjusted life years, disability-adjusted life years) have become more widely applied in health-care planning and evaluation.

Some methodological and data considerations

International comparative studies using the indicators summarized above depend upon assumptions about lack of bias arising from data-quality issues. Cancer incidence data, published in the *Cancer Incidence in Five Continents* series (Parkin *et al*., 2002) have been peer-reviewed for data quality and completeness of coverage of the population at risk. The mortality data available through the WHO statistical information system (http://www3.who.int/whosis/menu.cfm), based on information received from national statistical offices, may be biased by different practices in death certification, and, for some countries, may be quite incomplete, as far as population coverage is concerned. These sources of bias should be checked, using the tables showing estimated completeness of coverage (http://www3.who.int/whosis/mort/table3.cfm?path=whosis,inds,mort,mort_table3&language=english), before the rates are used for comparisons between populations or over time.

These caveats apply to all comparative studies, but two issues are specific to studies of cancer of the cervix. The principal one relates to the categories in the international classification of disease (ICD) which has, since its 7th edition (1955), provided for the coding of cancers of the uterus as 'Cervix', 'Corpus' or 'Uterus, part unspecified'. The proportion of uterine cancer cases and deaths ascribed to the third of these categories, generally referred to as 'not otherwise specified' (NOS), varies widely both between countries and over time. The problem is much worse for mortality statistics than for incidence. The NOS category comprises more than 10% of uterine cancers in less than 10% of the cancer registries reporting in *Cancer Incidence in Five Continents* (Parkin *et al*., 2002). For mortality, in contrast, the proportion of deaths certified as due to cancer of 'Uterus NOS' can be very high—well over 50% in France and Italy, for example, in 1995 for women aged over 30 years (http://www-depdb.iarc.fr/who/menu.htm). As a result, comparative studies using data without correction for NOS may yield highly misleading assessments of geographical (Figure 1a) and temporal differences (Figure 1b) in mortality. For example, much of the apparent increase in the mortality rate from cancer of the cervix in Spain is due to a reduction in the rate for 'Uterus NOS' through better specification of cause of death (Figure 1b). Before comparative studies can be performed, therefore, some form of 'reallocation' of these NOS cases and deaths to the more specific categories is required. Several methods have been proposed (Arbyn & Geys, 2002; Bray *et al*., 2002). When the percentage of NOS cases is



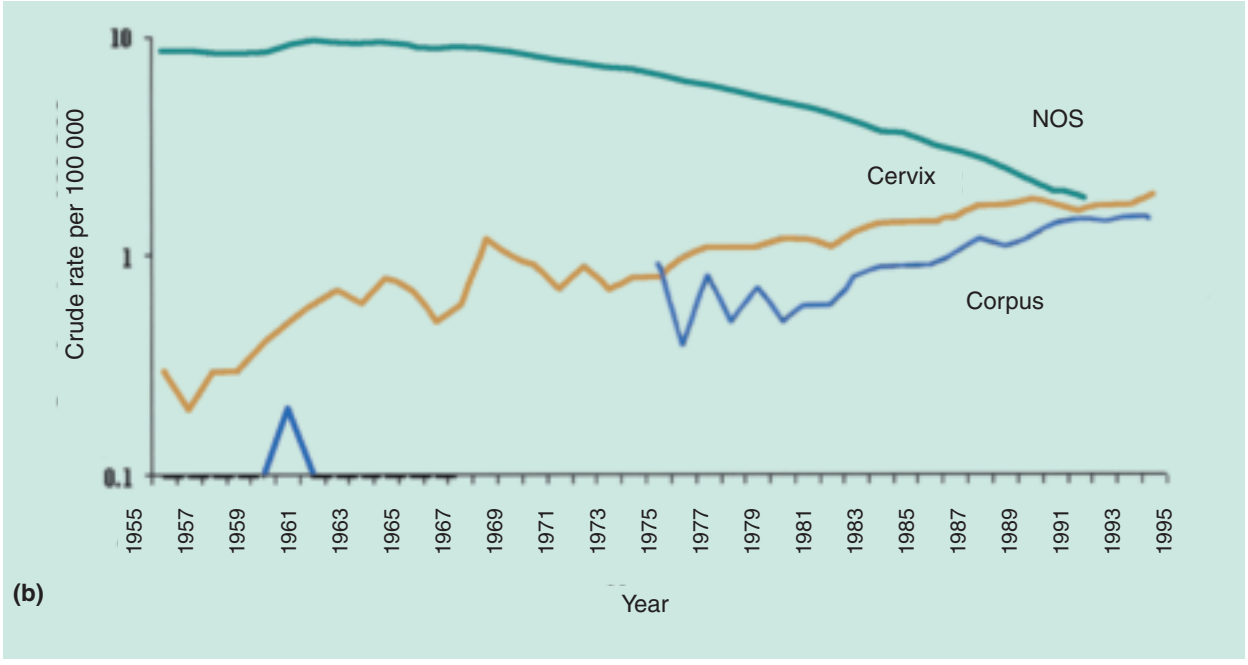


Figure 1 (a) Mortality from cancer of the cervix uteri in nine European countries, 1998; (b) Trends in mortality from cancer of the uterus, Spain
From http:www-depdb.iarc.fr/who/menu.htm

relatively small (< 25%, say), this reallocation can be according to the proportions of cases in the series with specified site, by age group. When a larger fraction of the cases have the precise site missing, it is preferable to use proportions from a different (reference) population which has data of better quality and is thought to be epidemiologically similar.

The second issue specific to cervical cancer epidemiology is that incidence and mortality rates are calculated using the entire female population as the 'population at risk', although women who have had a total hysterectomy for reasons other than the presence of cervical neoplasia are not at risk for the disease. Such women ought to be excluded from the population at risk, but the prevalence of hysterectomy is generally not known. Hysterectomized women may constitute a sizeable proportion of the population in some age groups and countries and this proportion may vary over time as well as by place and age. For example, in Ontario, Canada, the incidence of hysterectomy reached a peak in the early 1970s and then declined until 1990 (Snider & Beauvais, 1998). Rates were greatest in women aged 40–44 years. The self-reported prevalence of hysterectomy in 1994 varied from 13% to 28% of women aged 15 years and over by region of Canada; overall, 30% of women had had a hysterectomy by the time they attained age 65 (Snider & Beauvais, 1998). In England and Wales, the prevalence of hysterectomy was estimated as 21.3% at ages 55–59 in 1995 (Redburn & Murphy, 2001). Correction of the population at risk could therefore have a substantial impact on the estimated incidence and mortality rates, especially in older age groups, although little effect on the observed trends in Ontario (Marrett *et al.*, 1999) and England and Wales (Redburn & Murphy, 2001) was seen.

Cervical cancer: world patterns

Cancer of the cervix uteri is the second most common cancer among women worldwide, with an estimated 471 000 new cases (and 233 000 deaths) in the year 2000 (Parkin *et al.*, 2001). Almost 80% of the cases occur in developing countries, where, in many regions, it is the most common cancer among women, responsible for about 15% of all new cancers. The highest incidence rates are observed in Latin America and the Caribbean, sub-Saharan Africa, and south and south-east Asia (Figure 2). Cervical cancer is less common in the developed countries, where it was estimated to comprise about 4%

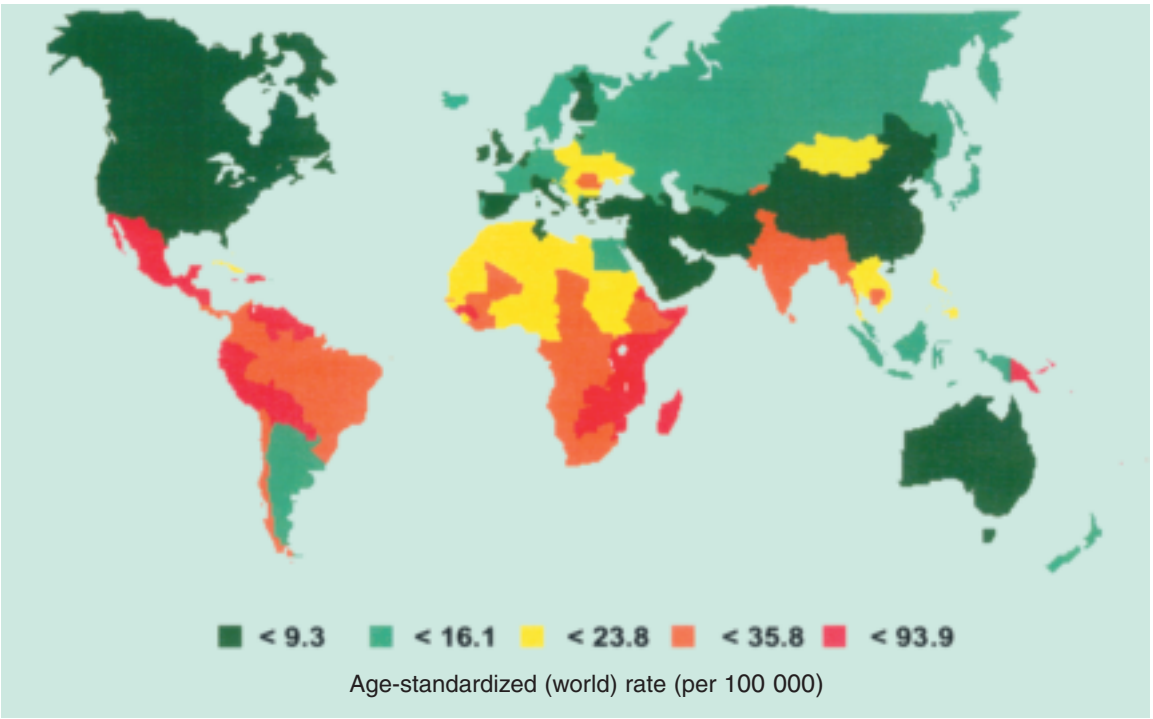


Figure 2 Incidence of cancer of the cervix uteri
From Ferlay *et al.* (2001)

of cancers in women in the year 2000, ranking sixth in importance.

Figure 3 shows incidence rates recorded in cancer registries around 1995 (Parkin *et al.*, 2002). These rates vary by at least 20-fold. The lowest (less than 14 per 100 000) are, in general, found in Europe (excluding some eastern European countries), North America and China. Incidence is generally higher in the developing countries of Latin America (average age-standardized incidence rates [ASR], 33.5 per 100 000) and the Caribbean (ASR, 33.5), sub-Saharan Africa (ASR, 31.0) and south-central (ASR, 26.5) and south-east Asia (ASR, 18.3) (Ferlay *et al.*, 2001). Very low rates are observed in China and in western Asia (Figure 2); the lowest recorded rate is 0.4 per 100 000 in Ardabil, north-west Iran (Sadjadi *et al.,* 2003).

This pattern is relatively recent, however; before the introduction of screening programmes in the 1960s and 1970s, the incidence in most of Europe, North America and Japan was similar to that seen in many developing countries today (Gustafsson *et al.*, 1997b): for example, it was 38.0 per 100 000 in the Second National Cancer Survey of the USA (Dorn & Cutler, 1959), 37.8 per 100 000 in Hamburg, Germany, in 1960–62, 28.3 per 100 000 in Denmark in 1953–57 and 22.1 per 100 000 in Miyagi, Japan, in 1959–60 (Doll *et al.*, 1966).

The majority of cases of cervical cancer are squamous-cell carcinomas.

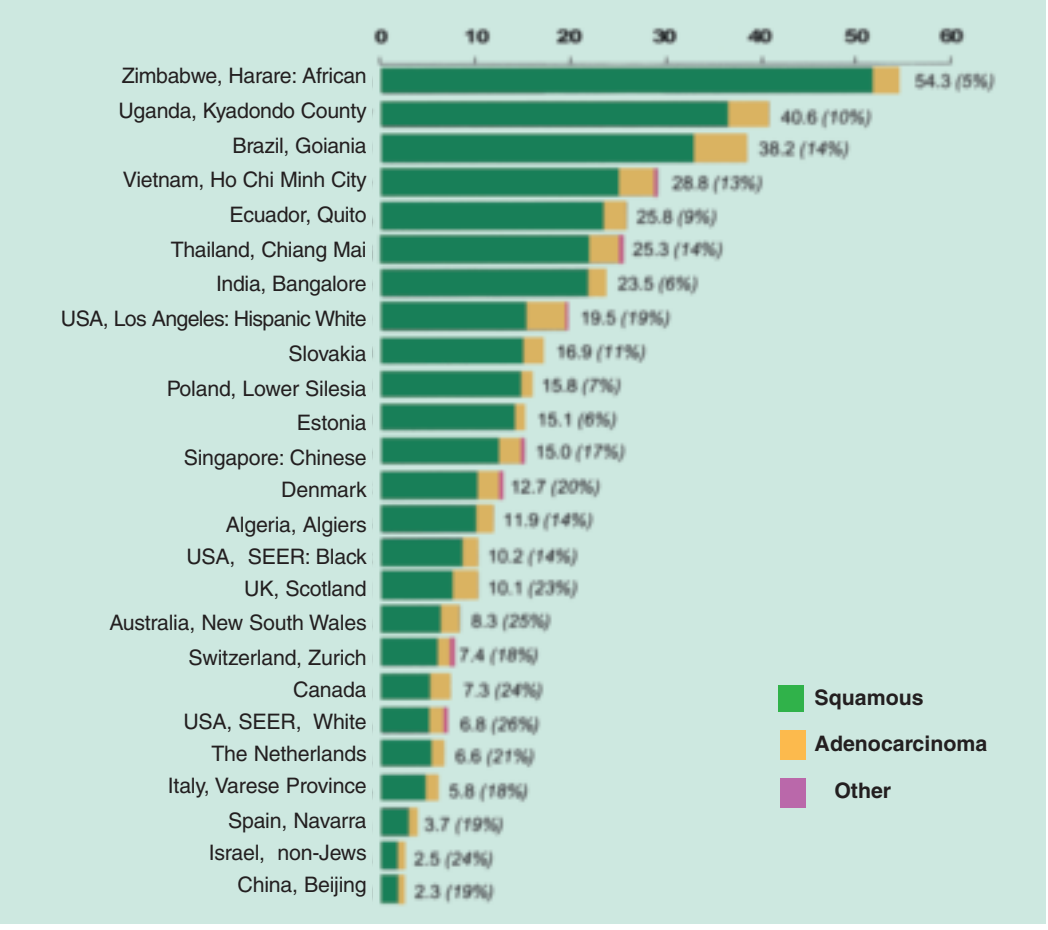


Figure 3 Age-standardized incidence rates (per 100 000) in selected cancer registry populations, around 1995), and the percentage of registered cases (of known histology) that are adenocarcinomas

Incidence rates by histological type were estimated by reallocating cases without specified histology (<10% of the total cases) to the three histological subtypes shown, according to observed proportions, by age group. From Parkin *et al.* (2002)

In general, the proportion of adenocarcinoma cases is higher in areas with a low incidence of cervical cancer (Figure 3). This probably relates to the presence of screening programmes, since cytological screening has, at least in the past, probably had little effect in reducing the risk of cervical adenocarcinoma (see Chapter 4). Adenocarcinomas occur within the cervical canal (from the glandular epithelium) and are not readily sampled by scraping the epithelium of the ectocervix (Fu *et al.*, 1987; Sigurdsson, 1995); a case–control study (Mitchell *et al.*, 1995) suggested that the risk of invasive adenocarcinoma was not reduced by screening.

There were an estimated 233 000 deaths from cervical cancer worldwide in 2000, 83% occurring in lower-resource areas, where this is the most common cause of cancer death (Parkin *et al.*, 2001). While mortality rates are much lower than incidence rates (the worldwide ratio of mortality to incidence is 49%), they correlate rather well with incidence patterns.

Demographic determinants of risk

It was noted at an early date that cervical cancer has quite marked differences in incidence according to classical demographic variables (age, social class, marital status, ethnicity, religion, occupation). Later, epidemiological studies (mainly case–control studies) showed consistent associations between risk and early age at initiation of sexual activity, increasing number of sexual partners of females or of their sexual partners, and other indicators of sexual behaviour (Muñoz *et al.,* 1992a,b). The part played by sexual behaviour of male partners in increasing risk was also the focus of interest in areas such as Latin America where cervical cancer is frequent, and where the median number of sexual partners of men is much greater than that of women, who are largely monogamous (Brinton *et al.*, 1987, 1989a,b). These findings strongly suggested a causative role for a sexually transmitted agent. The development of methods for detecting the deoxyribonucleic acid (DNA) of HPV in tissues allowed the central role of this virus in the etiology of cervical cancer to be confirmed (IARC, 1995) (see section on Etiology in this chapter).

It is likely that the observed associations of the classical demographic variables with risk of cancer of the cervix are largely the result of differences in exposure (and possibly response) to HPV, as well as to differences in patterns of screening. This can be investigated in analytical studies, where the independent effects can be investigated. Although of little interest from an etiological point of view, these demographic variables remain useful in a health services context, for example in monitoring the use of screening programmes.

The general form of the curve of incidence versus age shows a rapid rise to a peak usually in the fifth or sixth decade, followed by a plateau and a variable decline thereafter (Figure 4). This pattern with an early

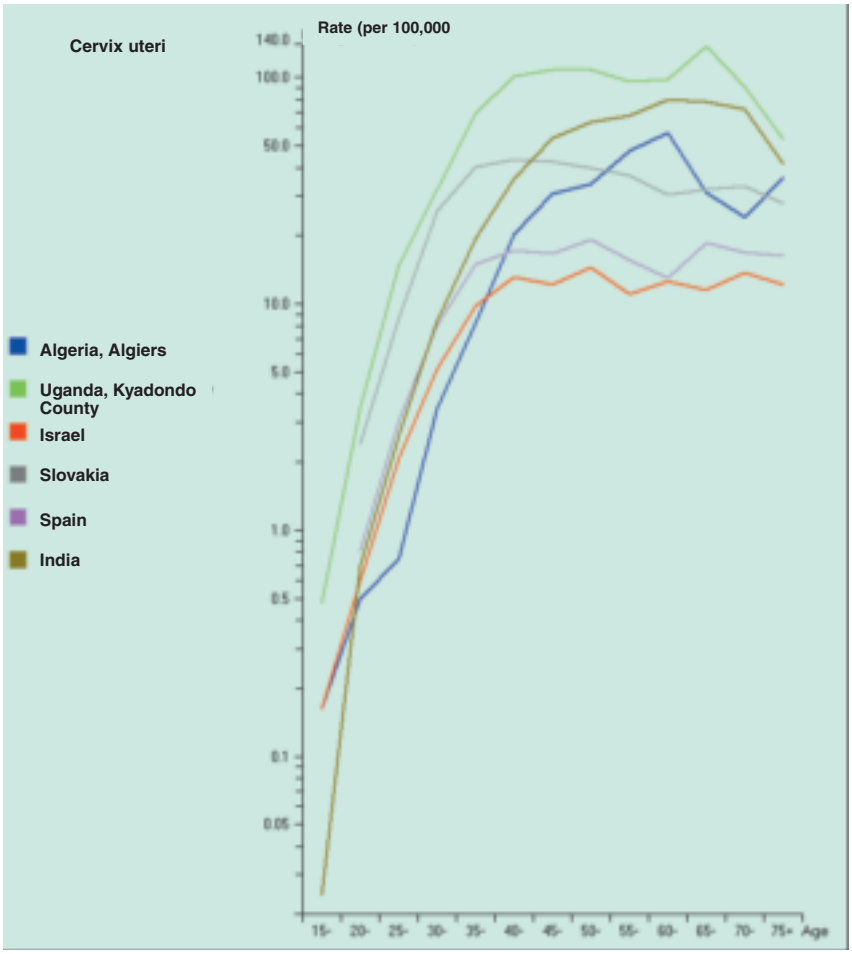


Figure 4 Age-specific incidence rates of cervical cancer in selected countries
From Parkin *et al.* (2002)

peak or plateau in risk is unique for an epithelial cancer, and reflects the natural history of infections with human papillomavirus (HPV) and the related carcinogenic mechanisms. The age profile is readily distorted by screening and also, when cross-sectional data (from a single time period) are examined, by birth-cohort-specific changes in risk (Ashley, 1966; Hakama & Penttinen, 1981). In an attempt to define the age-specific incidence patterns of cervical cancer in the absence of screening activity, Gustafsson *et al.* (1997b) compiled incidence data for 28 different populations for long periods of time between 1920 and 1989. After scaling (to permit direct comparison between countries with incidence rates of differing orders of magnitude), the rates for most populations fitted one of two reference curves used for descriptive purposes (Figure 5). The first group (*green line*), comprising Denmark, the former German Democratic Republic, the Federal Republic of Germany (before reunification), the Netherlands, Norway, Slovenia and Sweden, had an onset at about age 25, a rapid rise between 30 and 40 and a peak at ages 44–49 years. After the peak, the decline was fairly rapid, falling to half the maximum (the half peak value) at 70–75 years. The second group (*blue line*), comprising most American, Asian and African registries, plus Finland and Poland, had onset at about the same age but a slower rise to a peak at ages 50–65, followed by a decline similar to that in the first group. Data from the United Kingdom and China did not fit these curves. For the United Kingdom, this is almost certainly the result of long-term variation in risk by birth cohort (Hill & Adelstein, 1967; Osmond *et al.*, 1982), while in China it is probably due to a low level, and late onset, of exposure to etiological factors, especially HPV (IARC, 1995). Analysis of temporal changes in the curves for the Nordic countries revealed shifts in the peak incidence with time towards earlier ages. This is also probably an effect of increasing risk among successive birth cohorts, since cross-sectional analysis of age-specific incidence showed that a 3% annual increase in successive birth cohorts would move the shape of the curves for the second group of countries towards the shape seen for the first group (Gustafsson *et al.*, 1997b). This adds further weight to the other evidence that strong cohort effects exist that need to be taken into account in any analysis of incidence with respect to time.

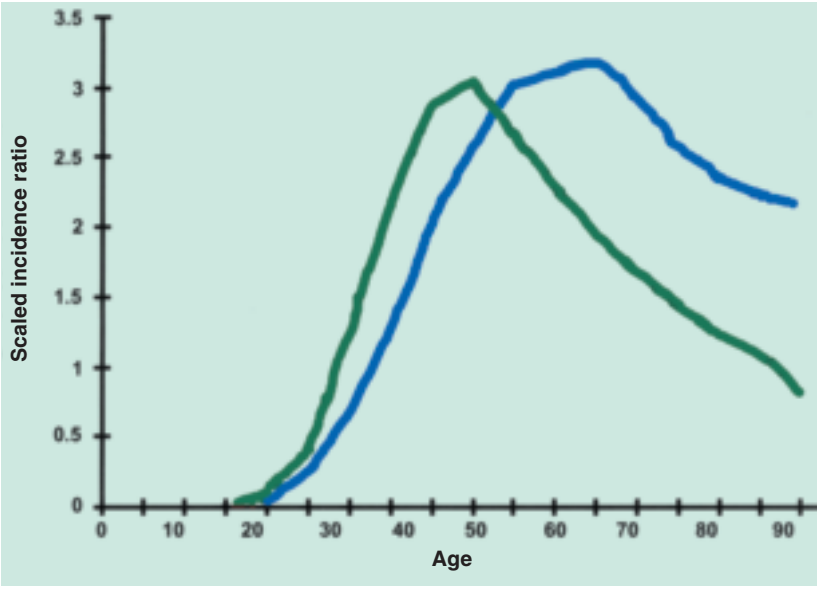


Figure 5 Scaled age-specific incidence ratios for cervical cancer for time periods prior to screening
Green line: weighted average from Denmark, Germany, Netherlands, Norway, Slovenia and Sweden.
Blue line: weighted average from Finland, Poland, Connecticut, Brazil, Colombia, Jamaica, Puerto Rico, USA, Hong Kong, India, Israel, Japan, New Zealand, Singapore, Thailand and Africa. Scaling is by dividing each value by the world-standardized rate for the same population.
From Gustafsson *et al.* (1997b) (reproduced by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

One of the earliest observations in cancer epidemiology was the rarity of cancer of the cervix among (unmarried) nuns (Rigoni-Stern, 1842), an observation that has been confirmed more recently (Fraumeni *et al.*, 1969). Risk is higher in women who are divorced or separated than in married women. The risk of cervical cancer is especially high among women marrying at young ages (Jones *et al.*, 1958; Boyd & Doll, 1964). These associations are related to other aspects of sexual behaviour such as number of sexual partners and age at initiation of intercourse (Terris *et al.,* 1967).

Women of lower socioeconomic status (defined by, for example, income, educational level or housing type) are at higher risk for cervical cancer (de Sanjose *et al.,* 1997). HPV infection appears to be more prevalent in women of lower educational and income levels (Hildesheim *et al.*, 1993; Varghese, 2000). Other correlates of social status such as nutrition, genital hygiene, parity, smoking, other genital infections and use of preventive ser-

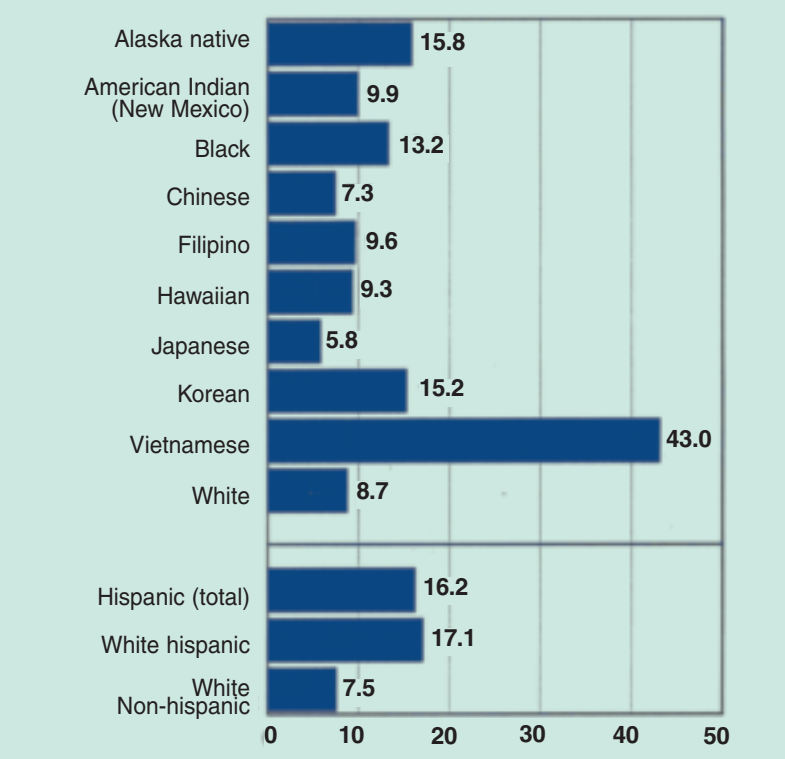


Figure 6 Incidence rates of cancer of the cervix uteri in the US SEER programme for 1988–92
From Miller *et al.* (1996)

vices (especially screening) may be responsible for the observed differences. Varghese (2000) found a significant association between social status and HPV infection in India, and social status remained a determinant of HPV infection even after adjustment for promiscuity.

In a review of data from the US Surveillance Epidemiology and End Results (SEER) programme for 1988–92, Miller *et al.* (1996) noted the highest incidence of cervical cancer among Vietnamese, with a rate some 7.4 times that in Japanese women (Figure 6). The incidence in black women was about 1.5 times that in whites. At least part of the racial differences is explicable by differences in terms of socioeconomic indicators, such as income and education; when adjustment is made for such factors, the black–white differences are greatly diminished (Devesa & Diamond, 1980). Other examples of striking differences between ethnic groups living in the same environment are the white and black populations of Harare, Zimbabwe (Bassett *et al.*, 1995), and the Chinese, Indian and Malay populations of Singapore (Lee *et al.*, 1988).

Certain religious groups in the USA, such as the Amish (Cross *et al.*, 1968) and Mormons (Lyon *et al.,* 1980), have been reported to have relatively low risks for cervical cancer compared with the general population. Jewish populations have also been noted to have lower risk than other religious groups among whom they reside (Boyd & Doll, 1964). Quite marked differences in incidence have been reported between different religious communities in Mumbai (Bombay), India (Figure 7) (Jussawalla & Yeole, 1984). The extent to which these different cancer risks reflect prevalence of HPV infection has not been studied.

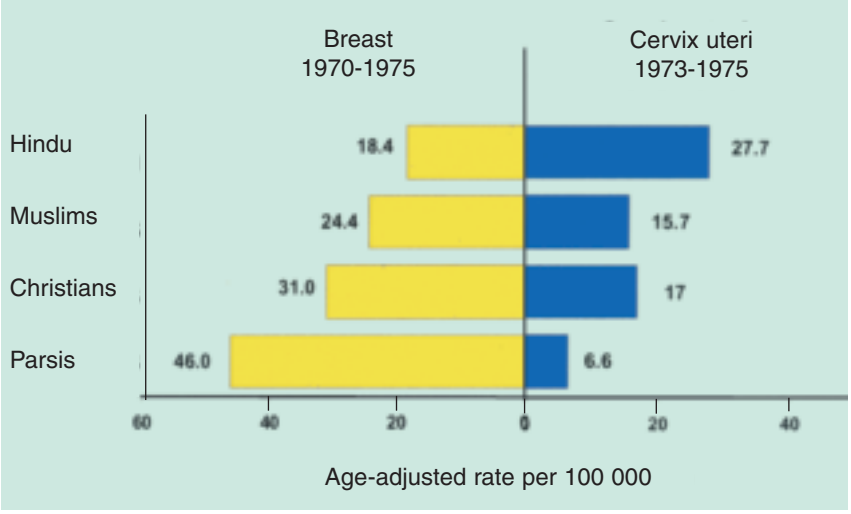


Figure 7 Incidence rates (per 100 000) of breast cancer and cancer of the cervix uteri among religious groups in Mumbai, India
From Jussawalla *et al.* (1981); Jussawalla & Yeole (1984)

High rates of cervical cancer have been reported among prostitutes (Rojel, 1952; Moghissi & Mack, 1968). Job/branch categories with excess relative risks for cervical cancer observed in studies using cancer registries or death certificates include hotel and restaurant personnel and waitresses (Williams *et al.,* 1977; Kjaerheim & Andersen, 1994; Pukkala, 1995), maids, nurses' aids (Sala *et al*., 1998), cleaners and cooks (Bulbulyan *et al*., 1992; Pukkala, 1995; Alterman *et al*., 1997) and woodworkers (Hall & Rosenman, 1991; Pukkala, 1995; Weiderpass *et al*., 2001). Exposure to various solvents has been found to be associated with increased risk (Blair *et al*., 1979; Berlin *et al*., 1995; Weiderpass *et al*., 2001). Women in agriculture seem to be at increased risk in some settings (Stubbs *et al*., 1984; Blair *et al.,* 1993; McDuffie, 1994), but at decreased risk in others (Andersen *et al*., 1999). A twofold increase in risk for cervical cancer in workers exposed to multiple pesticidal agents has been reported (Wesseling *et al*., 1996). There are also associations with occupations of husbands and partners, specifically those necessitating prolonged absences from home (Beral, 1974).

Survival and cancer control

Information on survival has long been recognized as an important indicator in monitoring cancer control activities (WHO, 2002), although it is not an adequate indicator of the effectiveness of cancer control on its own, but must be considered in context, together with incidence and mortality (Welch *et al*., 2000). Survival is usually studied to evaluate the effectiveness of treatment for cancer, and indeed, the availability and accessibility of high-quality treatment has a major influence on patient survival. It should be remembered, however, that population-based survival statistics from cancer registries reflect the outcome of the totality of cancer patients, including those who receive no treatment whatsoever. They are therefore the average result of the whole range of cancer-control activities, including screening and the organization of treatment services (Black *et al*., 1998). Estimates of survival in different populations may be influenced by a range of prognostic and other factors. Some prognostic factors, such as age and sex, are always available, and usually so too are tumour-related variables such as sub-site and histological type.

Stage of disease at diagnosis is generally the most important factor determining the survival of cancer patients, so that variations in the stage distribution of tumours among populations being compared are of particular concern. Table 1 shows a comparison of five-year relative survival, by stage, from several population-based series.

Many cancer registries attempt to collect data on extent of disease. However, there are known variations in the diagnostic techniques used to determine stage and in the adequacy of recording and abstracting the relevant data, which lead to considerable measurement error. Comparisons of stage-specific survival data between population-based registries should therefore always be performed with this potential problem in mind.

Although an improvement in survival from the cancer of interest is considered to be a necessary but non-sufficient indicator of the success of a cancer screening programme, an effective cervical cancer screening programme may, paradoxically, have the opposite result. Thus, in Finland, Dickman *et al.* (1999) observed that, although survival improved over time between 1955 and 1994 for almost all cancers, cervical cancer was an exception; for this site, survival decreased slightly from 1965–74 to 1985–94. This is because, when overall incidence decreases, due to screening, a greater proportion of cases are advanced cancers in women who have not participated in the screening programme. It is possible, too, that interval cancers may represent a length-biased subset of more aggressive tumours that were not detected by screening in preinvasive or early invasive stages.

There are two related approaches to the estimation of survival: the Kaplan–Meier and actuarial, or life-table, methods (Berkson & Gage, 1950; Kaplan & Meier, 1958). The former is particularly useful when exact survival times are available, since

Table 1. Five-year relative survival (%), by stage, from several population-based series

		Stage of cancer		
Reference	**Country, period**	**Local**	**Regional**	**Distant**
Ries *et al.* (2003)	USA: SEER (white), 1992–99	93	52	17
Dickman *et al.* (1999)	Finland, 1985–94	84	49	28
Carstensen (1993)	Denmark, 1978–87*	81	38	8
Yeole *et al.* (1998)	Mumbai, India, 1982–86	77	35	6

* Crude survival

smooth estimates of survival as a function of time since diagnosis can be obtained. The actuarial method requires a life-table with survival times grouped usually into intervals that permit calculation of the cumulative probability of survival at time t_i from the conditional probabilities of survival during consecutive intervals of follow-up time up to and including t_i. 'Observed survival' is influenced not only by mortality from the cancer of interest, but also by deaths from other causes. Relative survival takes into account the risk of death from causes other than the cancer under study (Ederer *et al.*, 1961). For comparisons between different populations, a further standardization of survival by age is necessary.

Factors influencing survival

Survival of cervical cancer patients varies by age. In the EUROCARE-3 study (Sant *et al.*, 2003), for example, relative survival of cases aged 15–44 years at diagnosis (74% at five years) was more than twice that of women who were aged 75 or more (34%), with a clear decreasing trend in survival with increasing age. The difference may be related to biological factors (tumour growth) or be the result of the higher prevalence of co-morbid disease such as hypertension and cardiovascular disease in the elderly, making the patient less likely to receive optimal treatment, or to have a favourable result from it.

Kogevinas and Porta (1997) summarized the results of ten studies that examined social class differences in survival from cancer of the cervix. In eight of these, patients of lower social class had poorer survival than those in high classes, although the differences were not great. The differences may relate to timing of diagnosis (patients of lower social class present later), in treatments applied, in the biological characteristics of the neoplasm, or in host factors. Staging procedures may be less intensive in patients of lower social class, so that there may be misclassification of more advanced cancer to earlier-stage disease. The life-tables (all-cause mortality) used to calculate relative survival only seldom allow for differences in competing causes of death between social classes. In general, however, it is thought that this is not an important source of error.

International comparisons of survival

Survival statistics for various periods from cancer registries in developed countries such as the USA, Canada, European countries, Japan and Australia have been published (Hakulinen *et al.*, 1981; Berrino *et al.*, 1995; Inoue *et al.,* 1998; Berrino *et al.*, 1999; Ries *et al.,* 2003; Sant *et al.*, 2003). Data on cancer survival from developing countries were sparse until 1995 (Nandakumar *et al.*, 1995; Sriamporn *et al.*, 1995). Sankaranarayanan *et al.* (1998a) summarized survival data from several registries in developing countries, and more recently, the first data from Africa have become available (Wabinga *et al.*, 2003; Chokunonga *et al.*, 2004).

Five-year relative survival rates vary between regions, with quite good prognosis in low-risk regions, but even in developing countries, where many cases present at relatively advanced stage, survival rates are fair: 49% on average (Sankaranarayanan *et al.*, 1998a).

Time trends in survival from cancer of the cervix

In the first half of the 20th century, there were major improvements in survival from cancer of the cervix, due in part to improving stage at diagnosis, and in part to better results of treatment within stage, particularly as a result of advances in radiotherapy (Pontén *et al.*, 1995; Sparén *et al.*, 1995). In most developed countries, there has, however, been little change in survival in recent decades. In Denmark, for example, five-year relative survival was 61.3% in 1958–62 and 63.9% in 1983–87 (Carstensen, 1993); in the USA, survival was 69.1% in 1974–76 and 71.3% in 1992–99 (Ries *et al.,* 2003).

Figure 8 shows time trends in relative survival for nine populations (Chia *et al.*, 2001). The series from Europe, the USA and Japan show little or no improvement in survival, while there has been a moderate improvement in Singapore, from 46% in 1968–72 to 63% in 1988–92. The relatively unfavourable trends in survival may be the result of a counterbalance between the effect of screening and improvements in treatment, as mentioned above. With the success of screening, the lesions that are diagnosed as invasive cancer between screenings will be those that are more aggressive and associated with poor survival.

Pathology of cervical neoplasia

The objective of cervical cancer screening programmes is to reduce the mortality from (and incidence of) the disease by identifying women with precancerous cervical lesions and early invasive cancers, and treating these women appropriately. Precancerous lesions are defined biologically as lesions that have, in principle, a capacity to progress potentially to invasive cervical cancer if left untreated. They are strongly associated with both morphological cellular changes and specific high-risk types of HPV, and continued expression of HPV-derived oncoproteins (e.g., E6 and E7) results in unregulated cellular proliferation. Phenotypically, precancers are characterized by intracellular high-risk HPV DNA, chromosomal instability with resulting aneuploidy, and monoclonality. Morphological appearances alone

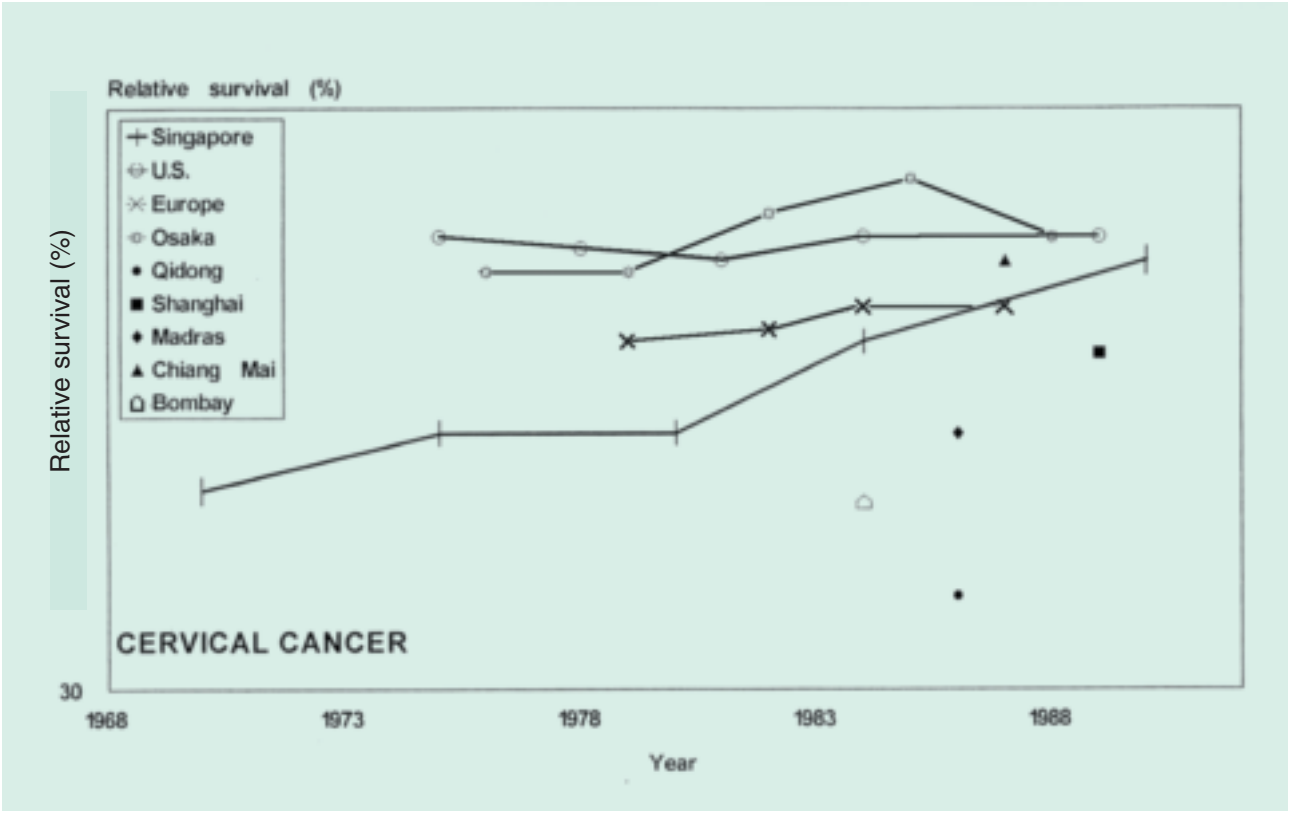


Figure 8 Relative survival of cervix cancer cases in nine populations
From Chia *et al.* (2001) Reproduced by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

often do not allow distinction of precursor lesions that have a substantial capacity to progress from those lesions that do not, contributing to uncertainty for both clinicians and epidemiologists. Nevertheless, until more precise methods are developed for use in day-to-day settings, histological appearance remains the basis for the definition of both precancerous and cancerous cervical lesions.

Intraepithelial squamous lesions

Terminology

The uterine cervix is the cylindrically shaped lower third of the uterus that extends into the vagina. The cervix has a central opening or external os that opens into the endocervical canal which communicates with the uterine cavity (Figure 9). The cervical epithelium is derived from two embryologically distinct sources. The part of the cervix that projects into the vagina, called the ectocervix or portio, is covered by non-keratinized stratified squamous epithelium similar to that of the vagina. This stratified squamous epithelium is derived from the urogenital sinus. In contrast, the endocervical canal is covered by tall, mucus-secreting columnar cells that are of Müllerian origin. The junction between these two epithelia is termed the squamocolumnar junction. The squamocolumnar junction is not fixed anatomically, but migrates throughout life. At the time of puberty, it is usually positioned towards the periphery of the ectocervix and with age, it migrates inward towards the external os (Figure 10). This migration occurs in large part by a process termed squamous metaplasia, in which the columnar endocervical-type epithelium is replaced by a stratified squamous epithelium. The area of the cervix where this transformation from columnar epithelium to stratified squamous epithelium takes place is referred to as the transformation zone (Figure 10). The metaplastic area adjacent to the receding squamocolumnar junction has, for unknown reasons, a unique susceptibility to HPV-induced neoplastic transformation, particularly in the anterior and posterior areas. These are the areas where most squamous-cell carcinomas of the cervix develop.

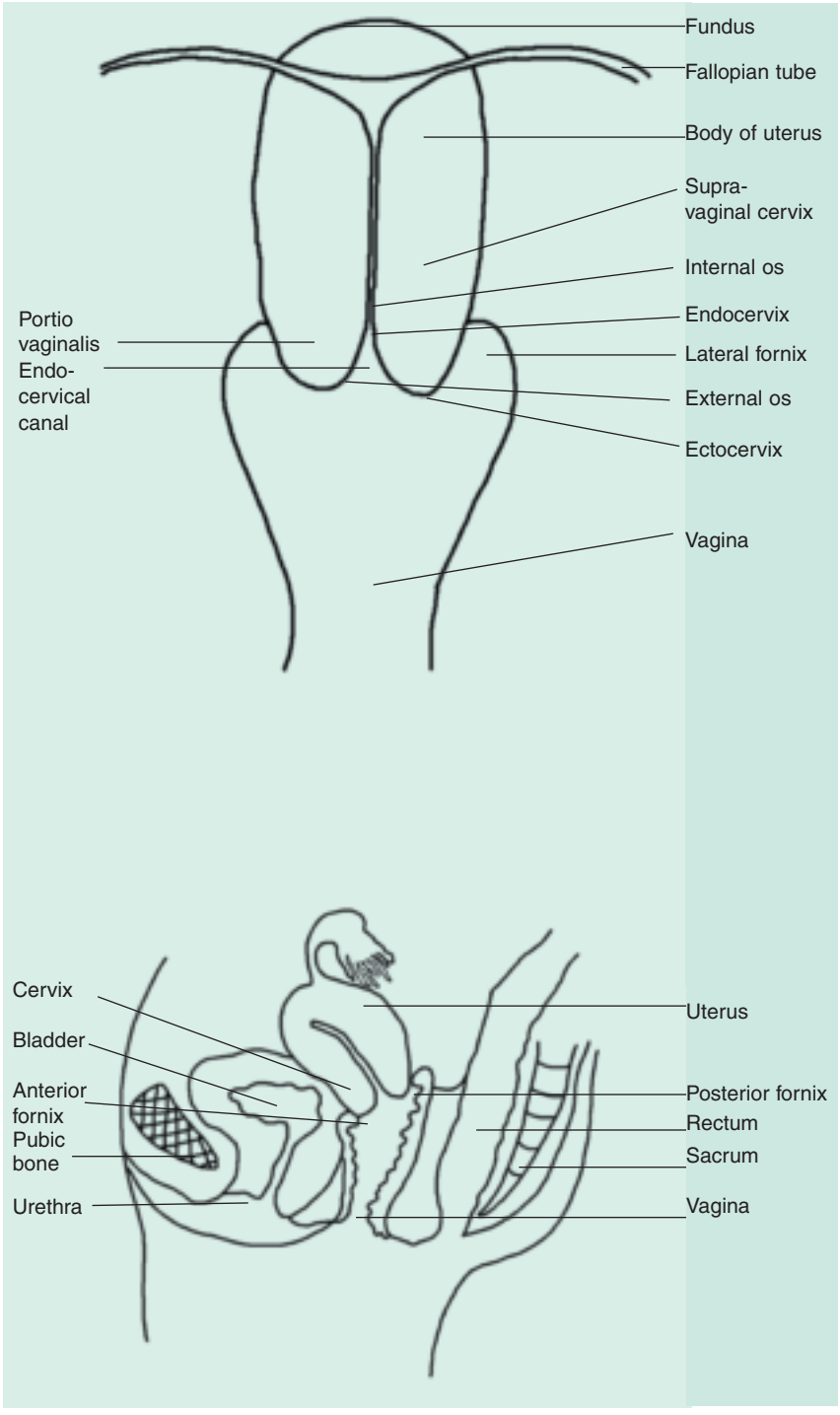


Figure 9 Gross anatomy of the uterine cervix
From Sellors & Sankaranarayanan (2003)

Cervical cancer and intraepithelial lesions that develop in the transformation zone can be visualized by colposcopy and diagnosed by histological examination of colposcopy-directed biopsies of areas that appear abnormal.

It is now generally accepted that squamous and glandular neoplasms of the cervix are caused by infection of cervical epithelium by specific HPV types (Bosch *et al.*, 1995; Muñoz *et al.*, 2003). HPV infection is associated with a wide spectrum of histological appearances, some of which may be readily identified by routine light microscopy. Terminology used to classify these cellular changes has undergone periodic revision to incorporate advances in the scientific and clinical understanding of cervical neoplasia.

At least three separate, but for the most part interchangeable, histopathological classifications are currently in use (Table 2). All recognize that persistent HPV infection of cervical squamous epithelium leads to two categories of intraepithelial squamous lesions: productive, self-limited HPV infections, and those with the potential, if left untreated, to progress to invasive squamous-cell carcinoma (Wright *et al.*, 2002b). Biopsies of productive HPV infections of the cervix have been classified as *koilocytotic atypia, koilocytosis, condyloma, mild dysplasia, cervical intraepithelial neoplasia grade 1 (CIN 1)* and *low-grade squamous intraepithelial lesion (LSIL).* CIN 1 lesions are heterogeneous with respect to their associated HPV types, clonality and ploidy status. The lesions can be associated with any of the anogenital HPV types, can be either monoclonal or polyclonal, and are aneuploid in only about one third of cases (Fu *et al.*, 1983; Lungu *et al.*, 1992; Park *et al.*, 1996; Hering *et al.*, 2000). They tend to be transient and are unlikely to act as cervical cancer precursors. Lesions more likely to represent cervical cancer precursors have

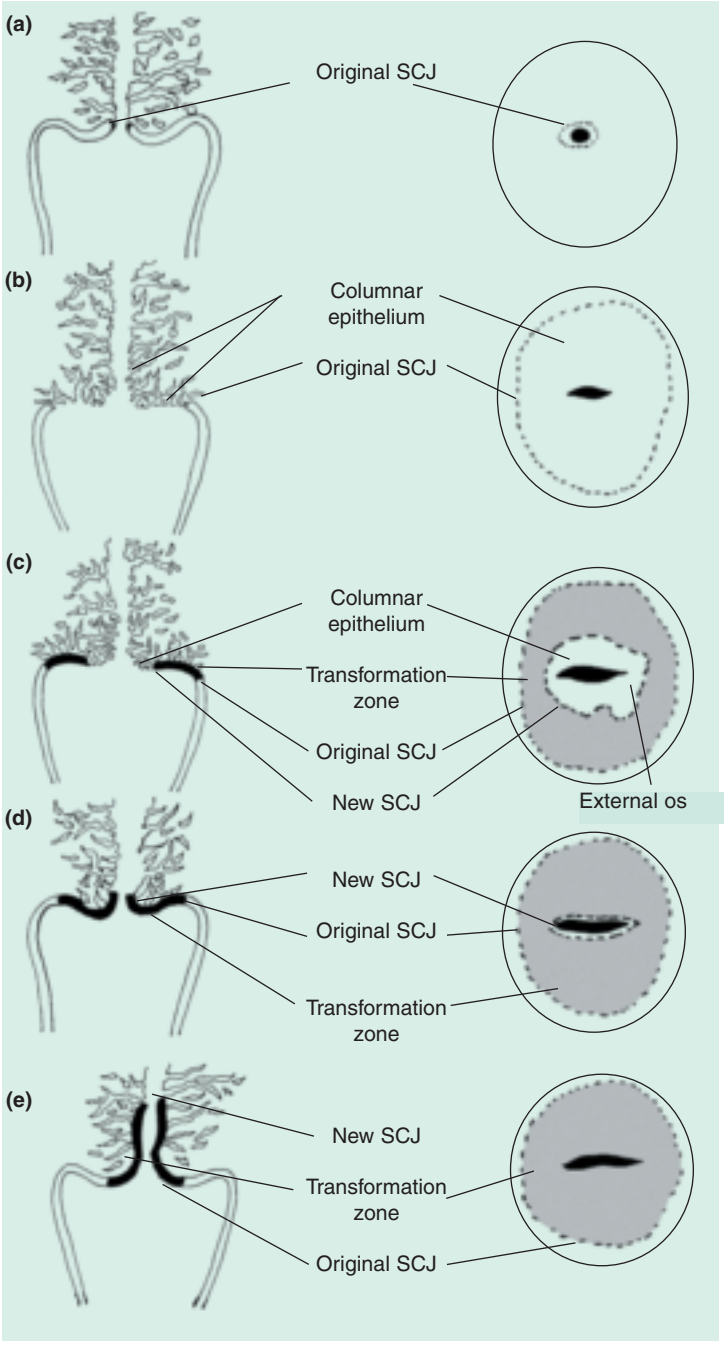


Figure 10 Location of the squamocolumnar junction (SCJ) and transformation zone: (a) before menarche; (b) after puberty and at early reproductive age; (c) in a woman in her 30s; (d) in a perimenopausal woman; and (e) in a postmenopausal woman

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been classified as *moderate dysplasia, severe dysplasia*, *CIN 2, CIN 3*, *carcinoma in situ,* and *high-grade squamous intraepithelial lesion (HSIL)*. CIN 2 and CIN 3 lesions are usually associated with high-risk types of HPV, are monoclonal and are usually aneuploid (Fu *et al*., 1983; Lungu *et al*., 1992; Park *et al*., 1996; Hering *et al*., 2000).

The designation carcinoma *in situ* was almost invariably used for full-thickness lesions of the uterine cervix by authors who adhered to the early WHO classification (Riotton *et al*., 1973). This was reflected in the early studies of the natural history of cervical cancer (see later in this chapter) and in the cases reported to cancer registries. Following Richart's (1980) description of the cervical intraepithelial neoplasia (CIN) terminology, there was an increasing tendency to include cases referred to earlier as carcinoma *in situ* within the CIN 3 designation; this tendency accelerated when the Bethesda System was introduced (National Cancer Institute, 1989). Thus, while most authors continue to use the CIN 3 designation for histological diagnoses, the carcinoma *in situ* designation has now almost completely disappeared. Because CIN 3 combines severe dysplasia, which has a defined probability of regression, with carcinoma *in situ*, which regresses less, care is required in comparing the findings from earlier studies that used the term carcinoma *in situ* with more recent studies that have not.

The traditional dysplasia/carcinoma *in situ* and CIN classifications recognize that intraepithelial squamous lesions of low, intermediate and high risk for progression to invasive cervical cancer can be identified and attempt to stratify these lesions accordingly. However, inter-observer and intra-observer studies consistently document poor reproducibility of the distinction between CIN 2 and CIN 3

Table 2. Grading schemes for preinvasive histological abnormalities of uterine cervical squamous epithelium

Dysplasia classification system	Cervical intraepithelial neoplasia (CIN)	Bethesda classification system
Mild dysplasia	CIN 1	LGSIL
Moderate dysplasia	CIN 2	HGSIL
Severe dysplasia	CIN 3	HGSIL
Carcinoma *in situ*	CIN 3	HGSIL

(Ismail *et al.*, 1989; Price *et al.*, 2003). Many pathologists report histopathological diagnoses using more than one classification scheme. In this *Handbook*, the CIN terminology is used when referring to specific histopathological entities except when directly reporting studies that used different terminology.

Pathological findings

Intraepithelial squamous lesions are characterized by abnormal cellular proliferation and maturation together with nuclear atypia. Neither ultrastructural nor immunohistochemical studies currently contribute greatly to the routine diagnosis of intraepithelial squamous lesions. The microscopic alterations that comprise intraepithelial lesions are semi-quantitatively classified into three categories. The grading of CIN lesions is prone to high rates of intra-observer and inter-observer variability (Ismail *et al.*, 1989, Robertson *et al.*, 1989a; Stoler & Schiffman, 2001). Inter-observer agreement is higher among CIN 3 lesions and lower among lower-grade lesions (Stoler & Schiffman, 2001). However, despite the poor reproducibility of a diagnosis of a given grade of CIN, separation of CIN into three subcategories (e.g., CIN 1, CIN 2, CIN 3) correlates to a general extent with rates of progression and of regression of the lesion (Mitchell *et al.,* 1996). With regard to microscopic morphological interpretation, poor reproducibility does not exclude accuracy (Renshaw, 2003).

CIN 1 (flat condyloma; koilocytosis; mild dysplasia): Neoplastic, basaloid cells and mitotic figures occupy the lower third of the epithelium in CIN 1 lesions. These lesions frequently show marked HPV cytopathic effects including perinuclear halos, multinucleation and nuclear membrane irregularities, and hyperchromasia (e.g., "koilocytosis") (Figure 11). Pathologists make frequent errors when attempting to distinguish reactive squamous proliferations from the HPV-induced lesions comprising this category. The most common error made in this category of lesions is 'overcall' of non-specific inflammatory or reactive lesions as productive HPV infections. In the National Cancer Institute's ASCUS-LSIL Triage Study (ALTS), 45% of biopsies initially classified as CIN 1 were downgraded to non-CIN when reviewed by a panel of expert gynaecological pathologists (Stoler & Schiffman, 2001). In particular, perinuclear haloes in the absence of significant nuclear atypia have been documented to be non-specific reactive features (Mittal *et al.*, 1990).

CIN 2 (moderate dysplasia): In CIN 2, neoplastic basaloid cells and mitotic figures occupy the lower two thirds of the epithelium (Figure 12). Although CIN 2 lesions usually show somewhat less HPV cytopathic effect than do CIN 1 lesions, koilocytes are often still identified in the epithelium. Distinction between CIN 2 and both CIN 1 and CIN 3 in biopsy specimens is complicated by the fact that the thickness of the epithelium occupied by neoplastic basaloid cells and mitotic figures often varies greatly within any given cervical biopsy specimen, while variations in the angle at which the epithelium has been cut during histological sectioning can also have an effect (Wright *et al.*, 2002b).

CIN 3 (severe dysplasia; carcinoma in situ): The characteristic histological feature of CIN 3 is the presence of neoplastic basaloid cells and mitotic

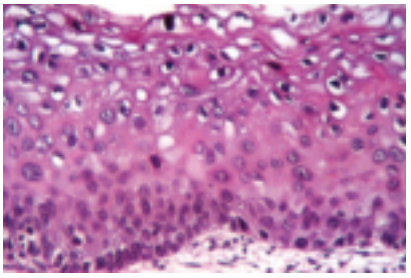

Figure 11 Cervical intraepithelial neoplasia 1 (CIN 1).
The upper two thirds of the epithelium shows maturation and focal koilocytosis. There is a mild atypia throughout.
From Tavassoli & Devilee (2003)

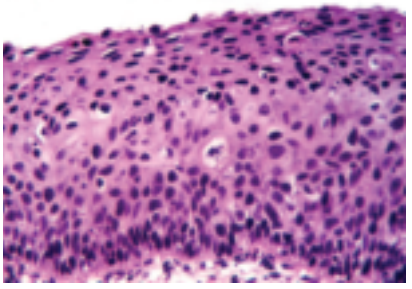

Figure 12 Cervical intraepithelial neoplasia 2
Nuclear abnormalities are more striking than in CIN 1 and mitoses are seen (centre). The upper third of the epithelium shows maturation.
From Tavassoli & Devilee (2003)

figures that occupy the full thickness of the epithelium. These cells have high nuclear:cytoplasmic ratios, with scant cytoplasm and dense, hyperchromatic nuclei having coarse clumped chromatin and irregular nuclear outlines (Figure 13). Although inter-observer variability among pathologists is moderate for histopathological diagnosis of CIN 2 and CIN 3 (Robertson *et al.,* 1989a; Stoler & Schiffman, 2001), overcall and undercall errors are not uncommon. Immature metaplasia (Crum *et al.*, 1983), atrophy and reparative processes are lesions without risk for progression to carcinoma that may be misinterpreted as CIN 2 and CIN 3. The distinction between CIN 2 or CIN 3 and atrophy in a postmenopausal patient can sometimes be established only after a repeat biopsy is taken after estrogen has been used to stimulate maturation of the cervical epithelium. Topical estrogen treatment induces maturation in atrophic cervical epithelium, but does not change the appearance of high-grade preinvasive lesions. In the future, immunohistochemical staining for various biomarkers such as p16 may be routinely usable to help distinguish CIN from its mimics. CIN 2 and CIN 3 lesions associated with extensive gland involvement may be confused with microinvasive squamous-cell carcinoma, resulting in overcall error.

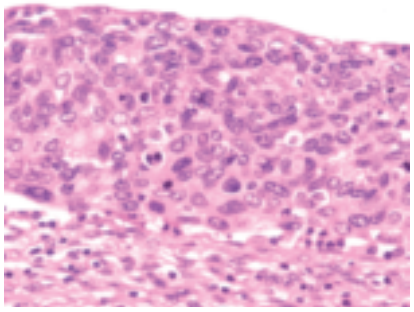

Figure 13 Cervical intraepithelial neoplasia 3

Squamous epithelium consists entirely of atypical basaloid cells. Note the moderate nuclear polymorphism, coarse chromatin and mitotic figures in the upper half of the epithelium.

From Tavassoli & Devilee (2003)

Intraepithelial glandular lesions

Terminology

Adenocarcinoma *in situ* (AIS) is the only well characterized preinvasive glandular lesion of the uterine cervix; it is much less common than its squamous counterparts. The US SEER database recorded 72 357 *in situ* cervical cancers with histology records between 1973 and 2001 (National Cancer Institute, 2004), of which only 2% were AIS. Terminology for intraepithelial glandular lesions with lower degrees of nuclear atypia and mitotic activity than AIS has been proposed; the proposed terms include *endocervical dysplasia, cervical intraepithelial glandular neoplasia and endocervical glandular atypia* (Bousfield *et al.*, 1980; Gloor & Hurlimann, 1986; Ayer *et al.*, 1987; Wright *et al.*, 2002b). Because of the rarity of biopsy-documented non-AIS preinvasive glandular lesions, the utility of non-AIS terminology has not been established.

Nearly two thirds of cases of AIS have coexisting preinvasive squamous lesions or invasive squamous-cell carcinoma (Colgan & Lickrish, 1990; Denehy *et al.*, 1997) and risk factors for AIS are similar to those for preinvasive squamous lesions (Ursin *et al.,* 1996). Because no natural history studies of AIS have been published, the evidence that AIS is the precursor lesions for invasive endocervical adenocarcinoma remains circumstantial (Wright *et al.*, 2002b). Like CIN 2 and CIN 3, AIS is associated with persistent infection with high-risk types of HPV (Tase *et al.*, 1989; Duggan *et al.,* 1994).

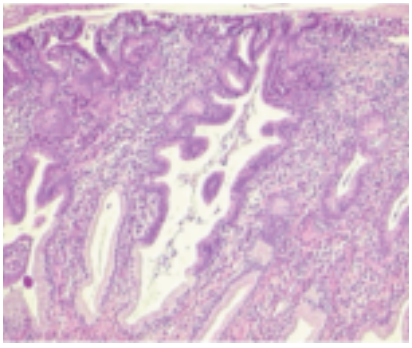

Figure 14 Adenocarcinoma *in situ*, coexisting with a normal endocervical epithelium (x 10)

From Sellors & Sankaranarayanan (2003)

Pathological findings and related errors

AIS is characterized microscopically by replacement of the glandular cervical epithelium by cytologically malignant epithelial cells. The cells of AIS have enlarged hyperchromatic nuclei that tend to stratify, have frequent mitotic figures and can form epithelial tufts (Figure 14). Glands involved by AIS do not extend into the stroma beyond the depth of glands not involved by AIS, nor by definition do they produce stromal desmoplasia. Neither ultrastructural nor immunohistochemical studies contribute to the diagnosis of preinvasive glandular lesions. Endocervical, intestinal and endometrioid subtypes of AIS have been described; of these, the endocervical subtype is the most common (Jaworski *et al.,* 1988). AIS must be distinguished from invasive adenocarcinoma, Arias–Stella reaction, glandular atypias due to inflammation and/or radiation, endometriosis, tubal metaplasia, microglandular hyperplasia and mesonephric remnants (Kurman *et al.*, 1992).

Invasive lesions

The World Health Organization Classification for tumours of the uterine cervix recognizes three general categories of epithelial tumours: squamous tumours and precursors, glandular tumours and precursors, and

Table 3. WHO histological classification of tumours of the uterine cervix

Epithelial tumours	
Squamous tumours and precursors	
Squamous cell carcinoma, not otherwise specified	8070/3
Keratinizing	8071/3
Non-keratinizing	8072/3
Basaloid	8083/3
Verrucous	8051/3
Warty	8051/3
Papillary	8052/3
Lymphoepithelioma-like	8082/3
Squamotransitional	8120/3
Early invasive (microinvasive) squamous cell carcinoma	8076/3
Squamous intraepithelial neoplasia	
Cervical intraepithelial neoplasia (CIN) 3 /	8077/2
squamous-cell carcinoma *in situ*	8070/2
Benign squamous cell lesions	
Condyloma acuminatum	
Squamous papilloma	8052/0
Fibroepithelial polyp	
Glandular tumours and precursors	
Adenocarcinoma	8140/3
Mucinous adenocarcinoma	8480/3
Endocervical	8482/3
Intestinal	8144/3
Signet-ring cell	8490/3
Minimal deviation	8480/3
Villoglandular	8262/3
Endometrioid adenocarcinoma	8380/3
Clear cell adenocarcinoma	8310/3
Serous adenocarcinoma	8441/3
Mesonephric adenocarcinoma	9110/3
Early invasive adenocarcinoma	8140/3
Adenocarcinoma *in situ*	8140/2
Glandular dysplasia	
Benign glandular lesions	
Müllerian papilloma	
Endocervical polyp	
Other epithelial tumours	
Adenosquamous carcinoma	8560/3
Glassy cell carcinoma variant	8015/3
Adenoid cystic carcinoma	8200/3
Adenoid basal carcinoma	8098/3
Neuroendocrine tumours	
Carcinoid	8240/3
Atypical carcinoid	8249/3
Small cell carcinoma	8041/3
Large cell neuroendocrine carcinoma	8013/3
Undifferentiated carcinoma	8020/3
Mesenchymal tumours and tumour-like conditions	
Leiomyosarcoma	8890/3
Endometrioid stromal sarcoma, low grade	8931/3
Undifferentiated endocervical sarcoma	8805/3
Sarcoma botryoides	8910/3
Alveolar soft part sarcoma	9581/3

"other" epithelial tumours (Table 3). The staging system developed by the International Federation of Gynecology and Obstetrics (FIGO Committee on Gynecologic Oncology and IGCS Guidelines Committee, 2000) is widely accepted (Table 4).

Microinvasive squamous-cell carcinoma

Microinvasive squamous-cell carcinomas are clinicopathologically defined lesions with minimal stromal invasion, an excellent prognosis and an extremely low incidence of lymph node metastases (Benson & Norris, 1977; Fu & Berek, 1988; Creasman *et al.*, 1998).

The FIGO defines microinvasive squamous-cell carcinomas as those demonstrating stromal invasion less than 5 mm, as measured from the point of origin of the invasive tumour elements. In addition, the surface extent of microinvasive squamous-cell carcinomas must be less than 7 mm, and there must be no histological evidence of vascular space invasion.

Invasive squamous-cell carcinoma

Three major pathological variants of invasive squamous-cell carcinomas in the uterine cervix are recognized: keratinizing carcinoma, large-cell non-keratinizing carcinoma and small-cell carcinoma. However, histopathological tumour type and tumour grade (well, moderately and poorly differentiated) are less predictive of patient outcome than the depth of invasion and presence (or absence) of lymphovascular tumour embolization (Crissman *et al.,* 1987; Look *et al.*, 1996) (Figure 15). Unusual histological variants of cervical squamous-cell carcinoma include lymphoepithelial-like carcinoma (Mills *et al.*, 1985), verrucous carcinoma (Tiltman & Atad, 1982), papillary carcinoma (Randall *et al.,* 1986) and spindle-cell (sarcomatoid) carcinoma. Spindle-cell squamous carcinoma is rare in the uterine cervix (Steeper *et*

Table 3 (contd)

Mesenchymal tumours and tumour-like conditions (contd)	
Angiosarcoma	9120/3
Malignant peripheral nerve sheath tumour	9540/3
Leiomyoma	8890/0
Genital rhabdomyoma	8905/0
Postoperative spindle cell nodule	
Mixed epithelial and mesenchymal tumours	
Carcinosarcoma (malignant müllerian mixed tumour; metaplastic carcinoma)	8980/3
Adenosarcoma	8933/3
Wilms tumour	8960/3
Adenofibroma	9013/0
Adenomyoma	8932/0
Melanocytic tumours	
Malignant melanoma	8720/3
Blue naevus	8780/0
Miscellaneous tumours	
Tumours of germ cell type	
Yolk sac tumour	9071/3
Dermoid cyst	9084/0
Mature cystic teratoma	9080/0
Lymphoid and haematopoietic tumours	
Malignant lymphoma (specify type)	
Leukaemia (specify type)	
Secondary tumours	

[1] Morphology code of the International Classification of Diseases for Oncology (ICD-O) (Fritz *et al.*, 2000) and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /2 for in situ carcinomas and grade 3 intraepithelial neoplasia, /3 for malignant tumours, and /1 for borderline or uncertain behaviour.
[2] Intraepithelial neoplasia does not have a generic code in ICD-O. ICD-O codes are only available for lesions categorized as squamous intraepithelial neoplasia grade 3 (e.g., cervical intraepithelial neoplasia 3) = 8077/2, squamous cell carcinoma *in situ* = 8070/2, glandular intraepithelial neoplasia grade 3 = 8148/2 and adenocarcinoma *in situ* = 8140/2.

Fritz, A., Percy, C., Jack, A., Shanmugaratnam, K., Sobin, L.H., Parkin, D.M. & Whelan, S. (2000) *International Classification of Diseases for Oncology (ICD-O)*, 3rd edition, Geneva, WHO

From Tavassoli & Devilee (2003)

al., 1983) and this diagnosis should be established only after clinicoradiological evaluation of extrauterine sites, together with immunohistochemical analysis to exclude a diagnosis of melanoma.

The clinical behaviour of invasive squamous-cell carcinomas may be predicted by a variety of histopathological features and ancillary studies. Tumour size, depth of invasion, parametrial involvement and nodal status

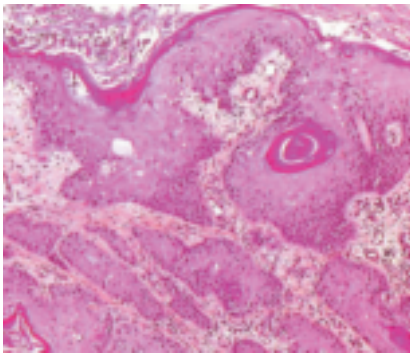

Figure 15 Keratinizing well differentiated invasive squamous-cell carcinoma (x 10)
From Sellors & Sankaranarayanan (2003)

are significant prognostic factors (Zaino *et al.*, 1992; Kristensen *et al.,* 1999). The presence of HPV type 18 in invasive squamous lesions may be associated with worse clinical outcome (Burger *et al.*, 1995; Rose *et al.,* 1995; Nakagawa *et al.*, 1996). However, neither tumour ploidy status (Atkin *et al.,* 1990) nor cellular oncogene expression (Riou, 1988) has been established as an independent prognostic marker in invasive squamous lesions.

Invasive glandular lesions of the cervix

During the past several decades, many though not all countries have seen an appreciable increase in the proportion of endocervical adenocarcinoma (Parazzini & La Vecchia, 1990; Ursin *et al.*, 1996; Vizcaino *et al.*, 1998; Alfsen *et al.*, 2000). Risk factors for invasive glandular lesions overlap with those for invasive squamous lesions, and invasive glandular lesions are associated with preinvasive squamous lesions in more than 50% of cases (Maier & Norris, 1980). Approximately 90% of invasive glandular lesions of the cervix are associated with high-risk HPV types, in particular HPV 18 (Lizano *et al.*, 1997; Pirog *et al.,* 2000). Use of immunohistochemical methods to distinguish endocervical glandular lesions

Table 4. FIGO staging for cervical cancers

Stage	Description
Stage 0	Carcinoma in situ, preinvasive carcinoma
Stage I	Invasive carcinoma strictly confined to cervix
Stage IA	Invasive carcinoma identified microscopically (all microscopically visible lesions, even with superficial invasion, should be assigned to stage IB)
Stage IA1	Measured invasion of stroma 3.0 mm or less in depth and 7.0 mm or less in horizontal spread
Stage IA2	Measured invasion of stroma more than 3.0 mm but no greater than 5.0 mm in depth and 7.0 mm or less in horizontal spread
Stage IB	Clinically visible lesion confined to cervix or microscopic lesion greater than stage IA2
Stage IB1	Clinical lesions of 4.0 cm or less in size
Stage IB2	Clinical lesions more than 4.0 cm in size
Stage II	Carcinoma extending beyond cervix but not to pelvic sidewall; carcinoma involves vagina but not its lower third
Stage IIA	No parametrial involvement
Stage IIB	Parametrial involvement
Stage III	Carcinoma extending onto pelvic wall; the tumour involves lower third of the vagina. All patients with hydronephrosis or non-functioning kidney are included unless known to be the result of other causes
Stage IIIA	Involvement of lower third of the vagina; no extension of pelvic sidewall
Stage IIIB	Extension to pelvic sidewall and/or hydronephrosis or non-functioning kidney
Stage IV	Carcinoma extends beyond true pelvic or clinically involves mucosa of bladder or rectum. Bullous oedema does not allow a case to be designated as stage IV
Stage IVA	Spread of growth to adjacent organs
Stage IVB	Spread to distant organs

From FIGO Committee on Gynecologic Oncology and IGCS Guidelines Committee (2000)

from endometrial glandular lesions revealed that 100% of AIS and 94% of endocervical adenocarcinomas were associated with high-risk HPV types (Zielinski *et al.*, 2003). The term microinvasive endocervical adenocarcinoma has been applied to invasive tumours less than 5 mm in thickness (Lee & Flynn, 2000). However, this term may not refer to a reproducible and distinctive histopathological entity (Zaino, 2000).

Endocervical adenocarcinomas exhibit several histological patterns, and different patterns often coexist in the same lesion. Histological classification of endocervical adenocarcinoma is based on the predominant pattern. The most common subtypes are mucinous and endometrioid adenocarcinoma (Saigo *et al.*, 1986; Kleine *et al.*, 1989) (Figure 16). No significant prognostic differences have been detected between the more common histological subtypes of endocervical adenocarcinoma (Alfsen *et al.*, 2001). Less common histological patterns include clear-cell adenocarcinoma (Noller *et al.*, 1974), which may occur in young women with a history of in utero exposure to diethylstilbestrol (DES), minimal deviation adenocarcinoma (also referred to as adenoma malignum) (Steeper & Wick, 1986), papillary serous adenocarcinoma (Zhou *et al.*, 1998), mesonephric

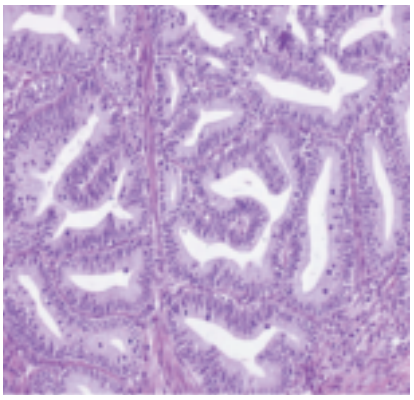

Figure 16 Well differentiated invasive adenocarcinoma (x 20)
From Sellors & Sankaranarayanan (2003)

carcinoma (Valente & Susin, 1987), villoglandular adenocarcinoma (Hopson *et al.*, 1990), glassy-cell carcinoma (Maier & Norris, 1982), adenoid cystic carcinoma (Mazur & Battifora, 1982), adenoid basal carcinoma (Baggish & Woodruff, 1966), adenocarcinoma with carcinoid features (Albores-Saavedra *et al.*, 1979) and adenosquamous carcinoma (Yazigi *et al.*, 1990). The villoglandular and adenosquamous subtypes have been reported to confer a more favourable prognosis (Chen *et al.,* 1998). The glassy-cell and papillary serous subtypes have been reported to be associated with less favourable prognosis.

Ancillary studies are generally required to distinguish primary endocervical adenocarcinoma from primary endometrial and metastatic adenocarcinomas. Immunohistochemical staining for carcinoembryonic antigen (CEA) (Kudo *et al.,* 1990), estrogen receptor protein (ER) (Staebler *et al.*, 2002), progesterone receptor protein (PR) (Staebler *et al.,* 2002), vimentin, 1C5 (Kudo *et al.*, 1990) and mucus-specific antigens M1, M2, and M3 (Maes *et al.*, 1988) have demonstrated utility in differentiating endocervical from endometrial origin for uterine adenocarcinomas. HPV in situ hybridization analysis has also been demonstrated to be useful in making this distinction (Staebler *et al.*, 2002).

Other cervical neoplasms

Unusual cervical neoplasms include leiomyosarcoma (Abell & Ramirez, 1973), endocervical stromal sarcoma (Jaffe *et al.*, 1985), embryonal rhabdomyosarcoma (sarcoma botryoides) (Brand *et al.*, 1987; Daya & Scully, 1988), alveolar soft-part sarcoma (Flint *et al.*, 1985), osteosarcoma (Gilmore *et al.*, 1956), malignant schwannoma, liposarcoma, malignant fibrous histiocytoma (Clement, 1990), malignant mixed mesodermal tumour (Gersell *et al.*, 1989), Wilms tumour (Bell *et al.*, 1985) and primary melanoma (Hall *et al.*, 1980).

Diagnosis and treatment of cervical preinvasive and invasive disease

Quality assurance is of critical importance to successful cervical cancer prevention (Miller, 2002a), and the pathologist is responsible for detecting and analysing errors that routinely occur in screening programmes by comparing histological findings with those from previous screening tests. The aim of this process of cytological/biopsy correlation (Table 5) is to ensure that women in target demographic groups receive appropriate clinical management.

Preinvasive cervical lesions

Diagnosis

Exfoliative cytology is the commonest way of diagnosing preinvasive disease; other methods, described later in this volume, include HPV DNA testing, screening colposcopy, visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI) and the newer and still experimental methods based on real-time imaging and tumour markers. A recent revision of the cytological classification criteria—the 2001 Bethesda system—(Solomon *et al.*, 2002), although keeping the classification of LSIL and HSIL to refer to the low and high grades in respect of preinvasive precursor lesions, subdivides the lower degrees of abnormality of so-called atypical squamous cells (ASC) into two categories (i.e., atypical squamous cells of undetermined significance, ASCUS, and atypical squamous cells that cannot exclude HSIL, ASC-H). The latter term (ASC-H) recognizes the high risk associated with these apparently minor cytological abnormalities containing undiagnosed HSIL lesions.

In general, women with abnormal cytological findings are referred for colposcopic evaluation, the referral criteria varying from country to country. After colposcopic diagnosis, a punch biopsy may be taken to confirm the histological diagnosis or immediate treatment may be instituted without prior histological confirmation of disease, the so-called "see and treat" approach.

Management

The atypical squamous cell classification described above comprises two categories defined as ASCUS and ASC-H. The former is associated with a relatively low risk of discovering underlying high-grade disease (in 5–17%), while the latter group has a much higher risk (Wright *et al.,* 1995; Manos *et al.*, 1999; Solomon *et al.,* 2001). The ASCUS-LSIL Triage Study (Solomon *et al.*, 2001; Stoler & Schiffman, 2001) confirmed that HPV DNA testing was helpful in identifying women with underlying high-grade histological disease in both groups. The high negative predictive value of HPV DNA testing (99%) immediately excludes the risk of underlying high-grade lesions in women with a nega-

tive result. Those with a positive result would be candidates for referral to colposcopy. An alternative to employing HPV DNA testing is repeated cytology at six- to twelve-month intervals. However, it seems that all diagnostic modalities (i.e., cytology, HPV DNA analysis and colposcopy) are fairly equivalent in the management of women with atypical squamous cells (Wright *et al.*, 2002a).

Histological confirmation of CIN 1, the most minor histological state of the three-stage CIN classification, presents problems in respect of consistency of diagnosis. In the ASCUS-LSIL Triage Study of low-grade disease, biopsies originally classified as CIN 1 were reclassified as CIN 1 in 43% of cases, downwards in 45% of cases and upgraded to CIN 2/3 in 12% (Stoler & Schiffman, 2001). Another problem is the presence of high-grade disease in up to 50% of excised specimens from women presenting with minor referral cytological abnormalities, i.e., mild dyskaryosis or LSIL (Massad *et al.*, 1996).

Rates of regression of CIN 1 appear to be up to 60%, with progression rates of only 10% (Ostör, 1993; Melnikow *et al.*, 1998) and it is not clear whether these lesions should be treated or not. Two studies (Flannelly *et al.*, 1994; Shafi *et al.*, 1997) indicated that it is safe to monitor the women with cytological follow-up rather than the commonly employed practice of immediate excision when the colposcopist assumes that underlying high-grade intraepithelial disease (CIN 2 or 3) is present. Indeed, the rate of confirmed CIN 2 or 3 in excised specimens is low (Luesley *et al.*, 1990). The Bethesda consensus conference (Wright *et al.*, 2003) recommended that women could be followed by regular cytological surveillance with no increased risk of severe preinvasive lesions being undetected. Conservative management can also be supplemented by an HPV DNA test after 12 months, when a finding of a high-risk HPV type will indicate referral for colposcopy. Likewise, after two negative cytological tests over one year and a negative HPV DNA result, a woman can be safely returned to routine screening.

Lesions histologically confirmed as CIN 3 have been found to have an average likelihood of 12% of progression to cancer, while those with milder changes (CIN 2) had an average rate of progression to carcinoma *in situ* of 22% (Ostör, 1993). Such estimates vary substantially (Mitchell *et al.*, 1996; Melnikow *et al.*, 1998), but treatment is indicated for both CIN 2 and CIN 3 lesions.

CIN in pregnancy

Regression of CIN 2 and 3 during pregnancy is minimal, but there is significant spontaneous regression post partum (Yost *et al.,* 1999). A patient with a cytological diagnosis of high-grade disease should undergo colposcopy. Punch biopsy to confirm a high-grade preinvasive lesion is not contraindicated in pregnancy. Excisional procedures, however, should be performed only where there is a definite risk of microinvasion (Wright *et al.*, 2003).

CIN in immunosuppressed women

Immunosuppressed women have an elevated incidence of persistent HPV infection, which in turn is associated with cervical cancer and its precursors. Women infected with human immunodeficiency virus (HIV) have increased rates of low- and high-grade epithelial lesions and atypical squamous cellular cytological abnormalities. (Massad *et al.*, 2001). There seems to be a high rate of recurrence and persistence of CIN 2 and 3 after treatment in HIV-positive women, with failure rates approaching 25% (Holcomb *et al.,* 1999) (see Chapter 4).

Treatment techniques

The object of treating histologically confirmed preinvasive CIN disease by any technique is to effectively eradicate the lesion, with minimal associated morbidity. Two categories of treatment techniques are available, namely destructive and excisional.

Techniques which involve destruction of the whole atypical transformation zone can be applied only if strict criteria are employed to ensure that no evidence of invasive cervical cancer lesions is present. These techniques, which include CO_2 vaporization, cryotherapy, electrocauterization and cold (thermo) coagulation, all have success rates of around 90%. Pre-treatment biopsy is mandatory and the level of destruction by these techniques extends to 7 mm depth; Anderson & Hartley (1980) showed that crypt involvement by CIN extends on average to about 3 mm. A meta-analysis has found that there is very little to choose between these techniques with regard to success or complications of treatment (Martin-Hirsch *et al.*, 2004).

Excisional techniques involving surgical removal (followed by histological analysis) range from CO_2 laser excision through the cold knife technique to the rare application of hysterectomy. However, electrosurgical excision of the transformation zone using an electrosurgical unit which produces a constant low voltage, with the ability to blend the cutting and coagulation characteristics of the current, is now the most popular technique. It must be performed after a comprehensive colposcopic examination and the intention is to remove the entire transformation zone with an adequate margin of normal squamous epithelium surrounding the abnormal area, with minimal artifactual damage (Prendiville, 2003a). Various types of morphological excision have been popularized by Prendiville (2003b), who proposed three parameters of

Table 5. Principles of error analysis through cytological/biopsy correlation (adapted from Suba *et al.*, 2004)[a]

Previous Pap smear result	Current biopsy result	Correlation requirement	Post-correlation error category	Potential action
NIL	None/missing	None		
	<CIN 1	None		
	≥CIN 1	Review of previous Pap and current biopsy	Cytology and biopsy interpretations accurate; clinical sampling error possible	Correlate with smear collector ID and monitor
			Cytology screening undercall	Correlate with cytotech ID and monitor
			Biopsy interpretive overcall	Correlate with pathologist ID and monitor
ASC-US	None/missing	Locality-specific		
	<CIN 2	None		
	≥ CIN 2	Review of previous Pap and current biopsy	Cytology and biopsy interpretations accurate; clinical sampling error possible	Correlate with smear collector ID and monitor
			Cytology interpretive undercall	Correlate with pathologist ID and monitor
			Biopsy interpretive overcall	Correlate with pathologist ID and monitor
LSIL	None/missing	Locality-specific		
	<CIN 1	Review of previous Pap and current biopsy	Cytology interpretive overcall	Correlate with pathologist ID and monitor
			Biopsy interpretive undercall	Correlate with pathologist ID and monitor
			Cytology and biopsy inter-pretations accurate; clinical sampling error possible	None (presumed spontaneous regression)
	≥ CIN 1	None		
AGC	None/missing	Alert programme manager	Clinical and/or systems errors	Clinical and/or process changes
	>CIN 1	Review of previous Pap and current biopsy	Cytology interpretive overcall	Correlate with pathologist ID and monitor
			Biopsy interpretive undercall	Correlate with pathologist ID and monitor
			Cytology and biopsy interpretations accurate; clinical sampling error possible	Alert colposcopist of possible incomplete clinical evaluation
	≥ CIN 1	None		
HSIL	None/missing	Alert programme manager	Clinical and/or systems errors	Clinical and/or process changes
	< CIN 1	Review of previous Pap and current biopsy	Cytology interpretive overcall	
			Biopsy interpretive overcall	Correlate with patholo-gist and monitor
			Cytology and biopsy interpretations accurate; clinical sampling error possible	Alert colposcopist of possible incomplete clinical evaluation
	≥ CIN 1	None		

Table 5 (contd)

Previous Pap smear result	Current biopsy result	Correlation requirement	Post-correlation error category	Potential action
Malignant	None/missing	Alert programme manager	Clinical and/or systems errors	Clinical and/or process changes
	< Malignant	Review of previous Pap and current biopsy	Cytology interpretive overcall	Correlate with pathologist ID and monitor
			Biopsy interpretive undercall	Correlate with pathologist ID and monitor
			Cytology and biopsy interpretations accurate; clinical sampling error possible	Alert colposcopist of possible incomplete cllinical evaluation
	Malignant	None		

[a] These are not practice guidelines. Rather, this table is intended to summarize the general principles of error detection and analysis through cytological/biopsy correlation studies. Previous Pap smear result(s), whenever and wherever available, should be reviewed before finalizing the histological analysis of any cervical biopsy specimen. When comparison of previous Pap smear results with current biopsy results indicates a discrepancy (correlation requirement), the previous Pap smear(s) should be retrieved and reviewed in conjunction with the biopsy. When possible, the previous Pap smear should be reviewed by a cytologist other than the one who initially examined the Pap smear.

Abbreviations: NIL, negative for intraepithelial lesion; ID, identity; CIN, cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; AGC, atypical glandular cells; HSIL, high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance

transformation-zone morphology that should be considered before undertaking excisional treatment—these are the size of the transformation zone, the position of the upper limit of the transformation zone and the visibility of this upper limit. These characteristics then identify the transformation zone as being completely ectocervical, fully visible but with an endocervical component or not fully visible. Classification of transformation zones into these three types allows simple documentation and comparison.

Martin-Hirsch *et al.* (2004) conducted a meta-analysis of 28 individual trials of various treatments for CIN. Comparison of the ablative and excisional techniques revealed no technique superior to any other. However, the authors noted that the excisional technique using an electrosurgical unit for so-called large loop excision of the transformation zone (LLETZ) produced the least morbidity and the most favourable surgical specimen for histological analysis, and concluded that LLETZ seemed to be the ideal method for treating CIN.

The effectiveness of LLETZ was analysed in two studies by Flannelly *et al.* (1997, 2001), who categorized women into three distinct groups with respect to risk of recurrence. Women younger than 50 years and without margin involvement in the excised specimen had a 92% chance of a normal cytological result in subsequent follow-up, while those with margin involvement had an 86% chance of a normal result. In contrast, older women (aged 50 and over) with margin involvement had only a 57% chance of a normal result. Therefore the latter group, who comprised only 3% of the women treated, are at high risk of recurrence and should be more intensively followed up by cytology and colposcopy than the younger group. Other studies have confirmed the relatively low failure rate with the LLETZ procedure (Tables 6 and 7).

In the 'see-and-treat' prodedure, a woman seen at a first visit can be treated on the basis of the colposcopic diagnosis, without histological confirmation. This approach obviates the need for a second visit but may result in overtreatment or unnecessary treatment of some women. In addition, some women require more time to consider their options with regard to treatment and potential complications.

New antiviral treatments using various therapeutic agents have been tested in an attempt to reduce the need for surgical intervention. Imiquimod, a non-specific immune response modulator, has been used in limited trials to treat low-grade lesions. Results suggest a variable clinical response

but with associated troublesome systemic side-effects (Cruickshank, 2003). HPV vaccines have been tried as an alternate immunotherapy regime for treating low-grade lesions. These therapeutic vaccines to eliminate HPV from the basal cells have been used in limited trials. Difficulties in assessing end-points following treatment in conjunction with the high regression rate of low-grade lesions are among the problems that hamper the acceptance of such vaccines (Fiander & Man, 2003).

Follow-up after treatment of CIN

There is a well recognized risk of recurrence of CIN and rarely of invasive cancer following both ablative and excisional treatment of CIN (Table 6). Follow-up can be by colposcopy, cytology or HPV DNA testing, or by a combination of any of these. Colposcopy at the first post-operative visit is beneficial to evaluate the physical state of the cervix and to determine the mode of follow-up. For example, if there is cervical constriction, an endocervical brush for cytological specimen collection would be recommended. The efficacy of post-operative cytological surveillance will be determined by the structure of the cervix.

Combining HPV DNA with cytological testing has been evaluated in two large meta-analyses. Zielinski *et al.* (2004) showed that the combined tests had increased sensitivity to detect persistent or recurrent CIN and increased negative predictive value to identify women at little or no risk for persistence or recurrence. The combination proved more effective than either test alone. It was recommended that women treated for CIN 3 should have this combined test after six months. If positivity on either test is found, then colposcopy and close surveillance are indicated. Women with a double negative test can be safely seen at 24 months and, if negative at this stage, should be referred back to routine screening. Paraskevaidis *et al.* (2004) drew similar conclusions, but highlighted that a positive HPV test, even in the presence of normal cytological results, can pick up treatment failures more quickly and accurately. However, they noted that cytology and colposcopy may still need to be performed to rule out false positive or negative results.

Table 6. Failure rates following loop excision (recurrence within one year)

Reference	Number of patients	Rate of residual disease
Prendiville *et al.* (1989)	102	3.0%
Murdoch *et al.* (1991)	721	4.6%
Bigrigg *et al.* (1994)	1000	5.0%
Flannelly *et al.* (1997)	1000	8.0%
Gardeil *et al.* (1997)	225	8.5%
Baldauf *et al.* (1998)	288	6.9%
Dobbs *et al.* (2000)	322	4.3%
Narducci *et al.* (2000)	505	3.7%
Paraskevaidis *et al.* (2000)	635	4.9%

Reproduced with permission from Prendiville (2003b)

Table 7. Findings of unexpected microinvasion or invasion in specimens after loop excision

Series	Unexpected		Cytology or punch biopsy findings (when known)
Prendiville *et al.* (1989)	1/102	(1%)	CIN 3
Bigrigg *et al.* (1990)	5/1000	(0.5%)	CIN 1 x 2, CIN 2 x 3
Guneskera *et al.* (1990)	1/91	(1%)	CIN 3
Luesley *et al.* (1990)	4/616	(0.6%)	–
Whiteley & Olah (1990)	0/80	(0%)	–
Halam *et al.* (1991)	8/1000	(0.8%)	–
Wright *et al.* (1991)	1/157	(0.6%)	CIN 2
Murdoch *et al.* (1992)	11/1143	(1%)	–

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Cervical glandular intraepithelial neoplasia and early invasive lesions

Diagnosis

Adenocarcinoma *in situ* (AIS) was first described in 1952. It is found in approximately 1% of all conizations performed for CIN. Coexisting CIN is found in approximately two thirds of AIS cases, while 10% of women with AIS have co-existing cancer of a glandular type. The Bethesda 2001 Conference (Solomon *et al.*, 2002)

reclassified atypical glandular cells (AGC) as atypical endocervical or endometrial cells or not otherwise specified. The management of patients with glandular abnormalities depends on the primary site. The percentage of cases of AGC associated with underlying high-grade disease is higher than for ASCUS. A cytological finding of AGC, favouring neoplasia, or of an adenocarcinoma *in situ* itself dictates referral for colposcopy. An associated CIN lesion would most likely be colposcopically diagnosed; a glandular preinvasive lesion is extremely difficult to confirm by this technique (Cullimore, 2003). Multi-focal disease is found in approximately 16% of AIS cases, with the glandular lesions themselves extending for a variable distance into the endocervical canal, although the majority (95%) progress less than 25 mm from the anatomical external os. Because of the poor sensitivity of colposcopy and even of cytology, endocervical sampling in women with these abnormal glandular cytological findings is an important part of diagnosis.

Treatment

It is important to note that colposcopy cannot adequately evaluate the endocervix and where glandular lesions are suspected, a cone biopsy will usually be required (see above). The LLETZ technique is unsatisfactory in procuring a large enough specimen for diagnosis and is associated with a higher frequency of marginal involvement (Wolf *et al.*, 1996) than with excision using the cold knife or CO_2 laser. A cylindrical base specimen with a width sufficient to encompass 5 mm on each side of the transformation zone is ideal (Cullimore, 2003). In women wishing to preserve fertility, the specimen taken usually extends for only 10 mm above the squamocolumnar junction of the cervix, but in older women it may include 20 mm of length of the endocervix.

There is a high risk of recurrence evidenced by finding residual AIS or even invasive adenocarcinoma in excisional samples. Soutter *et al.* (2001), in a study of 84 subjects from five hospitals, showed an incidence of residual disease at subsequent hysterectomy of 8/22 women in whom the margins of the initial excision specimen were involved by the glandular abnormality. In women in whom the excision margins are involved, a repeat conization is undertaken, especially in those wishing to maintain fertility, and hysterectomy in those who no longer desire fertility. Follow-up of these women by intense cytological screening and HPV DNA testing at six-monthly intervals with associated endocervical canal monitoring with either brush cytology or curettage is essential so as to check for recurrences.

Cervical cancer

Diagnosis and staging

A diagnosis of cervical cancer is uncommonly associated with an abnormal cytological result. More usually, the diagnosis is made when clinical symptoms develop. Confirmation is by biopsy taken from a suspicious lesion on the cervix or vaginal fornices. If colposcopy or a biopsy suggests early cancer in the form of microinvasion (Stage 1A), a mandatory and subsequent excisional biopsy (cone) must be taken which incorporates both epithelium and stroma, so that the depth and width of invasion below the basement membrane can be adequately assessed.

Staging for cervical cancer is based on clinical evaluation (bimanual digital pelvi-rectal examination), which should preferably be performed under general anaesthesia by an experienced examiner. The examination is designed to assess the extension of a tumour beyond the cervix into the parametria (transverse cervical ligaments), utero-sacral ligaments, the pelvic sidewall, the vagina and the bladder and/or rectum.

The clinical staging should not be changed in the light of findings of subsequent investigations. In case of doubt, the stage should be allocated to an earlier rather than a more advanced stage (FIGO Committee on Gynecologic Oncology and IGCS Guidelines Committee, 2000) (Table 4).

Additional routine investigations to supplement the staging of clinically evident cervical cancer include liver and renal function tests, chest X-ray, intravenous pyelogram/urography or ultrasound of the ureters to diagnose hydronephrosis secondary to ureteral obstruction by a tumour, cytoscopy to diagnose occult bladder invasion and proctoscopy if rectal mucosa involvement is suspected. Other investigations, such as bone scans or skeletal X-radiography, should be performed according to the clinical presentation of the patient. Endocervical curettage, hysteroscopy and colposcopy may be required to assess early microinvasive disease. Attempts to document extension of tumour to the uterine corpus should not be made.

Investigations such as computerized tomography or magnetic resonance imaging (MRI) scanning should not be used as the basis for clinical staging, but can provide useful information for planning treatment or further investigation, such as fine-needle aspiration of suspected lymph node involvement with metastases.

Diagnosis of stage IA1 and IA2 cervical cancer is based on microscopic examination of cervical tissue, provided by a large excisional biopsy (cone biopsy). The two most important dimensions are the depth of invasion (< 5 mm from the base of the epithelium from which the lesion originates) and the width of the invasive lesion (horizontal spread should be less than 7 mm). Vascular or lymphatic space

invasion does not affect the staging, but should be recorded, as it may influence treatment options (Figure 17).

It should be noted that if hydronephrosis is present, the stage is automatically allotted to stage IIIB even if the clinical staging is less advanced. The presence of bullous oedema of the bladder does not imply allocation to stage IVA unless there is histological confirmation of invasion into the bladder.

Treatment of different stages

Stage IA1 disease (depth of invasion < 3 mm and < 7 mm wide) has a risk of metastasis to regional lymph nodes of 1.2%, with a death rate of less than 1% (Sevin *et al.*, 1992; Benedet & Anderson, 1996). Where preservation of fertility is important, a cone biopsy may be considered a therapeutic procedure provided that (a) the woman is available for long-term follow-up, (b) the cervix is amenable to cytological and colposcopic evaluation, (c) the margins of the cone biopsy are free of both intraepithelial and invasive changes, and (d) there is no evidence of lymphatic or vascular invasion. Otherwise the optimal treatment is simple hysterectomy, which may be performed vaginally, abdominally or laparoscopically. Before hysterectomy, colposcopy should be performed to exclude occult extension to the vaginal fornices. If this is found, a cuff of vagina should be removed at the time of hysterectomy.

Stage IA2 (depth of invasion between 3 and 5 mm, width < 7 mm) has a risk of metastasis to regional lymph nodes of nearly 8% and a mortality rate of 2.4% (Sevin *et al.*, 1992; Benedet & Anderson, 1996). The recommended treatment is modified radical hysterectomy and bilateral pelvic lymphadenectomy, but in the absence of vascular or lymphatic invasion, a simple hysterectomy and bilateral node dissection is also considered adequate therapy (Elliott *et al.*, 2000). If preservation of fertility is important, a large cone biopsy with nodal dissection or trachelectomy with nodal dissection (extraperitoneal or laparoscopic) may be considered (Dargent *et al.*, 2000; Shepherd *et al.*, 1998).

Microinvasive adenocarcinoma seems to be the counterpart to microinvasive squamous carcinoma (Ostör *et al.*, 1997). Tumour involvement to at most 3 mm into the stroma is associated with minimal metastatic risk (Smith *et al.,* 2002a). However, measurement of such involvement and the distinction between truly invasive and intraepithelial disease remain difficult (Kaspar *et al.*, 1993). Treatment of early adenocarcinoma is contentious, but a recent meta-analysis reporting on 1170 cases treated by radical or simple hysterectomy and some even by conization showed that nodal metastasis was no more than 2.8% (Soutter, 2003). Disease-free survival rates for all treatment methods were just under 99%. Stage IA2 was not significantly different in prognosis from stage IA1 disease. The current recommendation for treatment of microinvasive adenocarcinoma is the same as for squamous-cell cancer.

Treatment strategies for stage IB and even early stage II invasive cancer include primary surgery with radical hysterectomy and pelvic lymphadenectomy or the option of primary radiation therapy with external beam radiation with either high-dose or low-dose rate brachytherapy. Published observational data indicate a five-year survival rate of 87–92% using either approach (Waggoner, 2003).

Radical hysterectomy and associated lymphadenectomy in younger patients involves ovarian conservation and avoids vaginal stenosis, which is a complication of radiotherapy. Surgical complications should be under 5% (Potter *et al.*, 1990). Radiotherapy involves a combination of external irradiation (using 40–50 Gy administered

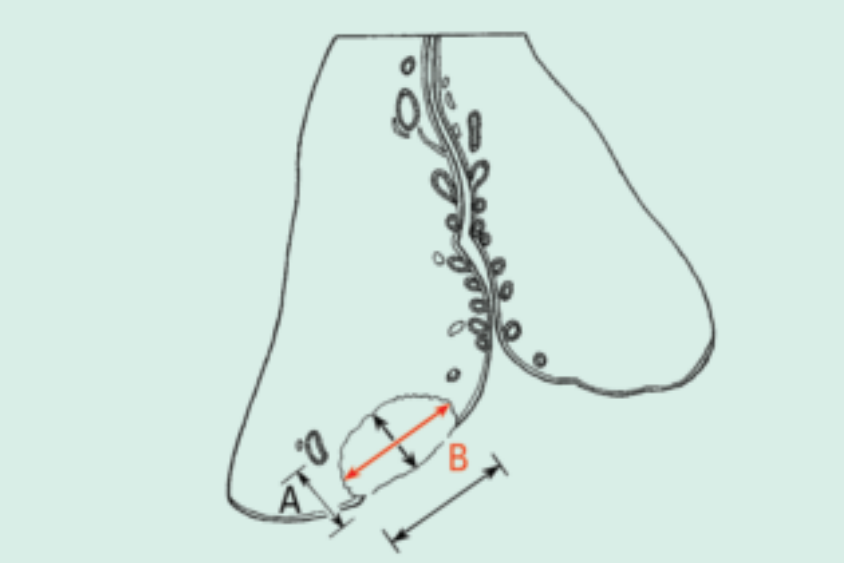

Figure 17 Schematic representation of the FIGO definition of Stage 1A carcinoma of the cervix. A: Depth of invasion no greater than 5 mm; B: Horizontal spread 7 mm or less

From Tavassoli & Devilee (2003)

over four to five weeks in daily portions) and intra-cavitary therapy (brachytherapy, with the intention of achieving a total dose of 80–85 Gy to point A and 50–55 Gy to point B), so as to treat the cervical disease, the parametrial sidewall tissues and pelvic nodes. Long-term complications involving the bladder and bowels may occur in up to 10% of cases.

Women with stage IB1 disease (under 4 cm in diameter) in whom fertility is important may be offered radical trachelectomy and laparoscopic lymphadenectomy, as described in relation to stage IA2 disease.

The treatment of stage IB1 cervical cancer (diameter < 4 cm) depends on the resources and the type of oncology services available and the age and general health of the woman. Multidisciplinary evaluation of women is recommended, to consider carefully the therapeutic options and toxicities. Dual modality treatments (surgery and radiotherapy) are more harmful, more expensive and associated with a higher rate of complications. Therefore, primary therapy should aim to use only one radical therapy, either surgery or radiation (with or without concurrent chemotherapy).

Recommended surgery involves radical hysterectomy (removal of the uterus, cervix, parametria, cuff of vagina, utero-sacral ligaments), bilateral pelvic lymph node dissection and ovarian suspension where appropriate.

If surgery is deemed inappropriate, primary radical chemo-radiation therapy is recommended. The standard radiation treatment is radical external beam radiation and brachytherapy. Concurrent chemotherapy is usually cisplatin given in a dose of 40 mg per m^2 weekly during external beam therapy.

When positive common iliac or para-aortic nodes have been identified as involved with metastatic disease, extended field radiation may be considered.

Five-year survival rates of generally 80–90% following either radical surgery or radical radiation as primary therapy for stage IB1 tumours have been reported (Hopkins & Morley, 1991; Landoni *et al.*, 1997; Waggoner, 2003).

For stage IB2 disease (diameter > 4 cm), five-year survival rates are reduced to approximately 65–75% (Hopkins *et al.,* 1991). Para-aortic nodes are commonly involved in this stage, as well as an increase in central and distant failures associated with recurrence.

Treatment options include (a) primary chemo-radiation therapy alone (Rose *et al.*, 1999a); (b) primary radical hysterectomy with bilateral regional lymph node dissection, usually followed by radical adjuvant radiation (with or without concurrent chemotherapy), determined by pathological criteria such as disease-free margins, lymph-vascular space involvement and metastases to lymph nodes (Keys *et al.*, 1999); and (c) neoadjuvant chemotherapy, followed by radical surgery as described above and the possible use of post-operative radiation (Sardi *et al.*, 1993).

The standard primary treatment of advanced cervical cancer (stages IIA, IIB, IIIA, IIIB, IVA) is primary radical radiation with a combination of external beam and intracavitary brachytherapy, with concurrent chemo-radiation therapy (Keys *et al.*, 1999; Rose *et al.*, 1999b; Whitney *et al.,* 1999). Results from five randomized trials on treatment of cervical cancer in the late 1990s prompted the US National Cancer Institute to recommend the incorporation of concurrent cisplatin-based chemotherapy in radiation therapy for cervical cancer treatment (National Cancer Institute, 2002).

If the surgical expertise and support services for post-operative care are available, pelvic exenteration may be considered for stage IVA disease, so long as there is no evidence of extension to pelvic sidewalls, in a patient with good general health.

Recurrent cervical cancer (stage IVB) may be in the pelvis, distant sites or both. The majority of recurrences occur within two years of diagnosis and the prognosis is poor, with most patients dying of their disease, with a mean survival time of seven months (van Nagell *et al.*, 1979).

Management of women with distant metastases and advanced recurrent cervical cancer requires the efforts of a multidisciplinary team, and includes palliative use of anti-cancer therapies (chemotherapy, radiation therapy for treatment of symptoms, including surgery such as colostomy for relief of symptoms related to recto-vaginal fistulae), control of symptoms (pain, bleeding, discharge, symptoms related to specific metastases), emotional, psychological and spiritual support of the patient and her family.

Invasive cervical cancer in pregnancy

Approximately 3.5% of cervical cancers occur in pregnancy. Survival rates for those with stage I disease are around 85–95% (Hopkins *et al.*, 1991). Individual management plans must be developed with proper consideration of the tumour size, the stage of the disease and the desire of women for continuation of the pregnancy.

Women with high-grade intraepithelial disease can deliver vaginally and be re-evaluated at four months post-partum. Those with early invasive disease (stage IA or B) may choose termination or continuation of the pregnancy until maturity of the fetus determines the date of delivery. The delay to accomplish fetal maturity, especially in patients with early invasive disease, leads to only a small degree of disease progression (Hopkins *et al.*, 1991). The mode of delivery depends on the stage of the lesion and its volume; it is unclear whether vaginal delivery

influences progression. Most patients with stage I disease prefer caesarean section at the time of planned radical surgery, with vaginal delivery reserved for those with preinvasive or stage IA1 disease. Radical surgery and radiation offer similar cure rates, with the former being used for stages IA2, IB and IIA. Such pregnancies have, surprisingly, been associated with low morbidity and high survival rates when surgery is used (Goff *et al*., 2000). Retention of ovaries in young women is indicated. However, women with stage IIB or more advanced disease or those not medically fit are candidates for definitive radiation therapy, which should be initiated immediately after delivery. The actual application of radiation requires adaptation to the anatomical distortions created by the pregnancy and patients opting for primary radiation therapy who intend to have a primary termination should have this before external therapy begins.

Follow-up

Women who have had cervical cancer require four-monthly follow-up for the first two years and then twice yearly for the subsequent five years. An annual cytological examination of the vaginal vault and chest X-rays on a regular annual basis for five years have been recommended (American College of Obstetricians and Gynecologists, 2002). However, the interpretation of cytology and/or colposcopy following irradiation requires special expertise.

Immunosuppression

Women infected with HIV are of particular concern, particularly as cervical cancer is very common in areas where HIV infection is endemic, such as sub-Saharan Africa. Women who are immunosuppressed, with CD4+ counts below 200 cells/μL, are at particular risk if treated with either radiation or chemotherapy, both of which have immunosuppressive effects. Where possible or appropriate, surgical treatment is preferred for HIV-positive immunosuppressed women. Women with CD4+ counts over 350 cells/μL appear to tolerate anti-cancer therapies as well as HIV-uninfected women (Lomalisa *et al*., 2000).

Cervical cancer in developing countries

In low-resource countries, many of the facilities for the treatment of cervical cancer do not exist, or if they do, the equipment is poorly maintained and will not provide either optimal or even suboptimal therapy. Chemotherapy may not be available, nor the resources or skills to provide radical surgical interventions. In these settings, even women with early cervical cancers will have a poor prognosis and the development of effective palliative care is essential.

In a low-resource environment, it will be difficult to apply the methods of diagnosis mentioned above to assist in defining the extent of malignant spread within the pelvis and allow accurate assessment of the stage of the cancer.

However, techniques such as cystoscopy, proctoscopy, radiography of pulmonary and renal systems with haematological and biochemical assessment can be performed within most settings. MRI and computerized tomography scanning may provide additional information but are not mandatory in assessing the FIGO staging. These sophisticated diagnostic techniques may provide valuable information for planning treatment but are extremely expensive. In low-resource settings, physical examination of the cervix, vagina, bladder and rectum sometimes offers the only feasible approach to staging.

Facilities for radical surgery, radiotherapy (in the form of external and intracavity radiation) and chemotherapy with platinum derivatives are available in many developing countries in Asia and Latin America (Sankaranarayanan *et al*., 1998a). However, this is not the case in large parts of sub-Saharan Africa.

Palliative care is inadequate in many regions of the world because of poor availability of medication, deficient health-care infrastructure, lack of training for health-care providers, associated with lack of counselling skills. Discomfort in discussing the diagnosis and management with patients and their families and a lack of awareness within the community of palliative care options are very common. Many health-care providers and policy makers lack awareness that there are inexpensive and effective ways to relieve advanced cancer symptoms (ACCP, 2003; WHO, 2001, 2002). WHO has developed a simple and inexpensive three-step analgesic ladder that can be easily incorporated into a treatment protocol (WHO, 2002).

The support of families and caregivers forms a most important part of the palliative management regime, and sometimes is the only source of social and psychological support. Patients and families must be informed and encouraged to believe that the cure of cervical cancer is feasible even in their sometimes deprived environment (Sankaranaranayan *et al*., 1998).

The etiology of cervical cancer

The epidemiological evidence relating HPV DNA to cervical cancer and its precursors includes a large and consistent body of data indicating, beyond any reasonable doubt, strong and specific associations between HPV infections and cervical cancer. The findings are consistent for all countries where investigations have taken place.

Once it was recognized that HPV represents a necessary cause of cervical cancer, reassessment of the role of co-factors in case–control studies was required by analysis restricted to HPV-positive women. Among persistently HPV-exposed women, some additional exposures further increase their risk of progression to advanced preinvasive lesions or invasive cancer. In the IARC studies, these co-factors were exposure to tobacco smoke, parity above five full-term pregnancies and use of oral contraceptives for five or more years. Presence of antibodies to *Chlamydia trachomatis* or to herpes simplex virus type 2 (HSV2) also modified the risk of progression significantly. Table 8 shows results pertaining to use of oral contraceptives, parity and cigarette smoking from the IARC multicentre case–control study (Castellsagué & Muñoz, 2003). The reported increases in risk for any of the co-factors were in general highly consistent within the range of 2.5- to 4-fold for the extreme categories of exposure.

Human papillomaviruses

Natural history and follow-up studies have clearly shown that HPV infection precedes the development of cervical cancer by a number of years and confirmed that sexual transmission is the predominant mode of HPV acquisition. These studies provided biological support for the long-known clinical and epidemiological observations that cervical cancer displays the profile of a sexually transmitted disease (STD). Case–control studies, case series and prevalence surveys have unequivocally shown that HPV DNA can be detected in adequate specimens of cervical cancer in 95–100% of cases, compared with a prevalence of some 5–20% in cervical specimens from women identified as suitable epidemiological controls.

The association has been recognized as causal in nature by a number of international review parties since the early 1990s, and the claim has been made that this is the first necessary cause of a human cancer ever identified.

The implications of the recognition that, in the absence of viral DNA, cervical cancer does not develop, are of considerable public health relevance. On the one hand, the concept of risk groups comes into focus. High-risk women can be redefined as those who are persistent HPV carriers. Operationally this represents substantial progress from previous definitions of high-risk women according to their exposure to a constellation of ill-defined factors (low socioeconomic status, high number of sexual partners, smoking, use of oral contraceptives, history of STDs or any combination of the above). Most of these factors are now viewed either as surrogates of HPV exposure or as relevant co-factors given the presence of HPV DNA. On the other hand, if indeed HPV is a necessary cause of cervical cancer, the implication is that specific preventive practices targeting some putative non-HPV-related cervical cancer cases are no longer justified. Finally, methods are now available to screen the general population for HPV-DNA positivity.

On the basis of epidemiological surveys, the 40 HPV types infecting the genital area can be subdivided into low-risk types, which are mainly found in genital warts and CIN 1, and high-risk types, which are frequently associated with invasive cervical cancer. HPV DNA can be found in virtually all cervical carcinomas, with HPV types 16, 18, 31 and 45 being the most frequent ones (Bosch *et al.*, 1995; Walboomers *et al.*, 1999; Bosch *et al.*,

Table 8. Association of relevant co-factors among HPV-positive women

Co-factor	HPV-positive women	
	Cases/controls	OR (95% CI)
OC use (status and years)		
Never	1071/163	1
Ever	605/92	1.13 (0.80–1.59)
1–4 years	274/64	0.66 (0.45–0.98)
5 years	331/28	2.35 (1.44–3.85)
Full-term pregnancies (status and no.)		
Never	57/24	1
Ever	1616/229	2.45 (1.33–4.51)
1–2	279/59	1.79 (0.94–3.40)
3–4	450/70	2.61 (1.37–5.00)
5	887/100	3.88 (1.99–7.55)
Smoking (status and amount)		
Never	1265/218	1
Ever	409/36	1.99 (1.29–3.07)
1–5 cigarettes/day	181/17	1.72 (0.98–3.01)
6 cigarettes/day	211/18	2.16 (1.18–3.97)

OC, oral contraceptive
ORs adjusted for centre, age (<37, 37–45, 46–55, 56+), educational level (none, primary, secondary or higher), smoking amount (never, 1–5 cig./day, 6 cig./day+), age at first sexual intercourse (<17, 17–18, 19–22, 23+), lifetime number of sexual partners (1, 2–3, 4+), OC use (never, 1–4 years, 5–9 years, 10 years+), lifetime number of Pap smears (0, 1–5, 6+), and parity (0, 1–2, 3–4, 5–6, 7+).
Adapted from Castellsagué & Muñoz (2003)

2002; Clifford *et al.*, 2003a; Muñoz *et al.*, 2003). HPV types 33, 35, 51, 52, 58 and 59 are the next most common types in cervical carcinoma, with some geographical variation (Bosch *et al.,* 1995; Clifford *et al.*, 2003a; Muñoz *et al.*, 2004). These are also the most frequent types in HPV-DNA-positive HSIL (Clifford *et al.*, 2003b). It has been proven beyond reasonable doubt that infection with a high-risk HPV is a necessary prerequisite for the development of cervical cancer and IARC has evaluated HPV16 and HPV18 as carcinogenic agents for humans (IARC, 1995).

Epidemiological criteria to evaluate the causality of any given association have been developed. To the classical criteria known as the Bradford Hill criteria, IARC has added special rules to interpret specifically associations between viral agents (and other biological agents) and human cancer (IARC, 1995). Table 9 presents the key criteria to be examined and a qualitative assessment of how they are fulfilled by the available evidence. A comprehensive evaluation of the association between HPV and cervical cancer has been published (Bosch *et al.*, 2002).

Systematic review of the causality criteria strongly indicates that the association of HPV and cervical cancer is causal in nature. The association is very strong, consistent, specific and universal. HPV infection precedes preinvasive disease and cervical cancer. Under optimal conditions, HPV DNA can be found in all cervical cancer cases worldwide. The biological plausibility of the association (reviewed elsewhere in this chapter) is consistent.

A brief selection of key studies is reviewed below, outlining their contribution to the fulfilment of the currently accepted causality criteria: temporality and strength of the association, and exclusion of alternative explanations.

HPV and cervical neoplasia

Temporality

Cross-sectional studies have repeatedly found that sub-clinical HPV infections are highly prevalent in young individuals, whereas invasive cervical cancer typically develops in the third decade or later (Figure 18). The cross-sectional prevalence of HPV DNA decreases spontaneously to a background level of 2–8% in most populations in groups aged 35 years and above. In countries where intensive screening of young women takes place, part of the reduction in HPV prevalence may be attributable to aggressive treatment of HPV-related cervical lesions. Women who remain chronic HPV carriers are now considered the true high-risk group for cervical cancer. In some populations, a second peak of HPV DNA prevalence has been observed for older women (i.e., 50 years and above) and a second peak in the incidence of CIN 3 lesions and of invasive cervical cancer has also been reported (Herrero *et al.*, 2000; Lazcano-Ponce *et al.*, 2001). In all settings investigated, the point prevalence of HPV DNA in the young age groups is strongly related to the dominant sexual behaviour patterns in the population (Bauer *et al.*, 1993; Melkert *et al.*, 1993; Bosch *et al.*, 1994; Kjaer *et al.*, 1997; Jacobs *et al.*, 2000; Schneider *et al.*, 2000).

These population studies provide support for the concept that HPV infections precede the development of cervical cancer by some decades. Data from most cancer registries, including those in the USA, have established that the age-specific incidence of cervical cancer has a rising trend in the age interval 20–40 years, and shows a plateau or continues to increase smoothly after that age. Only occasionally do cases of invasive disease occur at earlier ages. Figure 18 shows the age-specific cross-sectional prevalence of high-risk HPV DNA in a screening programme in The Netherlands and the corresponding age-specific incidence rates of cervical cancer in that country. Very similar curves can be seen for other high- and low-risk countries (Bosch *et al.*, 1992; Muñoz *et al.,* 1992; Parkin *et al.*, 1997; Jacobs *et al.,* 2000).

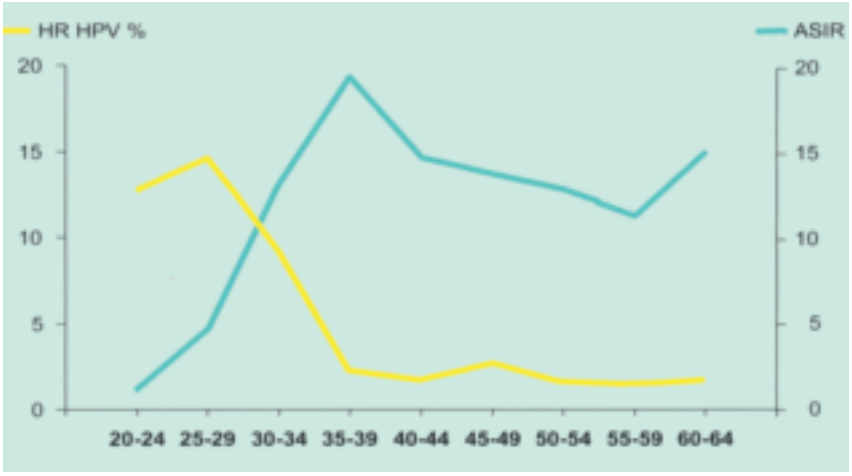

Figure 18 Age-specific prevalence of high-risk HPV DNA in 3700 women entering a screening programme and age-specific incidence rate (ASIR) of cervical cancer in The Netherlands

Sources of data: Jacobs *et al.* (2000); Parkin *et al.* (1997)

From Bosch *et al.* (2002) (reproduced with permission from the BMJ Publishing Group)

Table 9. Causality criteria and their fulfilment by the association of HPV DNA and cervical cancer

Criterion	Concept	HPV and cervical cancer	
		Type of evidence	Evaluation
Time sequence	Exposure must precede disease	Cohort studies to CIN 2/3	+++
Experimental (prevention)	Reduction of disease following reductions in exposure	Early vaccination trials	+
Strength and consistency	High OR/RR. Robust association in different settings	Case-control studies	+++
Biological plausibility and coherence	Mechanisms. Consistent with previous knowledge	Experimental	+++
Dose–response	Risk of disease is related to levels of exposure	Studies on number of partners	+
Qualification of causality			
Necessary	Exposure is present in all cases	Detailed investigation on 'HPV-negative' cervical cancer speciments. Exclusion of alternative explanations	++
Sufficient	Exposure always leads to disease	Natural history of transient infections	_

OR: Odds ratio; RR: Relative risk; CIN: cervical intraepithelial neoplasia
Table adapted from Bosch *et al.* (2002)

For the relationship between cervical cancer and HPV, compliance with the temporality criteria has been established by numerous cohort studies that monitored women from cytological normality to the stage of high-grade cervical intraepithelial neoplasia (HSIL or CIN 2 and 3). Continuing to monitor women to invasive disease is not acceptable on ethical grounds and thus information is not available.

Repeated sampling of women being followed for viral persistence and cervical abnormalities has shown that the median duration of infections detected at study enrolment is around eight months for high-risk HPV types (HPV16 in particular), compared with 4.8 months for the low-risk HPV types. In two unrelated studies, the time estimates were fairly consistent (Ho *et al.,* 1998; Franco *et al.,* 1999). In one study of incident HPV infections in Brazil, the mean duration of HPV detection was 13.5 months for high-risk HPV types and 8.2 months for the non-oncogenic types. HPV16 tended to persist longer than the average for other high-risk types (Franco *et al.*, 1999). The results were remarkably similar in student populations in the USA and the United Kingdom (Ho *et al.*, 1998; Woodman *et al.*, 2001). The self-limiting course of most HPV infections is consistent with the cross-sectional profile displayed in Figure 18. However, the currently observed time intervals may still suffer from imprecision in the estimates of time at first exposure, from variability in the end-point definition and from censoring due to treatment of early lesions.

Follow-up studies of women with and without cervical abnormalities have indicated that the continuous presence of a high-risk HPV is necessary for the development, maintenance and progression of progressive CIN disease (Koutsky *et al.*, 1992; Ho *et al.*, 1995; Remmink *et al.*, 1995; Ho *et al.*, 1998; Nobbenhuis *et al.*, 1999). A substantial fraction (15–30%) of women with high-risk HPV DNA who are cytomorphologically normal at recruitment will develop CIN 2 or CIN 3 within the subsequent four years (Koutsky *et al.,* 1992; Rozendaal *et al.*, 1996, 2000). Conversely, among women found to be negative for high-risk HPV DNA and cytologically identified as having either ASCUS, borderline or mild dysplasia, CIN 2 or 3 is unlikely to develop during a follow-up of two years and their subsequent cytological results are likely to return to normal (Nobbenhuis *et al.*, 2001a; Zielinski *et al.*, 2001a). Women found positive for low-risk HPVs rarely become persistent carriers and their probability of progression to CIN 2 or 3 is extremely low (Manos *et al.,* 1999; Zielinski *et al.*, 2001a).

As existing cohorts extend their follow-up time, more precise estimates are being obtained on the predictive

value of viral persistence as defined by repeated measurements of viral types and variants. In one such cohort in São Paulo, the incidence of cervical lesions in women who were HPV-negative twice was 0.73 per 1000 woman-months. The corresponding incidence among women with repeated HPV16- or HPV18-positivity was 8.68, a 12-fold higher incidence. The OR for HPV persistence among women who were twice HPV-positive for the same oncogenic types was 41.2 (95% CI 10.7–158.3) (Schlecht *et al.*, 2001). Retrospective assessment of HPV DNA status using archival smears from cases of cervical cancer and controls has provided evidence that HPV infection preceded the development of invasive disease, and showed its value in signalling false negative cytological results (Zielinski *et al.*, 2001a). It was also suggested that clearance of high-risk HPV in otherwise established cytological lesions is a marker associated with regression of CIN lesions (Nobbenhuis *et al.*, 2001a; Zielinski *et al.*, 2001b). Finally, persistence of HPV DNA detection after treatment for CIN 2 or 3 is an accurate predictor of relapse (Nobbenhuis *et al.*, 2001b).

These results are useful in defining the clinical role of HPV testing. However, most observations on preinvasive disease have limitations for making inferences on cervical cancer causality. This is because even in controlled settings, observations cannot be allowed to continue beyond the stage of HSIL/CIN 3 or carcinoma *in situ.*

A valuable approach to conducting follow-up studies of invasive cancer (as opposed to studies of CIN 3) without ethical and time constraints is provided by nested case–control studies. These are studies initiated several years in the past that assembled and stored large banks of biological specimens from healthy individuals. Linkage of a serum sample bank to cancer registry data can then identify cases of cervical cancer (or any other condition) that have occurred in the interval; the original specimens can be analysed for the presence of HPV biomarkers. HPV DNA prevalence can then be compared with the corresponding prevalence in specimens from epidemiologically suitable controls (individuals from the same cohort who did not develop the condition under otherwise equivalent exposures). Such studies have documented the existence of HPV exposure years before the development of the disease, thus reproducing the conditions of a longitudinal study. With this approach, a relative risk estimate of 16.4 (95% CI 4.4–75.1) was observed for invasive cervical cancer in Sweden using DNA extracted from stored Pap smears (Wallin *et al.*, 1999) and of 32 (95% CI 6.8–153) in The Netherlands (Zielinski *et al.*, 2001a). In a study of similar design, an OR of 2.4 (95% CI 1.6–3.7) was obtained using serological markers of HPV exposure (Dillner *et al.*, 1997).

Strength of the association

Figure 19 shows the HPV DNA prevalence in cervical cancer cases and controls in eight countries, from the IARC multicentric case–control study (Bosch *et al.,* 2002). Table 10 shows the numbers of subjects in the study, the prevalence of HPV DNA in each relevant group, and the OR estimates. The first two studies, conducted in Spain and Colombia, used for HPV detection early versions of the MY09/11 PCR system that identified HPV DNA in some 75% of the cases. The rest of the studies were analysed using the GP5+/6+ PCR system and its modifications, which resulted in an almost 20% increase in the HPV DNA

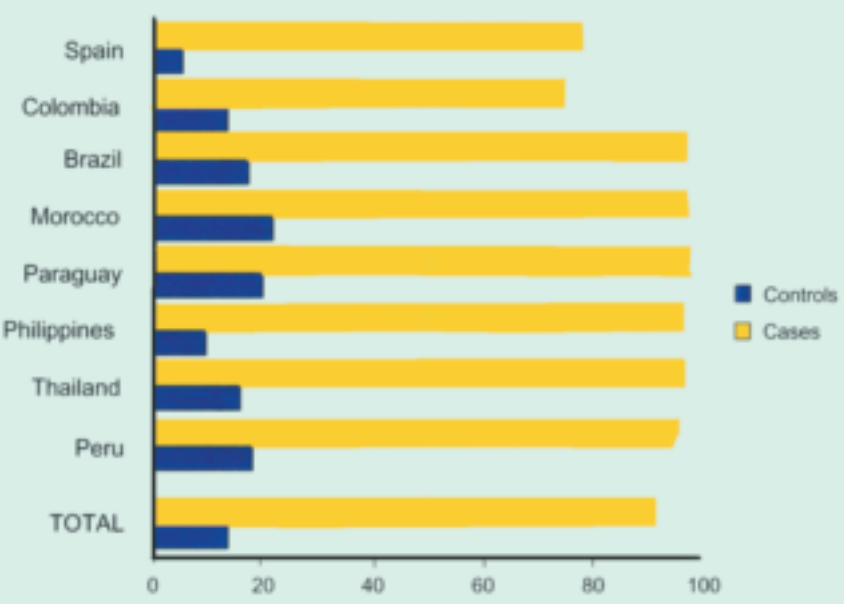

Figure 19 Prevalence of HPV DNA in cases and controls in the IARC multicentric case–control study

Source of data: Spain and Colombia: Muñoz *et al.* (1992), Brazil: Eluf-Neto *et al.* (1994), Morocco: Chaouki *et al.* (1998), Paraguay: Rolón *et al.* (2000), The Philippines: Ngelangel *et al.* (1998), Thailand: Chichareon et al. (1998), Peru: Santos *et al.* (2001).

From Bosch *et al.* (2002) (reproduced with permission from the BMJ Publishing Group)

detection rate (Muñoz *et al.*, 2003). The ORs for squamous-cell carcinomas were statistically significant and very high. Restricting the analyses to studies that used the GP5+/6+ HPV detection system, the adjusted OR for HPV DNA detection (the factor by which the reference risk of cervical cancer is multiplied if HPV DNA is detected) was 158.2 for any single type (95% CI 113.2–220.6). The risk of adeno- or adenosquamous-cell carcinoma in eight countries (Algeria, Brazil, India, Morocco, Paraguay, Peru, The Philippines, Thailand) was estimated to be 77.2 (95% CI 41.2–144.8) (F.X. Bosch, personal communication).

The pool of IARC studies was large enough to provide type-specific risk estimates for 18 HPV types. Type-specific risk estimates and confidence limits are displayed in Figure 20 (Muñoz *et al.*, 2003). These studies led to the conclusion that HPV types 16, 18, 31, 33, 35, 39, 45, rf51, 52, 56, 58, 59 and 68 should be considered high-risk carcinogenic types. Some evidence was also reported on a significant risk for HPV73 and 82. A second group of HPV types rarely found in cases was classified as low-risk, including HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108.

Figure 20 shows that the risk for any given high-risk type was not statistically different from the risk reported for HPV16. Likewise, the risk related to the presence of multiple HPV types in the specimen was no different from the risk linked to a single HPV type. The standard estimates of the attributable fraction (AF %; the proportion of disease that is related to HPV DNA) derived from these and most other studies range from 90 to 98%.

Under extremely rare circumstances, HPV of the low-risk group (HPV6 or 11) was found as the only type in specimens of invasive cervical cancer. Although statistically the increases in risk are largely non-significant, it should be considered that low-risk types also show carcinogenic capacity under special but as yet unidentified conditions, at a very low level. It is plausible that a minute fraction of the population harbours a special susceptibility to HPV and even the presence of a low-risk type is capable of initiating a neoplastic process.

The results of the multicentre study are consistent with findings on invasive cervical cancer and preinvasive disease in Costa Rica (Herrero *et al.*, 2000), Thailand (Thomas *et al.,* 2001b), Norway (Olsen *et al.*, 1995), Denmark (Kjaer *et al.*, 1996) and virtually all other countries in which such studies have been conducted (Figure 21).

The proportion of specimens containing multiple HPV types varies across studies and particularly in relation to the HPV detection method used. Table 11 (adapted from Bosch *et al.*, 2002) presents data on the proportion of specimens from cases and from the general population that showed multiple types. The table suggests that populations at high risk of cervical cancer and with high rates of HIV positivity tend to show higher proportions of multiple types as compared to populations not belonging to these groups. Longitudinal studies have suggested that the one-time, cross-sectional detection of type-specific HPV may underestimate the cumulative lifetime diversity of exposures to HPV (Woodman *et al.*, 2001). However, in all studies of invasive carcinoma, the risk linked to multiple HPV types does not vary significantly from the risk linked to single HPV types.

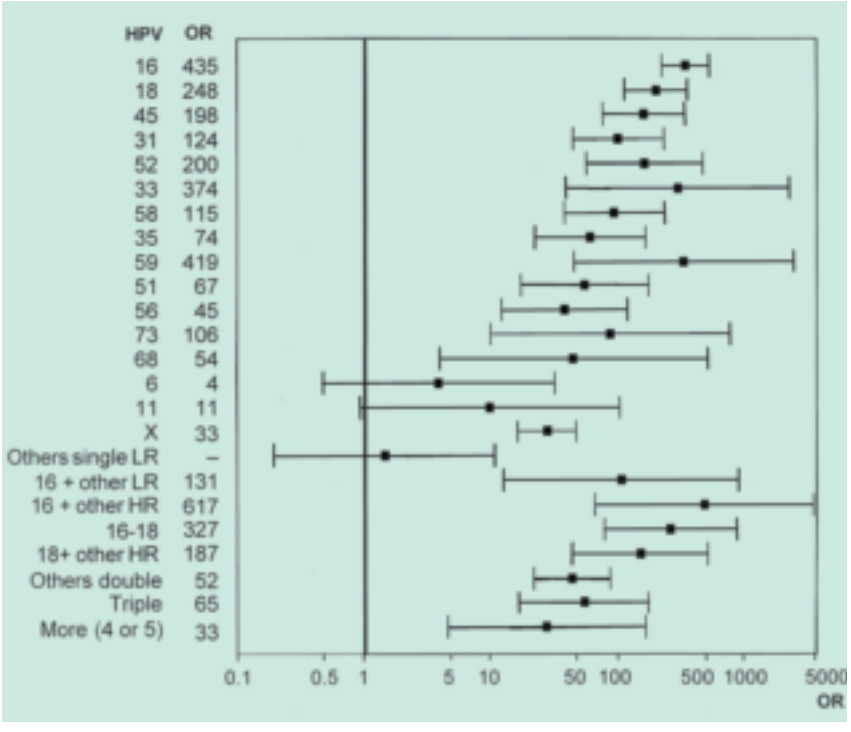

Figure 20 HPV type-specific odds ratios and 95% confidence intervals for cervical cancer.
Data from Muñoz *et al.* (2003)

Table 10. Risk of squamous-cell cervical cancer associated with HPV DNA

Country	Cases		Controls		OR[a]	95% CI
	No.	% HPV +	No.	% HPV +		
Brazil	169	97.0	196	17.3	177.0	65.5–478.3
Mali	65	96.9	12	33.3	109.2	10.6–1119.0
Morocco	175	97.1	176	21.6	113.7	42.3–305.3
Paraguay	106	98.1	91	19.8	208.1	46.4–932.8
Philippines	331	96.4	381	9.2	276.8	139.7–548.3
Thailand	339	96.5	261	15.7	163.5	82.0–325.9
Peru	171	95.3	175	17.7	115.9	48.6–276.4
Total invasive[b]	1356	96.6	1292	15.6	158.2	113.4–220.6
Spain	316	77.8	329	5.2	63.4	36.4–110.6
Invasive	159	82.4	136	5.9	75.7	32.9–174.2
Carcinoma *in situ*	157	73.2	193	4.7	58.9	27.4–126.7
Colombia	246	74.4	307	13.4	19.1	12.3–29.6
Invasive	111	78.4	126	17.5	17.7	9.1–34.3
Carcinoma *in situ*	135	71.1	181	10.5	21.1	11.5–38.8

[a] OR adjusted for age
[b] OR adjusted for age and centre
From Muñoz *et al.* (2003)

Table 11 Prevalence of multiple HPV types in cervical cancer cases and women without cervical cancer

Reference	Study	Cases	Non-cases	
		% of all specimens	% of HPV+	% of all
Muñoz *et al.* (2000)	IARC multicentric	4–20%	10%	1–3%
Herrero *et al.* (2000)	Rural Costa Rica	32%	38%	4%
Castellsagué *et al.* (2001)	Rural Mozambique		41%	15%
de Sanjosé *et al.* (2000)	Imprisoned women, Spain		71%	20%
Palefsky *et al.* (1999)	HIV+, USA HIV–, USA		42% 16%	– –

Table adapted from: Bosch *et al.* (2002)

Figure 22 shows the estimated percentages and numbers of cases of cervical cancer attributable to each HPV type, in all world regions combined. These were calculated by taking into account the estimated region-specific HPV genotype distribution and the number of incident cervical cancer cases (Munoz *et al.*, 2004). Most of the cancer cases (70.7%) are accounted for by the two HPV types 16 and 18; the percentage rises to 87.4% when five other types (45, 31, 33, 52, 58) are considered. The 13 HPV types currently used for screening purposes seem to be responsible for 91.6% of all cancer cases.

Exclusion of alternative explanations

In the majority of studies of HPV and cervical cancer, a small fraction of cases is labelled as HPV-negative and these have been examined to assess whether HPV-negative cervical cancer is a true biological entity (Riou *et al.*, 1990; Viladiu *et al.*, 1997). The proportion of such cases tends to be greater in studies of preinvasive neoplasia (Burger *et al.*, 1996; Tabrizi *et al.*, 1999). In the IARC studies, it was clear that in broad terms, 'HPV-negative' cases retained the same epidemiological risk factor profile as the rest of the cases (i.e., similar age, high number of sexual partners, young age at first sexual intercourse, long-term use of contraceptives, high parity and similar prevalence of HPV16 antibodies). These results strongly suggested that the apparently HPV-negative cases were also STD-related; however, none of the sexually transmitted agents that had occasionally been associated with cervical cancer satisfied the causality criteria outlined in Table 9. In the evaluation of the putative HPV-negative cases, it was demonstrated that lack of identification of HPV DNA could be attributed primarily to the poor quality of the specimen (tumour necrosis, lack of cancer cells in the specimen, poor preservation) and to the quality of the amplification system used. Walboomers *et al.* (1999) showed that using histological verification of the specimen and the GP5+/6+ testing system, HPV DNA could be

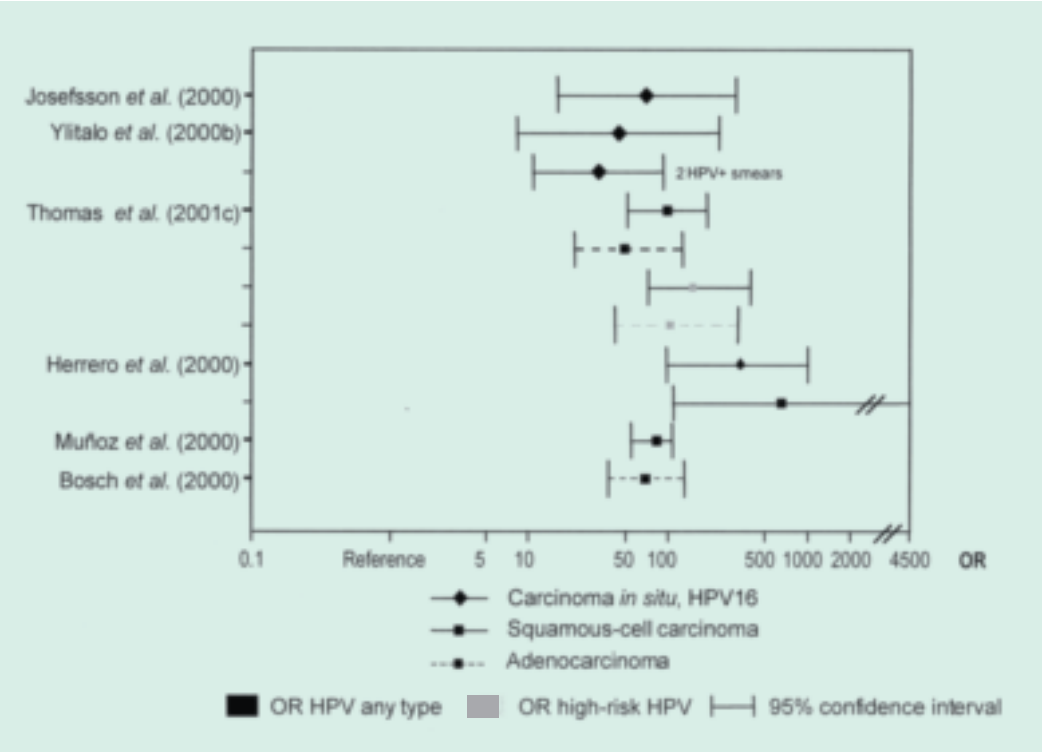

Figure 21 Odds ratios and 95% confidence intervals for associations found in case–control studies after 2000
Sources of data: Bosch *et al.* (2000), Herrero *et al.* (2000), Josefsson *et al.* (2000), Muñoz *et al.* (2000), Thomas *et al.* (2001c), Ylitalo *et al.* (2000a)
From Bosch *et al.* (2002) (reproduced with permission from the BMJ Publishing Group).

detected in 99.6% of cases of cervical cancer worldwide, supporting the concept that HPV is indeed a necessary cause of the disease.

In the last decade there has not been any hypothesis supported by sound epidemiological or biological data indicating an HPV-independent etiology of cervical cancer, but such a hypothesis should be retained as a scientific and research option, for four reasons. (1) Epithelial cells are capable of developing into cancer cells in all human tissues regardless of a known, viral or non-viral, cause, so cells in the human cervix might too. (2) Cellular genes involved in HPV-related carcinogenesis should be liable to spontaneous or induced mutations that could lead to cancer in the absence of HPV, though available evidence suggests that this event is rare within the life expectation of the human population. (3) It is likely that any non-HPV-related cancers occur very rarely and probably cluster in very old women, but relatively few cases of cervical cancer in very old women have been investigated. (4) Non-epithelial cancers do occur in the cervix at a low frequency.

HPV transmission

Several groups of studies have clearly shown that HPV is predominantly and largely transmitted through sexual intercourse. Other forms of transmission, especially from mother to child, are briefly outlined below, but their implications in cervical cancer are likely to be no more than marginal. The evidence for non-sexual transmission of HPVs (reviewed by Cason, 1996; Mant *et al.*, 2000), suggests that:

1. Genital HPV infections, including genital warts, may occur in populations without sexual experience, such as virgins, infants and children.
2. There is some evidence of non-genital transmission of low-risk HPVs.
3. Vertical and perinatal transmission of HPVs from mother to child does occur, although rates are generally very low.
4. High-risk genital HPVs have been detected in non-genital mucosa, such as that in the mouth, oropharynx and conjunctiva, and they have

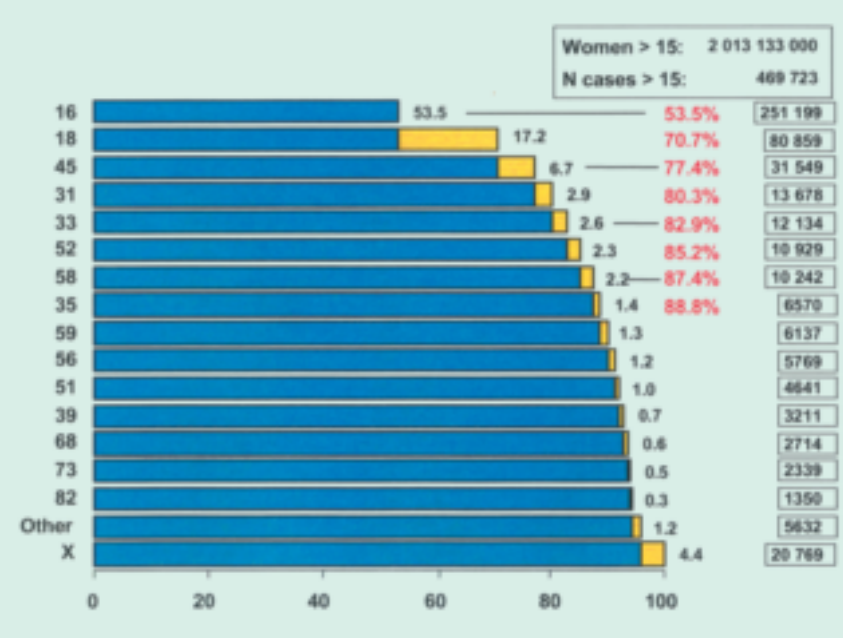

Figure 22 Distribution of HPV types in invasive cervical cancer: estimated number of cases attributable to each viral type
From Muñoz *et al.* (2004)
(reproduced with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

been associated with a fraction of cancers of the oral cavity and oropharynx and with conjunctival squamous-cell carcinoma.

5. There is low concordance of HPV types and HPV16 genomic variants between heterosexual partners.

HPV and sexual behaviour

Epidemiological studies of risk factors for HPV infection have clearly and consistently shown that the key determinants among both women and men are related to their sexual behaviour. The best studied risk factors are their lifetime number of sexual partners, the age at which sexual intercourse was initiated and the likelihood that at least one of the sexual partners was an HPV carrier as measured by his sexual behaviour traits (Bauer *et al.*, 1993; Hildesheim *et al.*, 1993; Wheeler *et al.*, 1993; Franco *et al.*, 1995; Muñoz *et al.*, 1996a; Kjaer *et al.*, 1997; Rousseau *et al.*, 2000; Silins *et al.*, 2000; Kjaer *et al.*, 2001). The role of males as possible vectors of HPV was measured in early epidemiological studies by questionnaires that addressed the sexual behaviour of the husbands or sexual partners of cervical cancer cases and controls. Later studies incorporated measurements of HPV DNA in exfoliated cells from the penile shaft, the coronal sulcus and the distal urethra (Barrasso *et al.,* 1987; Kjaer *et al.*, 1991; Bergman & Nalick, 1992; Bosch *et al.*, 1996; Castellsagué *et al.*, 1997). Figure 23 shows for both sexes the relationship between HPV DNA prevalence and either number of partners or age at first intercourse. In populations where female monogamy is dominant, the population of female sex workers plays an important role in the maintenance and transmission of HPV infections. Figure 24 shows the prevalence of HPV DNA in surveys of the general population in two European countries and in selected high-risk groups of sexual workers and imprisoned women from the same underlying populations. Figure 25 shows the correlation between the number of sexual partners and the prevalence of HPV DNA in the penis for husbands of monogamous and non-monogamous women.

The probability that a woman is an HPV carrier and her risk of developing cervical cancer have been consistently related to the presence of HPV DNA in the penis or the urethra of her husband or sexual partner (Kjaer *et al.*, 1991; de Sanjosé *et al.*, 1993; Bosch *et al.*, 1994; Juarez-Figueroa *et al.*, 2001; Thomas *et al.*, 2001a). These and many other observations have consistently confirmed in terms of HPV infections the century-old observations and the hypothesis formulated 30 years ago that male sexual behaviour is a central determinant of the incidence of cervical cancer (Rigoni-Stern, 1842; Beral, 1974; Skegg *et al.*, 1982).

Studies in couples have provided consistent evidence of the venereal nature of HPVs. Pridan and colleagues first showed an association between the number of sexual partners of the husband and the risk of cervical cancer among mostly monogamous Jewish women (Pridan & Lilienfeld, 1971). The male factor hypothesis was formulated soon after (Singer *et al.*, 1976; Skegg *et al.*, 1982). Geographical clustering of cervical and penile cancers (Smith *et al.*, 1980; Franco *et al.,* 1988; Bosch & Cardis, 1990) provides ecological support for the importance of men in the natural history of cervical cancer. Buckley *et al.* (1981) found that the risk of cervical cancer among monogamous women increased with the number of their husband's sexual partners, and with the husband's early age at first inter-

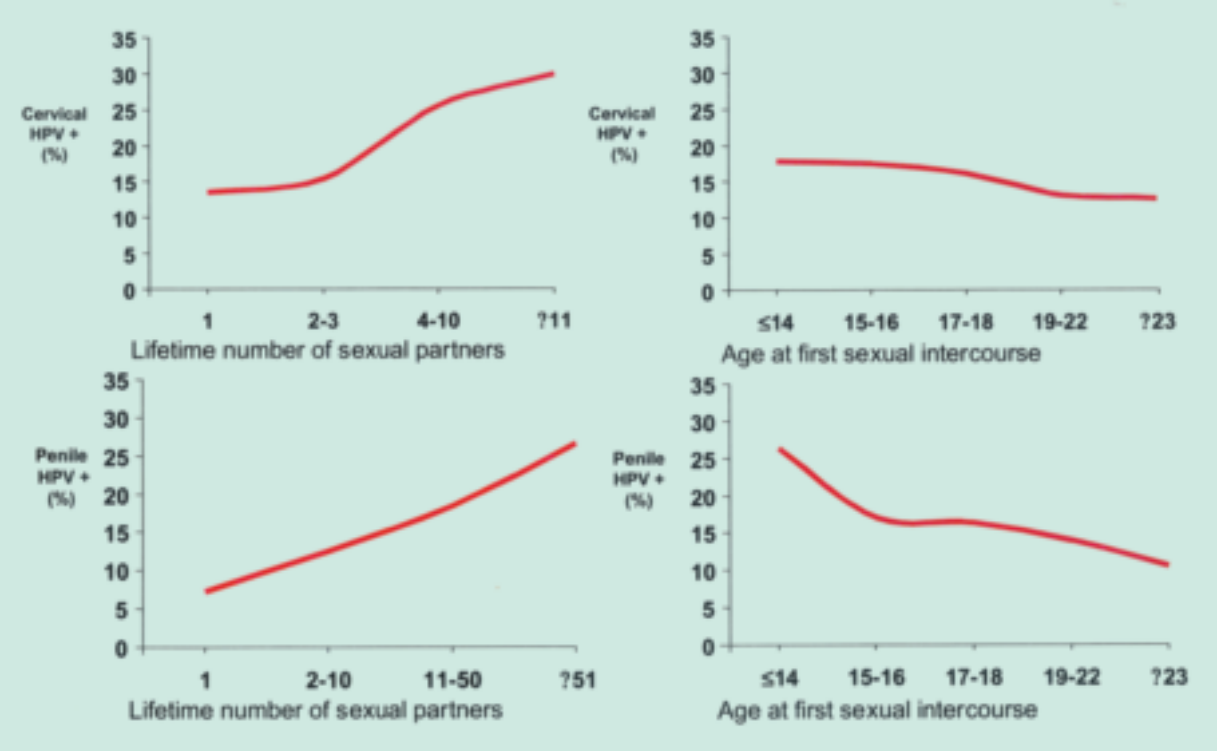

Figure 23 Prevalence of cervical and penile HPV DNA by lifetime number of sexual partners and age at first sexual intercourse in subjects without genital neoplasia (IARC studies)

Data from a series of 12 case–control studies of cervical cancer carried out by IARC in 10 countries. Data for females based on 2225 control women from studies in Algeria, Brazil, Colombia, India, Morocco, Paraguay, Peru, the Philippines, Spain and Thailand. Data for males based on 1140 men from studies in Brazil, Colombia, the Philippines, Spain and Thailand.

Adapted from Castellsagué & de Sanjosé (2003)

course, reporting of extramarital affairs and history of STDs.

The importance of the male role was also suggested by early studies of cancer clusters within couples. One study reported that subsequent wives of husbands whose previous wife developed cervical cancer had an increased risk of cervical neoplasia (Kessler, 1977), and other studies showed that wives of men with cancer of the penis had a high incidence and mortality due to cervical cancer (Martinez, 1969; Graham *et al*., 1979; Smith *et al*., 1980).

Data from the Swedish Family Cancer Database showed that husbands of women with *in situ* or invasive cervical cancer had an excess risk of anal cancer, a recognized HPV-related cancer (Hemminki & Dong, 2000). Anal cancer was also increased as a second primary cancer in women with cervical neoplasia (Hemminki *et al.,* 2000a). Of special interest is the excess risk of both tonsilar cancer and cancer of the tongue in husbands of cervical cancer patients, supporting the evidence that HPV may be etiologically involved in a fraction of these tumours (Hemminki *et al*., 2000b, Herrero *et al.,* 2003).

Quantitative evidence of the male role has been provided by formal case–control studies comparing either direct histories of sexual behaviour or clinical evidence of HPV-related lesions in male partners of women with and without cervical cancer (Zunzunegui *et al*., 1986; Brinton *et al.,* 1989b; Kjaer *et al*., 1991; Bosch *et al.,* 1996; Muñoz *et al.,* 1996b). Huynh *et al.* (2004) showed an increased risk among women in Viet Nam in relation to the presence of their husbands in the army stationed in the south.

There is some evidence that HPV types differ in sexual transmissibility, with oncogenic (high-risk) types and non-oncogenic (low-risk) types having somewhat different risk factor profiles

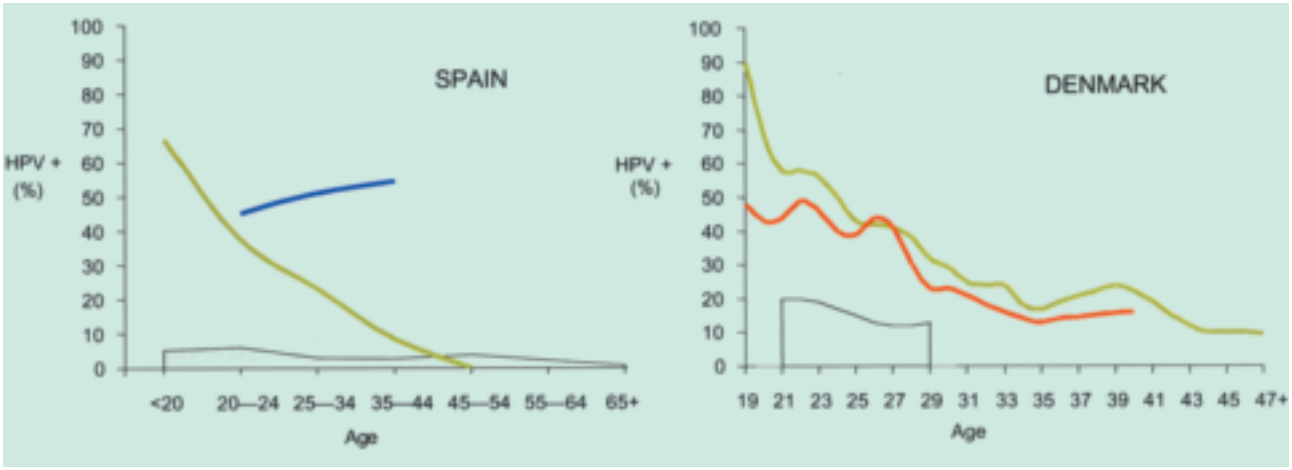

Figure 24 Prevalence of cervical HPV DNA in different risk groups in Denmark and Spain
Data for Denmark (adapted from Kjaer *et al.*, 2000) include 182 female sex workers (—), 187 female sexually transmitted disease clinic attendees (—) and 1000 women from the general population (▭) . Data for Spain (adapted from de Sanjosé *et al.*, 2000, 2003 and Touze *et al.,* 2001) include 187 female sex workers, 153 incarcerated women (—) and 1101 women from the general population (▭) (Adapted from Castellsagué & de Sanjosé (2003)

when considered as groups. The associations with number of sexual partners are stronger for the oncogenic types than for non-oncogenic types (Franco *et al.*, 1995; Kjaer *et al.*, 1997; Rousseau *et al.*, 2000).

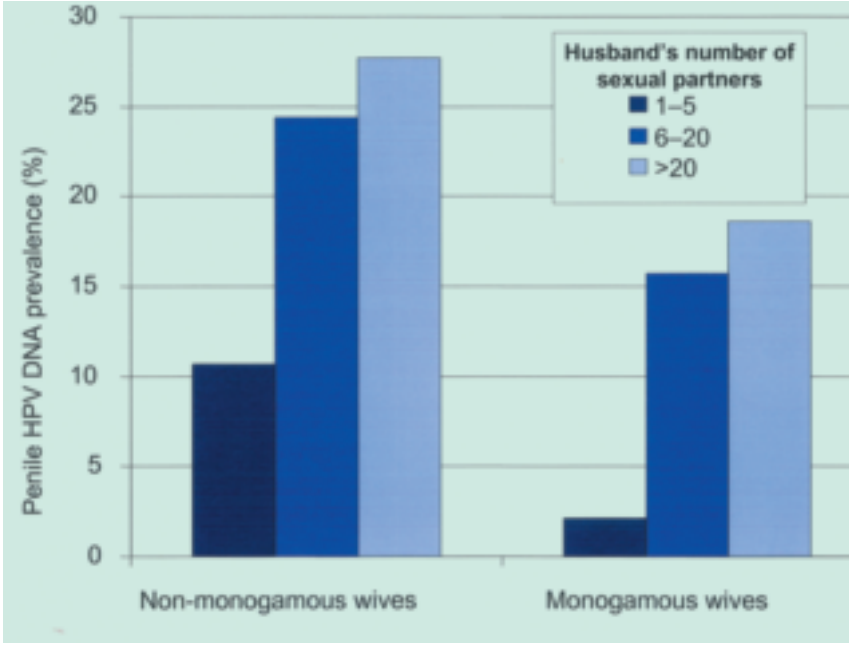

Figure 25 Penile HPV DNA prevalence by number of sexual partners of husbands of monogamous and non-monogamous women in Spain and Colombia.
Data (adapted from Castellsagué *et al.*, 1997) include 595 men who were husbands or stable coital partners of women with and without cervical cancer.

Most studies of concordance of genital HPVs in heterosexual couples, but not all (Baken *et al.,* 1995), have found a relatively poor correlation of HPV-positivity and HPV type in cervical and penile samples (Hippelainen *et al.,* 1994a; Kyo *et al.*, 1994; Strand *et al.,* 1995; Castellsagué *et al.*, 1997; Franceschi *et al.*, 2002). This is particularly important in case–control studies, in which a woman with cervical neoplasia is expected to be a long-term carrier of HPV DNA, whereas the husband is likely to have been a transient HPV DNA carrier (Hippelainen *et al.*, 1994b). Moreover, in some couples, the current partner may not be the relevant one in determining the woman's risk of HPV infection. Agreement in HPV findings, however, was also modest in couples where both the wife and husband reported only one lifetime sexual partner (Franceschi *et al.,* 2002). Among women with cervical neoplasia, the relevant infection may have occurred years earlier. The relatively low prevalence of penile

HPV infection in their husbands suggests that viral shedding of advanced cervical lesions is limited. In addition, cross-sectional detection of penile HPV DNA may measure relatively recent exposures to HPVs that may be unrelated to the initiation of cervical neoplasia in the wife. Finally, the low agreement may be partly due to technical reasons, since a smaller amount of penile exfoliated cells is usually obtained from men compared with the cellular yield obtained from the cervix.

Male circumcision, penile HPV and cervical cancer

The IARC multicentric study on cervical cancer compared penile HPV DNA prevalence in circumcised and uncircumcised men and estimated the woman's risk of cervical cancer according to the husband's circumcision status. Circumcised men were about three times less likely to harbour HPV DNA in their penis than uncircumcised men. Male circumcision also reduced the risk of both genital HPV infections and cervical cancer in the female partner, particularly and most strongly in women whose male consorts had a promiscuous sexual history (Castellsagué *et al*., 2002). Other studies have, however, failed to report a lower prevalence of HPV DNA in the penis of circumcised males (Weaver *et al*., 2004).

Mother-to-child and perinatal transmission of HPV

Non-sexual transmission of HPVs was first suggested in 1956, in a case report of a male child that developed symptoms of laryngeal papillomatosis and penile warts at three and six months after birth to a mother with condyloma (Hajek, 1956). Since then a large body of epidemiological data on perinatal transmission of HPVs has accumulated (reviewed in Cason, 1996; Mant *et al*., 2000). A carefully conducted large study concluded that the risk of perinatal transmission of HPVs, although present, is probably very low (<3%) (Watts *et al.,* 1998).

Perinatal HPV transmission has been unequivocally demonstrated for recurrent laryngeal papillomatosis, a rare, potentially life-threatening condition associated with HPV types 6 and 11, the types most commonly detected in genital warts.

Some evidence of intra-uterine infection with HPVs is available (Tseng *et al*., 1992; Armbruster-Moraes *et al*., 1994; Favre *et al*., 1998; Tseng *et al*., 1998).

Non-sexual transmission of HPV

Since Fleming *et al*. (1987) reported on a five-year-old boy with HPV2-positive warts on the anus and hand, a number of other case series have confirmed the possible non-sexual transmission of HPVs, particularly low-risk HPV types (reviewed in Lacey, 1996). These findings raise the possibility that patients with genital warts may transfer genital HPVs not only to their sexual partners by finger–genital contact but also horizontally to their children (Sonnex *et al*., 1999).

Finger–conjunctiva HPV transmission has been suggested by studies reporting the presence of HPV DNA, predominantly type 16, in human ocular surface squamous neoplasias, including conjunctival carcinomas (reviewed by Newton, 1996). A study in Uganda, a high-risk area for this tumour, found a statistically significant association between high titres of HPV16 antibodies and conjunctival squamous-cell carcinoma (Newton *et al*., 2002).

Transmission of HPV in blood, breast mllk and sperm

It is very unlikely that HPVs are transmitted via blood, as HPVs do not have a known viraemic phase, and no case of HPV detection in blood has been documented.

Transmission of HPV to infants via the process of breast-feeding has not been documented.

Evidence on the possible role of sperm as a vector for HPVs suggests that semen may be a transmitter of cell-associated HPV during the process of ejaculation (Ostrow *et al*., 1986; Nieminen *et al*., 1991; Chan *et al*., 1994; Kyo *et al*., 1994; Lai *et al.,* 1997; Olatunbosun *et al*., 2001).

Papillomavirus types

Early attempts to classify human papillomaviruses (HPV) were based on the rather strict tropism of certain HPV types for cornifying squamous epithelium (cutaneous types, e.g., HPV1, 4, 10) or mucosal epithelium (mucosal types, e.g. HPV6, 16, 31), with some types strongly linked to distinctive clinical presentations. However, this classification is over-simple and is incorrect in some cases, as demonstrated by the presence of the mucosal type HPV6 in cornifying genital warts. Another attempt to group papillomaviruses is the separation into skin types causing warts (e.g., HPV1) and genital types affecting primarily the anogenital area (e.g., HPV6, 16, 18). Again this classification is rather artificial, because HPV16 can also be found in nail-bed carcinomas on the hands. The modern classification into different HPV genotypes is based on DNA nucleotide sequence differences within the coding regions of the genes for the E6, E7 and L1 proteins, with different genotypes distinguished by having less than 90% sequence homology in these regions (de Villiers, 1994; Chan *et al*., 1995). By this definition, over 130 different HPV types have been described to date. Further subdivision into sub-types is based on sequence homology of 90–98% and variants with over 98% sequence homology.

HPV intratype variants are defined as having more than 98% nucleotide

sequence identity, determined over the E6, E7 and L1 open reading frames (ORFs), with the reference sequence (Van Ranst *et al.*, 1992; Myers *et al.,* 1996).

Because of the low prevalence of some genotypes, as detected by different genotyping methods, it has been difficult to categorize these according to risk, so that only 11 genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58) have been consistently classified as high risk (Lörincz *et al.,* 1992; Bosch *et al.*, 1995; Walboomers *et al.*, 1999). In a recent analysis using pooled data from 11 studies (Muñoz *et al.*, 2003), genotypes 59, 68, 73 and 82 were newly identified as high risk, while types 26, 53 and 66 were designated as probable high-risk types. Another possibility hindering precise risk classification is that the test sensitivities of genotyping methods may differ for distinct genotypes. For example, the most widely used genotyping systems based on polymerase chain reaction (PCR), such as the MY09/11 and GP5+/GP6+ systems (see Chapter 2), differ in their detection threshold levels for different HPV types. The sensitivity of the GP5+/6+ systems appears to be lower for HPV52, 53, 58 and 61 than that of the MY09/11 system, whereas GP5+/6+ has higher sensitivity for detection of HPV35 (Qu *et al.*, 1997).

Papillomavirus biology

Papillomaviruses are widespread among higher vertebrates, but have strict species-specificity; transmission from non-primates to humans has not been reported. In general they cause local epithelial infections, although with animal fibropapillomaviruses (e.g., bovine papillomavirus; BPV), infection can also be found in the dermis. Viral spread to distant body sites does not occur.

Papillomaviruses are small icosahedral particles with a diameter of 55 nm, belonging to the family of Papillomaviridae. They have no envelope and consist of a capsid composed of 72 capsomeres, which accommodates the viral genome. The capsomeres are composed of two structural proteins: the 57 kDa late protein L1, which accounts for 80% of the viral particle and is considered to be a group-specific antigen, and the 43–53 kDa minor capsid protein L2 (Pfister & Fuchs, 1994). Because of the absence of an envelope, papillomaviruses are relatively stable and resistant to desiccation, retaining viability extracellularly for at least one week (Roden *et al.,* 1997). They are also resistant to organic solvents and heat treatment to 56°C causes only a minor loss of infectivity.

Infections with papillomaviruses may cause local cell proliferation, which becomes apparent in the form of benign tumours such as common warts, condylomas and cervical intraepithelial neoplasia. The majority of benign tumours spontaneously regress in immunocompetent patients. Inherited or induced immune deficiencies lead to higher persistence of infections. In the case of the high-risk HPV types (see below), persistent infection confers a high risk for progression of the primary tumours into carcinomas (zur Hausen, 2000).

Structure of the HPV genome

The HPV genome consists of a double-stranded 8-kbp DNA molecule, which is associated with cell-derived histone proteins that produce a nucleosome-like superhelical twisted structure. The relative arrangement of the 9 to 10 ORFs within the genome is conserved within all papillomavirus types. One speciality of papillomaviruses is that the partly overlapping ORFs are arranged on only one DNA strand. To increase their coding capacities, HPVs make use of polycistronic transcripts and fusion proteins from different reading frames. The genome can be divided into three regions: the long control region (LCR), the region of early proteins (E1–E8) and the region of late proteins (L1 and L2). In accordance with this, two RNA poly-A addition sites, one for the early protein transcripts and one for the late protein transcripts, are always present (Pfister & Fuchs, 1994). A diagram of the genome organization of human papillomaviruses is given in Figure 26 and the functions of the different ORFs are summarized in Table 12.

The size of the long control region varies from 500 to 1000 bp in different HPVs. There are no ORFs in this area of the genome, but it does contain several control elements which regulate HPV DNA replication and gene expression (Iftner, 1990).

The proteins of papillomaviruses

Transcription of the genes E6 and E7 is a consistent feature in cervical carcinomas and was the first indication of an important role for these genes in HPV-associated tumorigenesis (Schwarz *et al.*, 1985; Androphy *et al.*, 1987; von Knebel Doeberitz *et al.*, 1988; Sedman *et al.*, 1991; Goodwin *et al.*, 2000; zur Hausen, 2002). The E6 and E7 genes of HPV16 and HPV18 have been confirmed as potent viral oncogenes; the transforming and immortalizing abilities of their products have been demonstrated in numerous experiments in tissue culture and animal models (Münger & Howley, 2002).

The E6 ORF encodes several small proteins of approximately 150 amino acids, with molecular weights of about 16–18 kDa. Because of the presence of a splice donor and two splice acceptor sites within the E6 ORF of high-risk anogenital HPV types, smaller E6 proteins (E6*I and E6*II) are produced, which may auto-regulate the E6 promoter itself (p97) that is responsible for their expression. No enzymatic

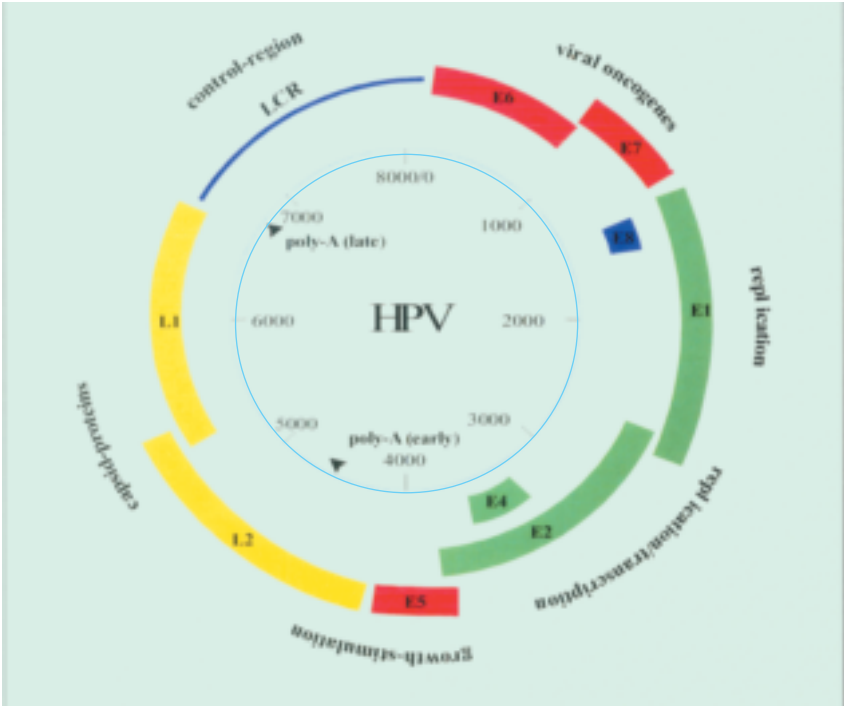

Figure 26 Genome organization of human papillomaviruses with the open reading frames (E1–E8, L1, L2) and the long control region (LCR)

function of E6 proteins has been demonstrated, but physical interactions with several cellular factors resulting in the deregulation of the cell cycle or interference with DNA repair have been described (Mantovani & Banks, 2001). The key activity of high-risk E6 proteins is their ability to inhibit the function of p53 (Scheffner *et al*., 1990; Werness *et al*., 1990). p53 is a sequence-specific transcriptional transactivator with a growth arrest function and is regarded as a tumour-suppressor protein, which is stabilized post-translationally (increase in protein half-life) in case of DNA damage (El Deiry *et al*., 1993). The binding of E6 to p53 proteins leads to enhanced ubiquitin-dependent degradation of p53. This results in a shortening of its half-life from 3 hours to 20 minutes, with a corresponding loss of its biological function (Scheffner *et al*., 1990; Mantovani & Banks, 2001). For the ubiquitination of p53, E6 needs a cellular protein called E6-associated protein (E6AP), which acts as an E3-ubiquitin-protein ligase and links ubiquitin to a lysine side-chain, forming a stable isopeptide bond (Huibregtse *et al.,* 1991). In non-infected eukaryotic cells, the ubiquitin-mediated proteolysis of p53 is triggered by the mdm-2 protein (Hengstermann *et al.,* 2001). However, in cells infected with high-risk HPV, the formation of the E6-p53-E6AP-complex replaces the normal regulation of p53 by mdm-2.

Independently of the E6AP-dependent degradation of p53, high-risk E6 proteins lead to a down-regulation of p53-dependent transcription, which can be explained by the targeting of CBP/p300, a p53 co-activator. Further, E6 appears to be able to activate the cellular enzyme telomerase in differentiated cells (Klingelhutz *et al*., 1996; Steenbergen *et al*., 1996; Mantovani & Banks, 2001). This enzyme counteracts the continuous shortening of the chromosome's telomeres which naturally occurs during replication of the cellular genome. Telomere shortening correlates with cell ageing and telomerase activity correlates with an increased life-span of the affected cell.

The E7 ORF encodes a small phosphoprotein of about 100 amino acids (10 kDa). E7 is a proliferation-inducing HPV oncogene and its activity is mediated through its ability to bind cellular proteins of the pRB family which, in concert with the E2F family of transcription factors, control the transition of the cell cycle from the G1- to the S-phase (Dyson *et al*., 1989; Münger *et al*., 2001). Binding of E7 to the hypophosphorylated, active form of pRB leads to the activation of E2F transcription factors, permitting progression of the cell into the S-phase of the cell cycle with subsequent cell replication (Chellappan *et al*., 1992; Boyer *et al*., 1996). Apart from this proliferative capacity mediated by specific sequences within the N-terminus of E7, the E7 proteins of 'low-risk' types possess a tenfold lower efficiency of binding to pRB than 'high-risk' E7 proteins and are very inefficient in cell transformation assays together with activated *ras* oncogene (Gage *et al.,* 1990; Münger *et al*., 2001). Chimeric substitution assays have attributed the difference in pRB-binding affinity and transforming capacity between low-risk and high-risk HPV types to the exchange of a single amino acid (Münger *et al*., 2001).

Forced entry into the S-phase is necessary for the virus to generate an environment that allows amplification of the viral DNA; this induces a number of cellular responses, like stabilization of the p53 protein which would lead to programmed cell death. To counteract

Table 12. Size and function of papillomavirus proteins

Viral protein/genomic element	Molecular weight/ size	Function
Non-coding elements		
Long control region (LCR)	500–1000 bp	Origin of replication and regulation of HPV gene expression
Early proteins		
E1	68–85 kDa	Helicase function; essential for viral replication and control of gene transcription
E2	48 kDa	Viral transcription factor; essential for viral replication and control of gene transcription; genome segregation and encapsidation
E3	Unknown	Function not known; present in only a few HPVs
E1–E4	10–44 kDa	Binding to cytoskeletal protein
E5	14 kDa	Interaction with EGF/PDGF receptors
E6	16–18 kDa	Interaction with several cellular proteins; high-risk HPV type E6 causes degradation of p53 and activate telomerase
E7	~ 10 kDa	Interaction with several cellular proteins, like with pRB and transactivation of E2F-dependent promoters
E8–E2C	20 kDa	Long-distance transcription and replication repressor protein
Late proteins		
L1	57 kDa	Major capsid protein
L2	43–53 kDa	Minor capsid protein

these cellular responses, high-risk papillomaviruses encode the E6 protein, which causes degradation of p53.

Replication cycle in the infected epithelium

The initial infection by HPV probably occurs in stem cells of the basal layer of stratified cervical epithelium or in associated hair follicles of the skin (Stanley, 1994; Schmitt *et al.,* 1996). HPV genomes are then established as extrachromosomal elements in the nucleus. Upon cell division, one of the daughter cells stays in the basal layer and provides a reservoir of viral DNA, while the other cell migrates away from the basal layer and initiates a programme of differentiation. This leads to amplification of the viral DNA, expression of capsid proteins and finally the production of progeny virus. Since HPVs rely on cellular enzymes to replicate their genome, one major consequence of an HPV infection is a blockage of cell cycle exit. HPV-infected suprabasal cells undergo an incomplete S-phase to replicate HPV genomes to high levels (Laimins, 1996). With the high-risk HPV types, the blockage of cell cycle exit and induction of S-phase in differentiated suprabasal cells is mediated by the E6 and E7 proteins (Halbert *et al.*, 1992; Cheng *et al.*, 1995; Ruesch & Laimins, 1998).

HPVs maintain their genome at 10 to 100 virus copies per infected cell over long periods of time *in vitro* and this is thought to reflect the replication of viral DNA in basal cells *in vivo* (Laimins, 1996). Disturbances in the control of replication of high-risk HPV may have implications for the progression of high-risk HPV-induced lesions *in vivo*, as the viral DNA is extrachromosomal in precursor lesions, but is frequently found integrated into the host chromosomes in invasive cancers (Cullen *et al.*, 1991). As no common integration site(s) have been identified, integration does not generally target proto-oncogenes or tumour-suppressor genes of the host cell. On the other hand, deletions and rearrangements of the integrated viral DNA occur. A model has been proposed which suggests that inactivation of the E2 gene releases E6/E7 oncogene expression from negative control (zur Hausen, 2002). However, no evidence has yet been presented that increased E6/E7 expression is indeed necessary for the progression of HPV-induced lesions. Viral DNA integration could simply be a consequence of an environment that does not support HPV

DNA replication. This is supported by fact that long-term extrachromosomal replication of high-risk HPV DNA has not been achieved in established HPV-positive or -negative tumour cell lines, but occurs almost exclusively in normal human keratinocytes (Frattini *et al.,* 1996; Del Vecchio *et al.*, 1992).

Immunity to HPV

Numerous studies have found a positive association between the detection of HPV antibodies and the risk of cervical neoplasia, in line with the notion that HPV antibody detection is a marker of cumulative exposure to HPV. Although these antibodies, particularly those directed against the virion capsid proteins L1 and L2, might be effective in preventing infection, it is commonly accepted that antibodies are not important effectors of the regression of established HPV infections and related cervical lesions. Neutralizing antibodies are generated by a type-specific conformational epitope in the viral particle. In contrast, disrupted or partially disrupted viruses expose epitopes that are broadly cross-reactive or even group-specific (Cowsert *et al.*, 1987; Christensen *et al.*, 1996). HPV types 6 and 11 are an exception, having been shown to contain shared epitopes and type-specific epitopes on intact capsids (Christensen *et al.*, 1994, 1996). Seroconversions against the HPV16 capsids are seen concomitantly with or within a few months after acquisition of HPV16 DNA (Andersson-Ellstrom *et al.*, 1994, 1996; Wikstrom *et al.*, 1995a, b; Carter *et al.*, 1996), but in a subset of patients can be delayed many months after the detection of viral DNA. In Sweden, the risk for seroconversion to the major oncogenic HPV type, HPV16, was found to increase linearly by about 4% for each life-time sexual partner up to a plateau of about 32% among women with an average of eight lifetime sexual partners (Dillner *et al.*, 1996). Low seroprevalence (2–7%) has often been found in monogamous women (Andersson-Ellstrom *et al.,* 1994, 1996; Carter *et al.*, 1996; Dillner *et al.*, 1996; Wideroff *et al.*, 1996; Viscidi *et al.*, 1997; Kjellberg *et al.*, 1999). HPV seropositivity in adult virginal women has not been reported, though the total number of virginal women tested is not large (Andersson-Ellstrom *et al.*, 1994, 1996). Large-scale surveys among children aged under 13 years found HPV seroprevalences of the order of 2% (Mund *et al.*, 1997; af Geijersstam *et al.,* 1999).

The major isotypes of serum antibodies against HPV capsids are IgG1 and IgA (Wang *et al.,* 2000). The serum IgA response is also HPV type-specific, as demonstrated by its correlation with the presence of type-specific HPV DNA (Wang *et al.,* 2000). Secretory IgA antibodies to HPV capsids are detectable in cervical mucus. In contrast to serum IgG, however, serum IgA correlates with the number of recent sexual partners and with the life-time number of partners, mostly among young women (Wang *et al.,* 2000), suggesting that the IgA response is less biologically stable over time than the IgG response. However, it is less clear whether antibodies against one HPV type protect against subsequent reinfection with the same or another closely related type and, if so, whether this protection is related to specific IgG and IgA subclasses. After the HPV infection has been cleared, serum antibody levels remain stable over time, even after 15 years of follow-up (Shah *et al.,* 1997).

The cellular immune response is an important effector mechanism for the clearance of established HPV infections. The first line of defence is the immune response with natural killer (NK) cells inducing apoptosis in virus-infected cells and in tumour cells. The specific activity of NK cells requires so-called killer immunoglobulin-like receptors (KIRs) which enable them to distinguish normal from virally infected or tumour cells. A direct antiviral cellular immune response is mediated by cytotoxic T-cells that recognize and kill infected cells presenting viral peptides, with the help of human lymphocyte antigen (HLA) class I molecules on their surface.

Viral and host risk factors

HLA haplotypes

The factors that determine whether an HPV infection is cleared or persists and that increase the risk for cervical cancer are not fully defined, but cellular immunity plays a major role. Altered HLA class I allele in cervical cancer has long been recognized and the presence of specific HLA class II alleles may be decisive for the risk for cervical cancer. In the case of HLA class I A2 (Montoya *et al.,* 1998), B44 (Bontkes *et al.,* 1998) and HLA-B7 (Duggan-Keen *et al.,* 1996), negative associations have been described. The most likely underlying mechanism is allele-specific down-regulation of these antigens during cervical carcinogenesis. In addition, the existence of HPV16 variants with E6 mutations affecting HLA-A2 and -B7 binding motifs suggests that lack of CD8-restricted epitopes may enable the virus to escape the immune response (Ellis *et al.,* 1995; Yamada *et al.,* 1995). Many studies in humans have focused on the association of HLA class II with SIL or cervical cancer and several HLA class II haplotypes were found to be associated with disease, such as DQw3 increasing (Wank & Thomssen, 1991) and DRB1*1301 decreasing (Hildesheim & Wang, 2002) the risk for cervical cancer in general. Some associations were found to be type-specific; thus, DR15 increases the risk for HPV16-carrying cancer and DR7 may be either protective or increase the risk (Konya & Dillner, 2001).

Cellular gene polymorphism

Another seemingly important marker of risk is a single nucleotide polymorphism in codon 72 of the p53 gene. There are two structurally different forms of wild-type p53, containing either a proline (Pro) or an arginine (Arg) at amino acid residue 72 (Matlashewski *et al.,* 1987). Storey *et al*. (1998) reported that the Arg p53 variant has higher susceptibility to HPV-E6-mediated degradation in tissue culture experiments, a discovery that could translate into a new cervical cancer risk factor. They also found that women with cervical cancer were more likely to be homozygous for Arg at position 72, rather than heterozygous or homozygous for Pro. It thus appeared that the Arg/Arg genotype at position 72 conferred susceptibility to HPV-induced cervical cancer and a few studies confirmed this association, but many more have failed to (reviewed by Koushik *et al.,* 2004). Inter-laboratory variability in p53 genotyping has been proposed as a possible explanation for the null results (Makni *et al.,* 2000). A recent meta-analysis using random-effects models that included 45 studies published since the original Storey *et al.* (1998) publication attempted to identify characteristics that significantly contributed to heterogeneity. For invasive cervical cancer with undefined histology, the Arg/Arg genotype was found not to affect risk (OR = 1.1; 95% CI 0.9–1.3). However, slightly increased risk was observed for squamous-cell carcinoma (OR = 1.5; 95% CI 1.2–1.9) and adenocarcinoma (OR = 1.7; 95% CI 1.0–2.7). Meta-regression analysis identified departures from Hardy–Weinberg equilibrium in the control group as the most important factor contributing to heterogeneity among results for invasive lesions. Summary ORs for studies in equilibrium were essentially null (Koushik *et al.,* 2004). This study suggests a possible role of p53 codon 72 polymorphism at a late carcinogenic stage in cervical cancer. However, further investigations are required with appropriate attention to design, sample size and methodological issues.

Loss of heterozygosity (LOH)

Many studies have considered chromosomal abnormalities in cervical cancer. Chromosomal alterations have been consistently identified, such as LOH at chromosome 3p, 6, 11, 13, 16, 17 and 19, and chromosomal amplifications at 3q (Southern & Herrington, 1998; Lazo, 1999; Kaufmann *et al.,* 2002); identification of the target genes (oncogenes or tumour-suppressor genes) affected in these areas is now required.

Viral variants

Although more than 100 HPV types have been identified, studies on variants in viral genes mainly relate to the E6 gene of HPV type 16 (Hecht *et al.,* 1995; Lizano *et al.,* 1997; Villa *et al.,* 2000; Chan *et al.,* 2002b). HPV16 variants with nucleotide alterations within the E6 gene, referred to as non-prototype-like variants, are reported to be more frequently associated with high-grade CIN and cervical cancer than wild-type genomes (Xi *et al.,* 1997; Zehbe *et al.,* 1998a), although this phenomenon could be population-dependent (Zehbe *et al.,* 1998b; Nindl *et al.,* 1999). On the basis of regional differences, HPV16 variants have been termed European (E), Asian-American (AA) , African (Af1 and Af2) and North-American (NA) (Ho *et al.,* 1991; Yamada *et al.,* 1997).

Interestingly, a significant over-representation of G/T variants at position 350 (350G/T) of the HPV16 E6 gene was detected in cervical cancers of women with a p53 Arg/Arg polymorphism; a possible differentially oncogenic effect of HPV16 350G/T variants which is influenced by the p53 genotype was therefore suggested (van Duin *et al.,* 2000). Another E6 variant (the 131G variant) was found to be present in 9.6% of cervical carcinoma patients (n = 94), of whom 78% had the HLA-B7 allele, already identified as a possible risk factor (Brady *et al.,* 1999). Most of the studies performed did not consider other variations that may occur in the E6/E7 region or in other regions of the HPV genome. Therefore the current risk observed, which is associated with viral variants in general, might be an underestimate. Furthermore, this risk might be influenced by other genomic alterations.

Viral load

A number of cross-sectional epidemiological studies using the semiquantitative Hybrid Capture 2 (HC2) technique (Iftner & Villa, 2003) have demonstrated an association between viral load of high-risk HPV types and cervical cancer risk. However, estimates of viral copy numbers depend directly on the total input of cells and adjustment for cellular load is an absolute requirement that is frequently not fulfilled, as in the case of HC2. Using type-specific real-time quantitative PCR, Swan *et al.* (1999) and Ylitalo *et al*. (2000b) found that high viral load of HPV16 was associated with risk for progression. The number of HPV16 copies per µg of cellular DNA in patients with a normal cytological result (approximately 2 x 10^7) increased with the severity of the lesions, reaching a 100-fold increase in CIN 2/3 patients (Swan *et al.,* 1999). Moreover, the risk of incident SIL increases with viral load and the progression or regression of a given cytological abnormality is correlated with higher or lower viral load, respectively (van Duin *et al.,* 2002; Schlecht *et al.,* 2003b); these associations have not been found for other HPV genotypes (Swan *et al.,* 1997; Abba *et al.,* 2003). Only few longitudinal data are available (Lörincz *et al.,* 2002; Ylitalo *et al.,*

2000b). While very low viral loads are associated with low subsequent risk, high viral loads are not necessarily associated with high risk of incident CIN 3 or cancer (Snijders *et al.,* 2003). Moreover, the main determinant of viral load measurements from women with small CIN 3 lesions is the extent of surrounding, virus-producing CIN 1 and CIN 2 (Sherman *et al.,* 2003c). Therefore, although HPV16 viral load appears to be correlated with cervical cancer, the utility of viral load for predicting progression from HPV infection to cancer remains unclear. In addition, little is known about the relationship of viral load of other types than HPV16 to cervical neoplasia.

Viral DNA integration

HPV DNA is maintained as an episome in benign infections, whereas integrated HPV genomes are frequently detected in CIN 3, cervical cancer and derived cell lines. It has been proposed that this integration event confers a certain growth advantage on the infected cells by activating the expression of the viral oncogenes (zur Hausen, 2000). The current model suggests that inactivation of the E2 gene as a consequence of integration releases E6/E7 oncogene expression from E2-mediated negative control. However, no evidence has yet been presented that increased E6/E7 expression is indeed necessary for the progression of HPV-induced lesions. In addition, a number of studies found exclusively episomal HPV16 DNA in 20–70% of cervical cancers (Cullen *et al.,* 1991; Fuchs *et al.,* 1989; Matsukura *et al.,* 1989; Pirami *et al.,* 1997) and in high percentages (75–97%) of CIN 3. Therefore it remains unclear whether HPV integration is simply a consequence of loss of normal epithelial cell differentiation capacity, without biologically conferring any further risk downstream, or whether the integration event does contribute to progression.

Epigenetic events

Epigenetic events are events that alter gene expression (e.g., phenotype) without a change in the DNA sequence; they include hypermethylation or hypomethylation of genes (e.g., the addition or the removal of a methyl group). The silencing of tumour-suppressor genes via promoter hypermethylation in HPV-infected host cells is a frequent human epigenetic event (Dong *et al.,* 2001; Virmani *et al.,* 2001). For example, silencing of the *TSLC1* (tumour suppressor in lung cancer) gene is frequently seen in the progression from high-grade lesions to invasive cervical cancer (Steenbergen *et al.,* 2004).

Oral contraceptives and parity

A recent meta-analysis showed a linear dose–response relationship of cervical cancer with hormonal contraceptives. The point estimate of the summary OR is between 2- and 3-fold; the duration of the effect after cessation of use of oral contraceptives remains to de determined (Smith *et al.,* 2003).

High parity has been found consistently in most case–control studies to be associated with both cervical cancer and carcinoma *in situ.* Most of the major studies restricting analysis to HPV-positive women also report an increased risk of HSIL or cancer with increasing number of pregnancies. In the IARC multicentric study, the OR for cervical cancer in women with seven or more full-term pregnancies was fourfold higher than in nulliparous women, and the risk increased linearly with increasing number of full-term pregnancies (Muñoz *et al.,* 2002). Risk of HSIL or cancer significantly increased with increasing number of live births in the Costa Rica study (Hildesheim *et al.,* 2001). A borderline association with CIN 3 was found in the Manchester study (Deacon *et al.,* 2000). The study in Denmark (Krüger-Kjaer *et al.,* 1998) and the US prospective study (Castle *et al.,* 2002a) did not find an association with the risk of HSIL and CIN 3 or cervical cancer, respectively. However, these results could be explained by the low parity of the study populations. In addition, in the US cohort, information on parity was obtained only at enrolment.

Smoking

The effects of smoking have been extensively studied in many case–control studies, which found moderate and statistically significant associations with cervical cancer, even after adjustment for the strong effects of HPV. These findings are strikingly consistent with those obtained in studies restricted to HPV-positive women. The ORs for ever smoking among HPV-positive women are in the range of 2 to 5. Furthermore, most studies reporting risk estimates according to intensity, duration or pack-years of smoking have shown an increased risk of cervical cancer with increasing exposure to tobacco smoking. A prospective study in the USA found a positive association with smoking status and smoking intensity (Castle *et al.,* 2002a).

Malignant transformation of HPV-16-immortalized human endocervical cells by cigarette smoke condensate has been proven (Yang *et al.,* 1996). The fact that nicotine and tobacco-specific carcinogens have been detected in the cervical mucus of smokers (Prokopczyk *et al.,* 1997) strengthens the hypothesis of a synergistic action between cigarette smoking and HPV for the development of HSIL and cervical cancer. In a prospective study, smokers were found to maintain cervical HPV infections significantly longer and to have a lower probability of clearing an oncogenic infection than women who never smoked (Giulian *et al.,* 2002). The significant association between the extent of smoking reduction and the reduction in lesion size found in an intervention study of

smoking cessation among women with minor-grade lesions further supports the role of tobacco smoking in HPV carcinogenesis (Szarewski *et al.,* 1996).

Herpes simplex virus type 2 and *C. trachomatis*

The IARC multicentric case–control study investigated the presence of antibodies against the common sexually transmitted agents to assess their effect on cervical cancer risk in the presence of HPV DNA. The results show that among HPV-positive cases and controls there is a residual 1.5- to-2-fold increased risk linked to herpes simplex virus type 2 (HSV2) and *C. trachomatis* exposure, suggesting an interaction with the oncogenic capacity of HPV.

The pooled analysis of seven case–control studies included 1262 cases of invasive cancer and 1117 matched controls. Western blot analyses were used to detect type-specific antibodies to HSV types 1 and 2. As expected, seroprevalence was higher in cases than controls and the risk of squamous-cell carcinoma was significantly higher in analyses restricted to HPV DNA-positive cases and controls and adjusted for other possible confounders (OR = 2.19; 95% CI 1.41–3.40). The association was consistent in adeno- and adenosquamous-cell carcinoma (Smith *et al.,* 2002b).

The prevalence of antibodies to *C. trachomatis* varies greatly by country and serum antibodies were associated with a 1.8-fold increased risk of squamous-cell invasive cervical cancer in all countries considered, except in Spain. The risk was higher in women with elevated *C. trachomatis* antibody titre and in women under 55 years of age. *C. trachomatis* and *C. pneumoniae* species-specific serum antibodies were differentiated using a microimmunofluorescence antibody assay. The increased risk of squamous-cell invasive cancer was found in women with *C. trachomatis* but not with *C. pneumoniae* antibodies. The study thus supports the possibility that *C. trachomatis* increases squamous-cell cervical cancer risk, after accounting for cervical HPV infection (Smith *et al.,* 2004).

Human immunodeficiency virus (HIV)

The evidence for an interaction between HPV and HIV in the origin of cervical cancer has led to the recognition of cervical cancer as one of the criteria of acquired immunodeficiency syndrome (AIDS) among HIV-positive women. Subsequent studies largely confirmed this evidence, although some major confounders of the epidemiological association tend to obscure the results. In brief, these refer to the powerful impact of screening in some populations, the medical surveillance of HIV carriers in developed countries and the short survival time of AIDS patients in many populations at high risk of cervical cancer compared with the time interval between HPV infection and cervical cancer (Bayo *et al.,* 2002; de Sanjosé & Palefsky, 2002; Gichangi *et al.,* 2002).

Massad *et al.,* (1999) reported on the baseline cervical cytology among 1713 HIV-positive and 482 HIV-negative women. Cervical cytology was abnormal in 38.3% of HIV-positive women versus 16.2% of the HIV-negative. High-grade lesions, low-grade lesions and ASCUS were all significantly more common among HIV-infected women. The risk factors for any abnormal cytological finding were a CD4+ count lower than 200 cells/μL (OR = 2.13; 95% CI 1.45–3.13), the presence of HPV DNA and a previous history of abnormal cytology.

Ahdieh *et al.,* (2000) identified a higher baseline prevalence of cervical abnormalities among HIV-infected women (13.4%) compared with HIV-negative women (2.4%). In this follow-up study, 11 women were identified with CIN in subsequent visits, all of whom were HIV-positive and who had a median CD4+ count of 253 cells/μL. The risk for CIN was related to HPV persistence in all cases.

Thomas *et al.* (2001a) studied a group of 251 sex workers in Thailand. The HPV DNA prevalence was similar in HIV-positive and HIV-negative women. However, the risk of high-grade lesions was two-fold higher in women infected with both HPV and HIV than in the HIV-negative, HPV-positive women and 20 times higher than in HIV-negative, HPV-negative women.

Mandelblatt *et al.,* (1999b) provided a pooled estimate of 15 studies on the association between HIV and CIN. HIV-infected women had an eight-fold increased risk of CIN (OR = 8.8; 95% CI 6.3–12.5). Sun *et al.,* (1997) reported that, compared with HIV-negative, HPV-positive women, women co-infected with HPV and HIV had a lower regression rate of low-grade lesions and higher rates of progression from infection to CIN.

Ellerbrock *et al.,* (2000) reported that HIV-positive women were 4.5-fold more likely than HIV-negative women to have or develop CIN within a 54-month follow-up interval. Among HIV carriers, transient HPV infections (RR = 7.4; 95% CI 1.0–57.4), persistent infections with HPV types other than 16 or 18 (RR = 8.9; 95% CI 1.2–66.2) and persistent infections with HPV type 16 or 18 (RR =11.0; 95% CI 1.4–88.7) were all significantly associated with CIN.

The International Collaboration on HIV and Cancer (2000) published cancer data from 23 prospective studies that included 47 936 HIV-infected subjects from North America, Europe and Australia for the period 1992–99. It was concluded that there had not been a significant change in the incidence of invasive cancer (rate ratio = 1.87; 99% CI 0.77–4.56) during this period.

An overview of early studies in Rwanda, South Africa and Uganda concluded that invasive cancer was not

related to exposure to HIV, with a summary OR of 0.8 (95% CI 0.5–1.4) (Newton *et al.,* 1999). However, more recent data from a hospital-based case–control study in South Africa show increased risk for cervical cancer (OR = 1.6; 95% CI 1.1–2.3) and for vulvar cancer (OR = 4.8; 95% CI 1.9–12.2) among HIV-infected patients (Sitas *et al.,* 2000).

Reports from the USA and Europe are generally consistent in detecting an increased risk for cervical cancer among HIV-infected women. Selik and Rabkin (1998) found a relative risk for cervical cancer of 5.5 among HIV-positive women in the USA. Frisch *et al.* (2000), using data from the US Cancer Match Registry for the period 1978–96, found an RR of 5.4 for invasive cervical cancer among HIV-positive women compared with the general US population. Similar increases in risk were observed for *in situ* cervical cancer (OR = 4.6), cancer of the vagina and vulva (OR = 5.8) and anal cancer (OR = 6.8). The risk showed no major change between the time before AIDS diagnosis and up to 60 months after diagnosis. Similar results are available for the population of New York (Gallagher *et al.,* 2001).

In southern Europe, a strong association between invasive cervical cancer and AIDS has consistently been found. In Italy, the linkage of the National AIDS Registry and the population cancer registries showed a 15-fold increased cervical cancer risk in women with AIDS (Franceschi *et al.,* 1998). The joint Italian–French follow-up study of HIV-positive women also showed a 13-fold increased rate of cervical cancer in HIV-positive women (Serraino *et al.,* 1999). In Spain, the Catalonian AIDS surveillance system detected 58 cases of invasive cervical cancer among 823 HIV-positive women, an 18-fold increased risk compared with the general population (Mayans *et al.,* 1999).

In summary, HPV and HIV share some behavioural traits that define a particularly vulnerable high-risk group. Progression of HPV infections to CIN lesions and cervical cancer in the context of limited or absent screening seems to be increased among HIV carriers and AIDS patients. Further-more, increasing evidence suggests that progression is related to the severity of immunosuppression, as indicated by CD4+ counts.

Dietary factors

Recent epidemiological evidence on the role of diet and nutrition on the risk of HPV persistence, SIL and invasive cervical squamous-cell carcinoma, taking into account HPV, has been systematically reviewed (Giuliano & Gapstur, 1998; Castle & Giuliano, 2003). Although there is some epidemiological support for a role of diet in cervical carcinogenesis, the evidence remains inconclusive. One of the most relevant new findings is a possible protective effect of fruit and vegetables on HPV persistence. In relation to nutrients, a protective effect against cervical neoplasia is considered probable for folate, retinol and vitamin E and possible for vitamins B_{12} and C and carotenoids. Conclusions for nutrients from studies taking HPV infection into account do not differ substantially from those that did not control for it. Overall, no clearly different patterns were observed for nutritional co-factors between low- and high-grade cervical lesions or between retrospective and prospective study designs.

No cohort studies on SIL and only few on HPV persistence have been reported which comprehensively assess suspected nutritional or dietary factors with control for HPV status.

Principles of screening

Screening was defined by the United States Commission on Chronic Illness (1957) as "the presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures that can be applied rapidly". Thus screening is the use of methods to detect unrecognized health risks or diseases in order to permit timely intervention. WHO pioneered the development of criteria for screening (Wilson & Junger, 1968) that have been the mainstay of research into and the application of screening ever since.

Screening tests are usually applied on a large scale. They are used to distinguish apparently unaffected people from those who may have a disease, or may develop it. A screening test is not intended to be diagnostic. Screening procedures are generally easier to perform and cheaper than diagnostic procedures. Their results require confirmation through definitive diagnostic tests; sometimes direct treatment is offered on the basis of a positive test. Even if the screening test is harmless, it can cause anxiety and the subsequent investigations and treatment can be hazardous. Ensuring the safety of screening is of importance because large numbers of individuals will be screened, creating a potential for many to be harmed by the process of screening.

Screening is based on three key principles:

1. It is a process of selection with the purpose of identifying individuals who are at a sufficiently high risk of a specific disorder to warrant further investigation or sometimes direct action. It is usually a preliminary process to offering a diagnostic test and if required, treatment;
2. It is systematically offered to a population of people who have not sought medical attention on account of symptoms of the disease for which the screening is being conducted. It is normally initiated by medical authorities and not following a patient's request for help on account of a specific complaint.

3. Its purpose is to benefit the individuals being screened.

These principles bring with them implications for an ethical approach to those participating in the screening process. In medical practice, the special nature of the relationship between a patient and her physician has resulted in the need to build up a core of ethical principles which govern this relationship. An important distinction between screening and normal medical diagnosis and care is that the encounter is not originated by the individual who is the subject of screening; rather the provider of screening initiates the process. This is true whether screening is initiated by governments or public health units, or whether screening is carried out by the physician in his or her office. When a patient goes to see a physician for diagnosis of and hopefully relief from a symptom, or for treatment of an established condition, the physician is required to exercise his or her skills only to the extent that knowledge is currently available. In screening, however, those who are approached to participate are not patients, and most of them do not become patients. The screener believes that as a result of screening, the health of the community will be better. This does not necessarily imply that the condition of every individual screened will be better, but in general this should be so. There is an ethical responsibility on those planning to introduce screening to be in a position to expect an overall benefit in the community. This has to be coupled with the responsibility to minimize by all possible means the harm and anxiety that will affect certain individuals.

These responsibilities imply that if valid evidence is not available from properly conducted research studies on the effectiveness of screening, screening programmes should not be offered other than in the context of a properly designed experiment with validly constituted informed consent.

Those responsible for screening programmes also have an ethical responsibility to ensure that quality control of the screening tests is maintained and that the effectiveness of proven, beneficial programmes is continually monitored (Hakama *et al.,* 1985).

Some other ethical issues are also important. The first is to reduce unnecessary anxiety to a minimum. This requires selecting screening methods that will provide the most attractive combination of negative predictive value (e.g., reassurance) and false positives that is attainable in a given setting. The second is to ensure that a useful remedy is available for all individuals identified as being true positive. There should be no one for whom either a definitive diagnostic test is not available or direct action cannot be offered. If this is not the case, screening will merely generate groups of anxious individuals for whom there is no benefit.

The process of screening should identify a test-negative group for whom no further action is warranted. For the test-positives, there should be a protocol which defines the diagnostic tests available and the treatment available for those with a true-positive test result.

For cervical cancer, the protocol should define those referred immediately for treatment, and those for whom surveillance and repeat testing is recommended.

To attain these objectives, there is a need to ensure that those who are test-positive return for diagnosis and for treatment if found to be true-positives. A screening programme must ensure that there is sufficient contact with the individuals being screened to make them aware of the implications of a positive test result. Some provision should be made to ensure that they have somewhere to return to for further medical advice and if necessary, counselling. A screening programme that fails to take these considerations into account is failing in its duty of care.

Equity of access to screening services is another important consideration. All those who stand to gain from screening should have access to the procedure. A screening service should not be a service that relies on individuals seeking out particular tests or procedures that they have heard may be of value. Instead, those who organize the service have an obligation to ensure that those who have not heard of the test or procedure but who stand to benefit from it are adequately informed and located to enable them to be screened.

A final ethical issue concerns the extent to which the offer of screening in a community could divert resources from other, more important, healthcare programmes. This is a particular problem for low-resource settings. There is an ethical responsibility to distribute limited resources equitably across the total community in order to obtain maximal health benefit. Under certain circumstances, the offer of screening could diminish the overall level of health in a community, if it resulted in fewer resources being available for other diseases. However, a well organized programme should promote equity through better use of resources.

Natural history of cervical cancer

For effective and efficient application of screening, "the natural history of the disease should be known" (Wilson & Junger, 1968). This is because screening is based on the expectation that the early detection of cancer, in the detectable preclinical phase (DPCP) (Cole & Morrison, 1980), will result in a reduction in mortality from the disease. If effective screening is directed primarily to the detection of precursors, the development of invasive cancer will be prevented.

Knowledge on the natural history of the disease will facilitate decisions on the appropriate ages to initiate and cease screening and on the optimal frequency of re-screening in those who test negative.

The concept that invasive squamous-cell carcinoma of the cervix arises from intraepithelial precursor lesions was first put forward over a century ago, on the basis of the histopathological identification of intraepithelial lesions immediately adjacent to frankly invasive cervical cancers that had morphological similarities to the invasive cancers. The recognition that there was a spatial relationship between certain intraepithelial squamous lesions and invasive cancers led to additional studies to define the temporal relationship between the intraepithelial and invasive lesions. A temporal relationship between carcinoma *in situ* and invasive cervical cancer was suggested by case reports of carcinoma *in situ* lesions of the cervix occurring several months to years before the subsequent development of invasive cervical cancer.

Putative precursor lesions can be identified through direct observation of the cervix with a colposcope after application of a solution of 3–5% acetic acid (see Chapter 2). This accessibility to direct observation using colposcopy and cytological/histological sampling has allowed the pathogenesis of cervical neoplasia to be intensively studied for the last 50 years. These investigations led to the identification of additional types of intraepithelial squamous lesions that are histologically less severe than carcinoma *in situ*. With our in-depth understanding of the central role of HPV in the pathogenesis of cervical cancer and the natural history of HPV infections (see above), we now have a coherent model for the natural history of cervical disease.

When cervical cancer screening programmes based on cervical cytology were being introduced, it was appreciated that the numbers of cases of presumed precursors were much larger than the numbers of invasive cancers occurring in the same population (Burns *et al.,* 1968; Fidler *et al.,* 1968; Coppleson & Brown, 1975). This led to a series of studies designed to clarify the natural history of the disease as identified by the abnormalities detected by cytology. The majority of these studies used post-biopsy histological diagnoses as their end-point. However, a few used cytological diagnoses, and these are specifically identified in the sections which follow. In interpreting the findings from these studies, it must be borne in mind that cytologically and histologically derived diagnoses are not necessarily identical, even if the same terminology is used (see earlier in this chapter). Because of the impossibility of directly observing the outcome of lesions treated surgically, their natural history has to be inferred by statistical techniques, usually by applying models of the presumed natural history, that are valid only to the extent that the assumptions that led to the model are valid.

In the 1960s, a variety of studies provided evidence interpreted as providing support for the existence of a spectrum of cytologically or histologically defined cervical cancer precursors. Studies using electron microscopy, time-lapse cinematography, ploidy analysis and DNA content led to a hypothesis that these intraepithelial lesions form a biological continuum from very early precursor lesions referred to as CIN 1 to more advanced lesions referred to as CIN 3. Although this morphology-based model of a continuum has now been supplanted by a more discrete theory of multistage carcinogenesis as described below, the CIN scale still merits consideration as the current basis of clinical management.

Findings in the 1970s that many CIN lesions presumed to be cervical cancer precursors had histological and cytological similarities to HPV-induced genital warts produced a profound change in the understanding of the pathogenesis of cervical neoplasia (Meisels & Fortin, 1976; Meisels *et al.,* 1977; Purola & Savia, 1977). Subsequent studies have clearly shown that infection of the cervical epithelium with specific high-risk types of HPV plays a basic role in the pathogenesis of cervical cancer and its precursor lesions (see above).

Morphological appearances alone frequently do not permit the distinction of intraepithelial lesions that are associated with persistent high-risk HPV infections and have a substantial capacity to progress to invasive cervical cancer from those lesions that do not. Although the majority of histologically defined CIN 1 lesions demonstrating marked cytopathic effects represent transient lesions, it is important to recognize that some are associated with high-risk types of HPV and have biological features indicating that they may be true cancer precursors (Lungu *et al.,* 1992). These features include monoclonality, aneuploidy and loss of heterozygosity (LOH) (Fu *et al.,* 1988; Park *et al.,* 1996; Chung *et al.,* 2000). Similarly, it should be recognized that lesions that have the histological features of CIN 2, and a smaller subset of lesions with the histological features of CIN 3, are not aneuploid and do not show specific LOH. Therefore it is not possible to predict biological outcome solely on the basis of histopathological appearance.

Low-grade lesions

HPV infection of young women is frequent, and in the large majority of women transient. Repeated sampling of women being followed for viral persistence and cervical abnormalities has shown that the median duration of

a prevalently detected HPV infection is typically about eight months for high-risk types of HPV and 4.8 months for the low-risk types (see earlier in this chapter). If detected during follow-up following a negative cytological test, the median durations are doubled (Richardson *et al.,* 2003).

Woodman *et al.* (2001) studied the natural history of HPV infections in a cohort of 1075 young women (15–29 years) in the United Kingdom, who agreed to undergo intensive cytology and HPV screening, and who on average had repeat tests every six months. This allowed accurate determination of the timing of HPV infections and the development of CIN that followed some of these infections. Somewhat surprisingly, of the 240 women who developed abnormal cytology and whose HPV status was known, 41% tested negative for HPV and another 34% only tested HPV-positive at the same visit as that in which the abnormal cytology was detected. Thus, for only 25% was a positive HPV test predictive of abnormal cytology, although this translated into a cumulative risk of 33% at three years.

Molano *et al.* (2003) showed in a cohort of women aged 13–85 years, based on 316 type-specific HPV infections, that HPV16 had a significantly lower clearance rate than low-risk types (RR = 0.47; 95% CI 0.32–0.72). HPV types related to HPV16 (types 31, 33, 35, 52 and 58) had intermediate clearance rates (RR = 0.62; 95% CI 0.47–0.94), while other high-risk types did not show evidence of slower clearance than low-risk types. Similar clearance rates were seen for infections with single and multiple HPV types and for women < 35 and ≥ 35 years of age.

Many women with transient HPV infections develop cytological abnormalities while they are actively shedding HPV particles (Figure 27). These occur because the viral life cycle is closely linked to the state of epithelial cell differentiation. When HPV is actively replicating in cells, it can produce characteristic cytopathic effects. These are nuclear enlargement, multinucleation, hyperchromasia and perinuclear cytoplasmic clearing or halos (e.g., "koilocytosis") (see earlier in this chapter). When observed in cervical cytology specimens, these cellular changes are interpreted as either low-grade squamous intraepithelial lesion (LSIL) or atypical squamous cells (ASC), depending on the number of cells showing such changes and the severity of the changes.

The predominant age-curve of HPV prevalence shows a peak at young age and a steady decline in subsequent age groups (Jacobs *et al.,* 2000; de Sanjose *et al.,* 2003). Some population-based surveys of HPV prevalence (Herrero *et al.,* 2000) and particularly the 12 areas included in the IARC Multi-Centre HPV Prevalence Survey have revealed, however, some variation in the age-pattern of HPV prevalence worldwide (Figure 28). Figure 28 is based on population-based surveys of several hundreds of cytologically normal women aged 15–74 years in areas where no organized screening programme and little or no opportunistic screening have existed. Furthermore, prevalence is reported only for high-risk HPV types.

In a few areas in Asia (Sukvirach *et al.,* 2003; Shin *et al.,* 2003) and Latin America (e.g., Concordia, Argentina, Matos *et al.,* 2003; Bogotá, Colombia, Molano *et al.,* 2003), the typical pattern again has a peak in women younger than 25 years of age, but a few additional types of age-curve are seen. In countries with very low HPV prevalence (e.g., Hanoi, Viet Nam, Anh *et al.,* 2003), no peak in HPV prevalence is seen in young women. In the only sub-Saharan area included in the IARC survey (Ibadan, Nigeria, Thomas *et al.,* 2004), there was very little decline in HPV prevalence across age decades between < 25 and > 64 years.

The interpretation of these different age-patterns is not entirely clear. The rapid rise of HPV prevalence in young women after start of sexual intercourse can be explained by the high prevalence of HPV in many populations and the high infectiousness of HPV.

The persistently high levels of HPV prevalence seen, for instance, in Nigeria point to the possibility that in countries where cervical cancer incidence is very high, HPV infection tends to persist and/or re-infection is substantial in every age-group.

In early studies, the end-point used was dysplasia and often the different stages of dysplasia were not distinguished. As these studies are uninterpretable with regard to the natural history of low-grade lesions, they are not summarized in this section. The authors' terminology is retained in the summaries that follow.

Hakama & Rasanen-Virtanen (1976) used data emanating from the Finnish Cancer Registry and mass screening registries to estimate the probability of a woman aged 30–59 years being diagnosed with a cervical lesion after the first [negative] test in the national programme of five-yearly cytological screening. The probability of being diagnosed with dysplasia of low degree after a [negative] first result was estimated to be 0.006.

Campion *et al.* (1986) followed for 19–30 months 100 women under the age of 30 years who had cytological and colposcopic evidence of mild cervical atypia consistent with CIN 1 on three consecutive tests over a 16-week interval. They were reviewed every four months by cytology and colposcopy. If cytological evidence of severe dyskaryosis and colposcopic evidence of advanced CIN consistent with CIN 3 was obtained, a biopsy was performed and treatment given. Of the 100 women, 67 showed persistent mild

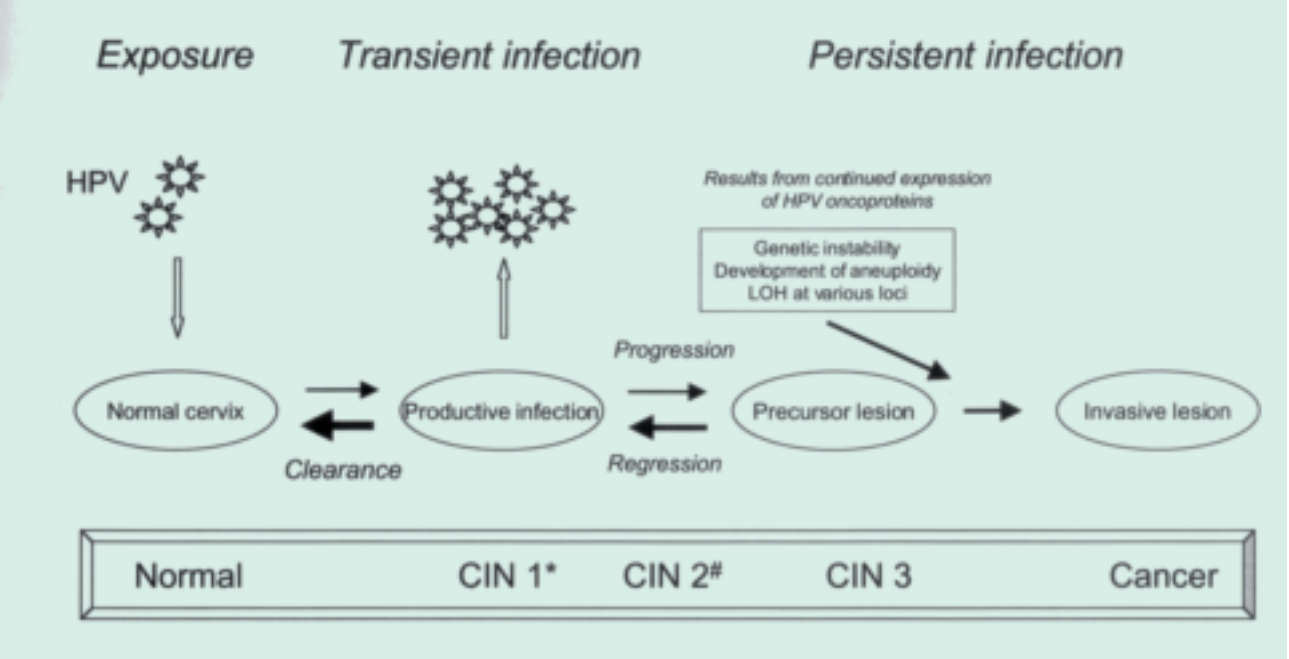

Figure 27 Natural history of preclinical abnormalities of the cervix
* Classical histological features of CIN 1 are uncommon among women who have transient infections
# This entity is not as well defined as CIN 3

disease (of which three regressed to normal but recurred during the study period), 26 progressed to CIN 3 (one regressed to normal but disease recurred and progressed to CIN 3 during the study period) and seven regressed to normal, and did not recur.

Luthra *et al.* (1987) followed 428 women with cytologically diagnosed dysplasia for up to 84 months, the majority for less than 30 months. Those who progressed within six months were excluded. Of 268 women with mild dysplasia, two developed carcinoma *in situ* or worse (computed actuarial rate 28.9%). [The Working Group noted the substantial loss to follow-up in this study.]

Syrjänen *et al.* (1992) followed 528 women with HPV-associated genital lesions for up to 10 years. Of 270 with an HPV-associated lesion without CIN, 67% showed regression as defined by a negative cytological test, colposcopy and punch biopsy, 27% persistence and 6% progression defined by punch biopsy. Of 106 women with CIN 1, 56% regressed over a period of six years and 14% progressed, while 3% showed recurrence after treatment. When the lesions were reclassified using the Bethesda terminology, 376 were LSIL, of which 64% showed regression and 8% progression. [The Working Group noted that the authors studied lesions associated with both low- and high-risk HPV types.]

Ostör (1993) reviewed reported studies that had considered the natural history of CIN 1. Only studies that employed cytology and/or cervical biopsy alone were reviewed (post-conization reports, and those where patients were treated by local destructive therapy, were excluded). Many were small studies (less than 60 subjects); 34 studies were included in the formal (summary) analysis. Only two of the studies of CIN 1 had over 1000 subjects. The total number of subjects with CIN 1 was 4504. After summarizing the data, Ostör concluded that 57% of CIN 1 regressed and 1% progressed to invasion. He suggested that, because of the small amount of cervical epithelium biopsied, it was probable that in many instances, more severe disease was not diagnosed. Therefore the proportion of cases of the various types that were estimated to have progressed was artifactually increased. [The Working Group noted that the author did not conduct a formal meta-analysis, but summarized the reported percentages without taking note of the person-years of observation in the various studies. It is also likely that there was an effect of cervical biopsy in influencing the subsequent risk of progression. This "intervention effect" must have

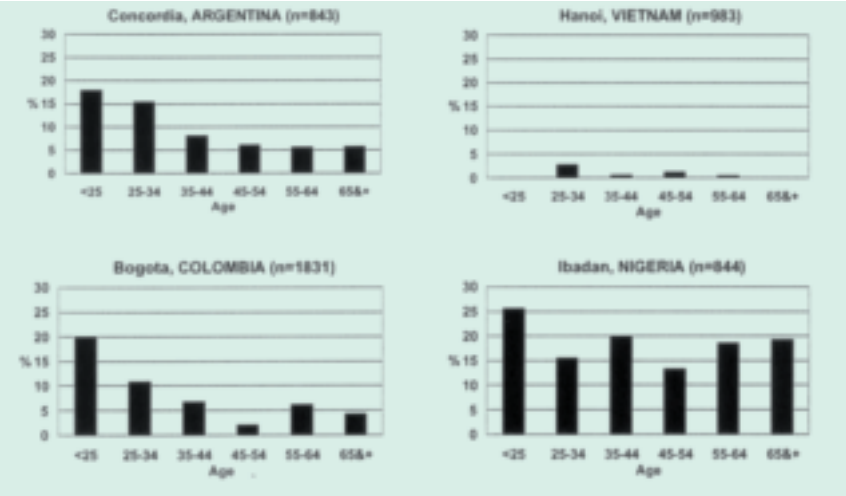

Figure 28 Prevalence of high-risk types of human papillomavirus (HPV)* among sexually active and cytologically normal women aged ≥ 15 years , in different countries. IARC multi-centre HPV prevalence surveys

* Includes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82

From: Anh *et al.* (2003), Matos *et al.* (2003), Molano *et al.* (2003), Thomas *et al.* (2004)

had some impact upon the estimated progression rates.]

A cohort of 17 217 women, identified through one pathology laboratory in Toronto and whose records of cytological examinations spanned many years, was studied by Holowaty *et al.* (1999). The pathologists serving this laboratory had made a consistent attempt to identify the different degrees of dysplasia by cytology, and only referred women for further assessment if there was cytological evidence of progression. By linkage of the records from the laboratory with those of the Ontario Cancer Registry, women who were subsequently diagnosed with carcinoma in situ or invasive carcinoma of the cervix were identified. The maximum extent of regression occurred in those with cytological evidence of mild dysplasia. Progression to severe dysplasia or worse within 10 years occurred in only 10% of those with mild dysplasia. Most of these progressions occurred within five years. There was even less progression, even within ten years, when invasive cancer was used as the endpoint (Table 13).

In prospective follow-up studies which used HPV testing, it was found that cytological abnormalities were diagnosed in only a minority of women who were HPV-positive at study entry. For example, cytological abnormalities (LSIL or worse) occurred within three years in 25% of women who were HPV DNA-positive at enrolment in one study (Ho *et al.,* 1998), in 28% (borderline dyskaryosis or worse) within three years in another (Woodman *et al.,* 2001), in 22% (LSIL or worse) within 56 months in a study in young women and adolescents (Moscicki *et al.,* 2001), in 17% (ASCUS or worse) over six years in another (Castle *et al.,* 2002b) and 5.4% in a study of 2404 women followed by cytology and PCR-based HPV testing every 4–6 months over a period of eight years (Schlecht *et al.,* 2003a). It was also noted in the latter study that the mean time for progression from ASCUS to LSIL or worse was less if the cytological specimen was positive for high-risk HPV (67 months), compared with 88 months in women with no HPV infection (Schlecht *et al.,* 2003a). The reason why cytological abnormalities are not identified in a greater proportion of HPV-infected women is probably related to the transient nature of many productive infections and the fact that the cytological manifestations of a productive infection can be subtle. The risk of cytological abnormalities is consistently observed to be greatest during the six months immediately after HPV DNA detection and diminishes quickly thereafter. If such women undergo colposcopy, they are frequently found to have apparently low-grade acetowhite lesions that on biopsy often demonstrate the characteristic histopathological features of CIN 1. These CIN 1 lesions are heterogeneous with respect to a number of important biological parameters in addition to the associated HPV types. These include ploidy, clonality and LOH at specific chromosomal loci that may represent tumour-suppressor genes. The majority of CIN 1 are either diploid or polypoid, most are polyclonal, and LOH at specific chromosomal loci is most commonly absent.

Liaw *et al.* (1999) followed a cohort of 17 654 cytologically negative women using the records of Kaiser Permanente in California. Enrolment in the cohort commenced in 1989, and women were followed to the end of 1994. On average, each woman had 0.6 tests per year, and 20% had no repeat test. Of a total of 380 incident cases of cytological abnormality identified during follow-up, 154 were ASCUS and 179 LSIL. Cervical lavages had been collected and stored at enrolment, and on diagnosis another lavage specimen was collected. Similarly, specimens were collected from up to three matched controls without abnormality and all specimens were tested

Table 13. Estimates of progression in women with cytological evidence of dysplasia

	Cumulative actuarial rate of progression (per 100 women) within 10 years to carcinoma *in situ* or worse (95% CI)
Mild dysplasia	2.8 (2.5–3.1)
Moderate dysplasia	10.3 (9.4–11.2)
Severe dysplasia	20.7 (17.0–24.3)
	Cumulative actuarial rate of progression (per 100 women) within 10 years to invasive cancer of the cervix (95% CI)
Mild dysplasia	0.4 (0.3-0.5)
Moderate dysplasia	1.2 (0.9-1.5)
Severe dysplasia	3.9 (2.0-5.8)

From Holowaty *et al.* (1999)

for HPV DNA using PCR. The data were analysed as a nested case–control study. Compared with women who were HPV DNA-negative on enrolment, those who were positive at enrolment had an OR of 3.8 (95% CI 2.6–5.5) for being diagnosed with LSIL. Infection with HPV16 was most likely to predict the development of SIL. The association was much stronger for HPV positivity detected at diagnosis.

Castle *et al.* (2002b) subsequently reported findings from a subcohort of 2020 women who were HPV-positive on enrolment but cytologically negative (from the same study as Liaw *et al.,* 1999), who had at least one additional visit after enrolment and were followed for 57 months on average. The cumulative incidence of cytologically detected ASCUS or worse was 16.8% and of LSIL or worse 6.4%. The cumulative incidence of ASCUS or worse was 4.2% among cytologically negative women.

High-grade lesions

Once HPV infections clear, they have tended not to recur within the follow-up in the existing cohort studies. It is unclear whether there is an important chronic state of HPV latency related to subsequent cancer risk in immunocompetent women. Evidence for viral latency and re-emergence due to failed immune surveillance comes mainly from women with immunosuppression secondary to HIV infection or immunosuppressive medication used for organ transplantation (Sun *et al.,* 1997).

A small proportion of women who become infected with HPV develop persistent HPV infections. The biological reasons why some women develop persistent infections are poorly understood, but probably include HPV type (with HPV16 persisting longer than other types) and differences in individual cell-mediated immune responses and other individual host factors, as well as environmental factors (e.g., possibly diet, smoking and co-infections with other sexually transmitted agents) (Sun *et al.,* 1997; Kjaer *et al.,* 2002b; Sedjo *et al.,* 2002, Richardson *et al.,* 2003). Persistent infections, for the most part, represent "abortive" viral infections. In abortive infections, no particles of HPV are produced within the infected cells and as a result, the cytopathic effects that are pathognomonic of productive HPV infections are greatly reduced. Follow-up studies have indicated that persistence of high-risk types of HPV is a prerequisite for the development of CIN 3 lesions and invasive cervical cancers (see earlier in this chapter) (Figure 27). For example, in a study of 1611 women with no cytological lesion on enrolment who were followed by HPV testing and cytology every 4–6 months for up to eight years, the incidence rate of SIL was 0.73 per 1000 women months (95% CI 0.5–0.9) among women free of HPV at the two initial visits and 8.68 (95% CI 2.3–15.1) among women with HPV type 16 or 18 infections persisting over both visits (Schlecht *et al.,* 2001). Further, clearance of high-risk HPV in otherwise established cytological lesions is a marker associated with regression of CIN lesions (Nobbenhuis *et al.,* 2001a; Zielinski *et al.,* 2001a; Schiffman *et al.,* 2002). Conversely, women with mild cytological abnormalities (e.g., ASCUS or LSIL) who lack identifiable high-risk types of HPV are at very low risk for developing CIN 2 or 3.

In the Liaw *et al.* (1999) cohort study of 17 654 cytologically negative women (see above), of a total of 380 incident cases of cytological abnormality identified, there were 47 with HSIL. Compared with women who were HPV DNA-negative on enrolment, those who were positive at enrolment had an OR of 12.7 (6.2–25.9) for HSIL. Infection with HPV16 was most likely to predict the development of SIL. The association was much stronger for HPV positivity detected at diagnosis.

Bory *et al.* (2002) reported results of follow-up of a sub-sample of 3091 women with normal cytological findings at first entry, among whom 659 (21%) had evidence of HPV infection. Of these 659 women, 241 (3% of the total studied) had a persistent HPV infection at 2–4 examinations, with a

final diagnosis of HSIL in 51 (0.7% of the total studied) within 4–36 months. The women who developed HSIL had a higher viral load than those with transient infections. All these women developed cytological abnormalities before or at the time of the colposcopy that led to the diagnosis. In contrast, of 2432 women who were negative for HPV and followed for a similar period as for the HPV-positive women, only two developed HSIL. Nobbenhuis *et al.* (1999) concentrated on CIN 3 as the end-point, using cytology and HPV testing by PCR to monitor for an average of 33 months 353 women who had been referred to gynaecologists with mild to moderate or severe dysplasia. Thirty-three women reached clinical progression, defined as CIN 3 covering three or more cervical quadrants, or a cytological result of suspected cervical cancer. All had persistent infection with a high-risk HPV type. The cumulative six-year incidence of clinical progression among these women was 40% (95% CI 21–59%).

Other data suggest that the intensity of an HPV infection (i.e., the viral load) is relevant to whether detectable disease develops. Thus, Cuzick *et al.* (1994) showed that in women with cytological abnormalities, a high viral load detected by a semi-quantitative PCR was strongly related to high-grade CIN. Subsequently, viral load determined with the Hybrid Capture technique has been reported to be directly related to the severity of the lesion (Clavel *et al.,* 1999; George *et al.,* 2000). Swan *et al.* (1999) reported a relationship between viral load and severity of CIN grade, which, however, appeared to be restricted to HPV16, and was absent for HPV18, 31 and 45. Ho *et al.* (1995) also suggested that women with SIL having a high viral load are more likely to have persistent SIL than those with a low level of HPV DNA. They conducted a study of 70 subjects with histopathologically confirmed cervical dysplasia, followed at three-month intervals for 15 months. Women with HPV type-specific infection and with a high viral load had the highest risk of persistent SIL compared with those having a low level of type-specific persistent infection or no type-specific infection (OR = 4.97; 95% CI 1.45–17.02). Josefsson *et al.* (2000) and Ylitalo *et al.* (2000b), in case–control studies nested within a cohort of screened women in Sweden, and using a fully quantitative PCR assay, demonstrated that cervical carcinoma *in situ* associated with HPV16 occurred mainly in HPV16-positive women who had consistently high long-term viral loads. These studies, based on tests of archived specimens, confirmed the long natural history of carcinoma *in situ,* as previously inferred from cytological observations. Thus, Ylitalo *et al.* (2000b) computed that the mean times from a first confirmed HPV infection before age 25 years to a diagnosis of carcinoma *in situ* in women with high and intermediate viral loads were 17 years and 19 years, respectively. Approximately 22% of women with evidence of a high viral load of HPV16 were eventually diagnosed with carcinoma *in situ*. Further, Josefsson *et al.* (2000) found that women in the highest 20% of the HPV16 DNA viral load distribution were at a 60-fold higher risk of being diagnosed with carcinoma in situ than women negative for HPV16. Subsequently, van Duin *et al.* (2002) found in a cohort study that in women with normal cytology, as well as those with abnormal cytology, an increased HPV16 viral load conferred an increased risk of developing a cervical lesion. This was confirmed by Schlecht *et al.* (2003b) in a cohort study of 473 women positive for HPV at the first two visits and followed by cytology and HPV testing for eight years. Compared with those having less than one viral copy per cell in specimens tested during the first two visits, the RR for incident SIL was 1.9 (95% CI 0.8–4.2) for those with 1–10 copies per cell and 4.5 (95% CI 1.9–10.7) for those with over 1000 copies per cell. The equivalent RR for HSIL for those with over 1000 copies per cell was 2.6 (95% CI 0.5–13.2).

The early studies frequently could not distinguish between different stages of CIN, or specifically between early lesions associated with transient HPV infections and those that represent persistent cancer precursors. Therefore, they are difficult to interpret in the light of our current understanding of the pathogenesis of cervical cancer. However, those that used histological diagnosis following biopsy and appropriate analytical methods contributed to our current understanding and these are summarized below.

In the early studies, the end-point used was carcinoma *in situ.* Subsequently, many authors used CIN 3 and more recently CIN 2/3 or HSIL (if cytology determined the end-point rather than histology). The authors' terminology is retained in the summaries that follow.

Data derived from several screening programmes in the USA were used to derive estimates of the average duration of carcinoma *in situ* varying from five years (Dunn, 1960) to 8.1 years (Dunn & Martin, 1967) and 11 years (Kashgarian & Dunn, 1970). Dunn & Martin (1967) pointed out the discrepancy between some of these studies in the amount of disease that appeared to be occurring when using the curve of age-specific incidence, and suggested that some of the precursor lesions would terminate in regression. They reported that the maximum incidence of carcinoma *in situ* occurred in women aged 25–29, with a decrease to a low level after 35 years.

Kasper *et al.* (1970) analysed the prevalence and incidence of gynaecological cancer detected cytologically in

175 767 women screened in Alberta, Canada. They concluded that the generation of new cases of carcinoma *in situ* begins in the 20–24 age group at a very significant rate and that carcinoma *in situ* develops six times more frequently before age 45 years than in older women. They estimated that in the average case, detectable carcinoma *in situ* was present 8–10 years before the development of invasive cancer.

Theoretically, if all cases of carcinoma *in situ* progressed to invasive cancer, studies that could determine the cumulative incidence of carcinoma *in situ* (not prevalence) over a lifetime and compare this with the expected cumulative incidence of invasive cancer in the absence of screening should find almost or complete equivalence of these two cumulative rates. Allowance has to be made for those cases of carcinoma *in situ* that remain without progression and these are measured by the prevalence of disease. The difference between the cumulative incidence of carcinoma *in situ* by age less the prevalent (non-progressed) cases of carcinoma *in situ* and the cumulative incidence of invasive cancer gives an indication of non-progressive disease.

The British Columbia cohort study evaluated explanations other than non-progression for that difference (Boyes *et al.,* 1982). The study utilized the records in the central cytology laboratory, the only one in the province. Records on all women born in 1914–18 (*N* = 52 452, Cohort 1) and 1929–33 (*N* = 66 701, Cohort 2) with follow-up to 1969 were extracted, and related to population data on invasive cancer, deaths, marriage and hysterectomy. The analysis concentrated mainly on corrected rates of prevalence and incidence of carcinoma *in situ* and of invasive cervical cancer. It was found that the gap persisted in spite of corrections for the false negative error and denominator error, and that the two cohorts 15 years of age apart had almost identical risks of carcinoma *in situ* at comparable ages. Thus the only remaining explanation for the gap was regression, which probably occurred in 40–60% of the detectable cases, especially at younger ages. Boyes *et al.* (1982) pointed out that when regression is part of the natural history, the 'observed' regression proportion is likely to be less than that which occurs. Therefore estimates of progression, made by the same process, are likely to be overestimates; however, they estimated that the proportion of carcinoma *in situ* that progressed ranged from 0.26 to 0.53.

The various models that had been applied earlier were reviewed by Prorok (1986). He concluded that the preclinical natural history of cervical cancer was complex, with a clear indication of regression of carcinoma *in situ,* as well as an age-dependence of transition probabilities and duration of disease status, that vary inversely by age. The mean duration of carcinoma *in situ* was model-dependent, with values in the 5–10 year range computed by authors who used early data, but values nearer 20–25 years were computed by authors who used longitudinal British Columbia data, which predated those subsequently analysed by Boyes *et al.* (1982).

In the Luthra *et al.* (1987) study (described in the section on low-grade disease above), of 138 women with cytologically diagnosed moderate dysplasia, 18 progressed (computed actuarial rate 29%) and of 22 with severe dysplasia two progressed (computed actuarial rate 36%). Of the total of 22 women who progressed during follow-up (including the two women originally diagnosed with mild dysplasia described above), 18 were diagnosed as carcinoma *in situ* and four early invasive cancer; there were no deaths. [The Working Group noted the substantial loss to follow-up in this study.]

An extension of the Boyes *et al.* (1982) study, with follow-up to 1985, included over 75 000 women in Cohort 1, over 100 000 in Cohort 2 and a younger cohort (Cohort 3) of nearly 140 000 women born in 1944–48 (Miller *et al.,* 1991b). Again there was little difference in incidence of carcinoma *in situ* at overlapping ages, confirming the absence of a cohort effect. The estimated proportions of cases of carcinoma *in situ* that regressed were 61% over ages 40–64 (Cohort 1), 70% over ages 25–54 (Cohort 2) and 77% over ages 15–39 (Cohort 3).

Syrjänen *et al.* (1992) defined regression by negative cytology, colposcopy and punch biopsy, and progression by punch biopsy. Of 68 women with CIN 2, 53% had regression over a period of six years, as did 14% of 42 with CIN 3. In contrast, 21% and 69%, respectively, showed progression, while 3% and 12% showed recurrence after treatment. When the lesions were reclassified using the Bethesda terminology, 376 were LSIL and 110 were HSIL. Of these HSIL lesions, 38% showed regression, 39% progression and 6% recurrence after treatment. [The Working Group noted that the authors studied lesions associated with both low- and high-risk HPV types.]

Koutsky *et al.* (1992) reported that after two years of follow-up of young college women, those initially HPV-positive had a cumulative risk of CIN 2 or 3 of 28%, compared with a cumulative risk of 3% for those initially HPV-negative. Risk was highest for those women infected with HPV16 or 18, the relative risk for those infected with type 16 or 18 being 11 (95% CI 4.6–26) for the development of any SIL compared with those without an HPV infection. Gaarenstroom *et al.* (1994) also showed that LSIL progressed to high-grade disease only if it contained high-risk HPV types.

In the Ostör (1993) review of the natural history of CIN described above for low-grade lesions, none of the studies of CIN 2 or 3 reviewed had over 1000 subjects. The total numbers of subjects in each of the categories were 2247 CIN 2 and 767 CIN 3. Ostör concluded that 43% of CIN 2 and 32% of CIN 3 regressed, and 5% and > 12% progressed to invasion.

Two more recent formal meta-analyses arrived at similar conclusions to those of Ostör (Mitchell *et al.,* 1996; Melinkow *et al.,* 1998).

Morrison *et al.* (1996) conducted a further update of the two cohorts originally studied by Boyes *et al.* (1982) to 1992, a follow-up period of over 40 years. The regression rate in the oldest cohort over this period was estimated to be 47% and in the younger cohort 72%.

In the Holowaty *et al.* (1999) cohort of 17 217 women, over 50% of those with cytological evidence of moderate dysplasia showed regression. Progression to severe dysplasia or worse within 10 years occurred in 32% of those with moderate dysplasia. Most of these progressions occurred within five years (Table 13).

In the Castle *et al.* (2002b) study of 2020 women who were HPV-positive but cytologically negative on enrolment and who were followed for 57 months on average, the cumulative incidence of high-grade lesions or worse was 2.2%.

In the Woodman *et al.* (2001) study of 1075 young women (15–19 years) who had intensive cytology and HPV screening, and who on average had repeat tests every six months, the risk of moderate or severe dyskaryosis (cellular evidence of dysplasia, in the UK terminology) was substantially greater in those who tested HPV-positive, and of the 28 women who developed high-grade CIN during follow-up, 82% had become HPV-positive after a median follow-up of 26 months. Nevertheless, compared with those who were HPV-negative during follow-up, the risk of moderate or severe dyskaryosis was maximal at six months after the first HPV-positive test (RR = 25.3; 95% CI 8.8–72.8) and declined rapidly thereafter (RR > 12 months = 6.4; 95% CI 2.1–19.6). In another study among women with normal cytology who were positive for high-risk HPV, 8% developed CIN 3 within a four-year interval (Rozendaal *et al.,* 1996, 2000).

Sherman *et al.* (2003a) studied a cohort of 20 810 women aged 16 years or more on enrolment who had satisfactory baseline cytology and samples suitable for HPV testing. They were screened annually by cytology for up to 122 months, during which period 171 women had CIN 3 or cancer diagnosed. The results of the HPV tests were not available for patient management, which was based on cytology according to standard practice. Of the women diagnosed with CIN 3 or worse, 123 had baseline cytology results of ASCUS or worse and/or a positive HPV test, 118 within the first 45 months of follow-up. During this period, the cumulative incidence of CIN 3 or worse was 4.5% among women with baseline cytology results of ASCUS or worse and/or a positive HPV test, compared with 0.16% among women negative on both tests.

Invasive cancer

Cervical cancer precursors can be defined in a variety of ways including virological measures, biological features and morphological terms. Persistence of infection with a high-risk type of HPV is now regarded as an absolute requirement for a lesion to be considered a precursor. Additional cellular events that occur in the majority of invasive cervical cancers and in many intraepithelial lesions include monoclonality, aneuploidy, genetic alterations which may result in the activation of oncogenes and inactivation of tumour-suppressor genes, and increases in telomerase activity. Monoclonality and aneuploidy are universally accepted features of malignancy at all tissue sites and are invariably found in invasive cervical cancers and in many CIN 3 lesions (Fu *et al.,* 1988; Hering *et al.,* 2000). Genetic alterations in cancers frequently appear to involve the inactivation of tumour-suppressor genes. Inactivation of tumour-suppressor genes is considered to be a two-hit process. One hit often involves a subtle mutation within one allele, whereas the other results in a gross deletion of the second allele (Weinberg, 1991) or methylation of that allele (Steenbergen *et al.,* 2004). Molecular studies of invasive cervical cancers and CIN 2 and CIN 3 lesions have shown that a multitude of chromosomal loci are often lost in cervical carcinomas and that similar losses are occasionally observed in CIN 2 and CIN 3 lesions (Mitra *et al.,* 1994a; Larson *et al.,* 1997; Chung *et al.,* 2000). High frequency of LOH, in more than 30% of cervical cancers studied, was found at 3p, 4q, 5p, 5q, 6p, 7q, 11p, 18p and 18q (Mitra *et al.,* 1994b; Mullokandow *et al.,* 1996; Rader *et al.,* 1996). Using comparative genomic hybridization (CGH) and LOH analysis, Steenbergen *et al.* (1996, 1998) demonstrated that HPV-mediated immortalization is associated with genetic gains at chromosomes 5p, 9q, 19 and 20 with LOH at 3p, 10p, 11p, 11q, 13q and 18q. In large part, these genetic alterations are thought to arise secondary to genetic instability induced in the cervical epithelium during the unregulated cell proliferation associated with expression of high-risk HPV-associated oncoproteins E6 and E7 in the basal layers, which is often associated with integration of the virus in the cellular genome.

In the study of Hakama & Rasanen-Virtanen (1976), the probability of being diagnosed with dysplasia

of high degree in the five-year interval after a negative cytological test was 0.010. For carcinoma *in situ* the probability was 0.012, while for microinvasive carcinoma it was 0.002. This compares with a probability of 0.010 for the development of frankly invasive carcinoma before the screening programme and 0.002 after the first [negative] test. Combining the probability of being diagnosed with *in situ* cancer with that for dysplasia of high degree, the authors estimated that 28–39% of such surgically treated preinvasive cases would otherwise have progressed to invasive cervical cancer, and that 21% of the frankly invasive cases are preceded by a preinvasive stage of shorter duration than the five-year interval between screenings, or have no preclinical stage.

Gustafsson & Adami (1989) used Swedish population-based incidence and mortality rates for cervical cancer to model the natural history of carcinoma of the cervix (including carcinoma *in situ*). They noted that the maximum incidence of carcinoma *in situ* occurred at 30 years. The proportion of cases of incident carcinoma *in situ* that progressed to invasive cancer was estimated to be 12.2%, with a mean duration of carcinoma *in situ* of 13.3 years, and the preclinical phase of invasive cancer without screening lasted on average for four years. They also estimated that 15–23% of prevalent carcinoma *in situ* progressed to invasive cancer. They did not estimate regression rates of carcinoma *in situ,* but concluded that the evidence was not compatible with two different types of cancer of the cervix with different natural histories. [The Working Group noted the differences between the estimates made by Gustafsson & Adami (1989) and those by other authors. One possible explanation is that a number of cases of CIN 3 were classified as carcinoma *in situ* in the data submitted to the cancer registry whose data these authors used, thus reducing the estimated proportion that progressed.]

The Holowaty *et al.* (1999) study of a cohort of 17 217 women found very little progression of moderate dysplasia, even within ten years, when invasive cancer was used as the end-point (Table 13).

Discussion

It is intuitively obvious that in order to gain knowledge of the natural history of the lesions discovered by screening, screening has to be performed and the detected abnormalities identified. However, as screening is performed in order to benefit the individual, it is rarely possible to avoid applying the currently best known therapy for the condition. For precursor lesions, this almost invariably involves surgical excision, resulting in complete, or almost complete, ablation of the cervical epithelium. Therefore, except in very unusual circumstances when observation rather than biopsy and treatment occurred (Barron & Richart 1968; Kinlen & Spriggs, 1978; McIndoe *et al.,* 1984), it is not possible to determine the natural history of the detected lesions by observing them. In that respect, the experience in New Zealand is salutary (McIndoe *et al.,* 1984). A total of 948 women with histologically confirmed carcinoma *in situ* diagnosed on punch biopsy but untreated were followed during 5–28 years. Of these, 817 had normal cytology on follow-up, 12 (1.5%) developed invasive carcinoma of the cervix. Of the remaining 131 women who continued to have abnormal cytology consistent with cervical neoplasia, 29% developed invasive carcinoma of the cervix or vaginal vault. McIndoe *et al.* (1984) estimated that patients with continuing abnormal cytology after an initial diagnosis of carcinoma *in situ* are 24.8 times more likely to develop invasive carcinoma than women who have normal cytology on follow-up.

There are a number of inherent problems in almost all of the prospective studies of natural history. These include differing lengths of follow-up; differing definitions for what constitutes 'disease' (e.g., confirmed by colposcopy, cytology or histology), the fact that cervical biopsy, or perhaps even repeat cytological sampling, might affect natural history, and importantly that little effort has been made to examine the natural history of individual lesions on the cervix, as opposed to sampling the cervix as a whole. The latter may be particularly important when considering persistence of CIN 1 lesions. Unless location-specific information and in-depth HPV type-specific information is obtained during follow-up, it is impossible to determine whether a lesion that appears to be a persistent CIN 1 lesion is actually persistence of the same lesion or instead multiple sequential lesions associated with different HPV types. The inherent variability associated with a histo-pathological diagnosis of CIN is also critical when considering the outcomes of the natural history studies. Almost half of biopsy-confirmed CIN 1 lesions are reclassified as non-CIN when re-reviewed by expert pathologists, so that an apparent rate of approximately 50% regression of biopsy-confirmed CIN 1 could be observed through simply re-evaluating the original slides. The problems inherent in predicting natural history based on histological appearance alone are evident from the wide variations in observed outcomes when CIN lesions of various grades have been followed in prospective natural history studies.

A number of observations have indicated that high-risk HPV infection precedes the development of high-grade CIN lesions (see section above on Temporality). In studies involving

women who had CIN 3, Remmink *et al.* (1995) and Nobbenhuis *et al.* (1999) suggested that only women who were infected with high-risk HPV types were likely to have progressive disease, and only in women with persistent infection was their disease likely to progress. Further, Zielinski *et al.* (2001a) suggested that infection with HPV precedes the development of a cytological abnormality. However, cross-sectional surveys (conducted across ages at one point in time), such as those just described, do not provide much information on natural history. It is necessary to follow groups of women for some time, and conduct repeated tests, to obtain information about progression and regression of cervical lesions.

Among women with normal cytology who were positive for high-risk HPV, 8% developed CIN 3 within a four-year interval (Koutsky *et al.,* 1992, Rozendaal *et al.,* 1996, 2000). More recent prospective follow-up studies have confirmed that HPV infection precedes the development of low- and high-grade CIN lesions (Schlecht *et al.,* 2001; Kjaer *et al.,* 2002b). The distinction whether an intraepithelial lesion is associated with a transient HPV infection or whether it is associated with a persistent HPV infection now appears to be the single most important factor in determining the lesion's potential for progressing into an invasive cervical cancer. Interestingly, in studies by Nobbenhuis *et al.* (2001a) and Zielinski *et al.* (2001a), it was shown that clearance of high-risk HPV in otherwise established cytological lesions is a marker associated with the regression of CIN lesions (Londesborough *et al.,* 1996).

In a retrospective study of women who developed cervical cancer, high-risk HPV was found to be present in cytologically normal smears taken more than 10 years before cervical cancer development (Zielinski *et al.,* 2001a). That HPV infections precede the development of cervical cancer by at least 15 years in most cases is further supported by cross-sectional data (Bosch *et al.,* 2002). In fact, in women over 30 years of age, the high-risk HPV prevalence declines to 2–4% (Jacobs *et al.,* 2000; Clavel *et al.,* 2001; Cuzick *et al.,* 2003; Petry *et al.,* 2003; Bulkmans *et al.,* 2004).

Among HPV-positive women, very low viral loads correlate with cytological normality and benign natural history (Josefsson *et al.,* 2000; van Duin *et al.,* 2002; Schlecht *et al.,* 2003b; Snijders *et al.,* 2003; Giuliano *et al.,* 2004; Hesselink *et al.,* 2004). However, high viral loads are not always associated with high risk of progression to cancer precursor lesions. Because of the great variability in viral load and lesion severity, there is currently no consensus that viral load, as identified in routine clinical samples, presents a clinically useful parameter.

In most developed countries, the cumulative incidence to age 65 of invasive cervical cancer is approximately 1.5%, so that a cumulative incidence in excess of 4% of carcinoma *in situ* indicates the extent to which the majority of these lesions do not progress or regress. The cytology-based studies showed that at least 50% of women with detectable carcinoma *in situ* will not progress, while the proportion that regress is at least of a similar order to the proportion that progress. It is likely that this is also true for the lesions identified by HPV testing (Cuzick *et al.,* 2003). However, the estimated progression rates in many studies may be overestimates, because what one observes (or can compute) in terms of regression is only a part of the regression that must be occurring, but is unobserved because of the intermittent nature of screening. Similar conclusions have also been reached concerning many of the dysplastic (SIL) abnormalities identified by cytology. The differences in extent of progression and/or regression reported in different studies may be due in part to differences in the application of similar terminology, or to differences in the way the classification was applied in different laboratories. Some authors have inferred that there can be direct progression to CIN 2 without passing through detectable CIN 1, but no data have been reported that directly addressed this issue. Although only one study has reported data using the Bethesda classification, it can be inferred that regression is an important part of the natural history of lesions classified both as HSIL (high-grade), as well as LSIL (low-grade) using this terminology.

The lessons from the HPV studies seem clear. Infection with HPV, especially the high-risk types, is predictive of subsequent development of squamous intraepithelial lesions, but such infection occurs far too frequently compared with the amount of cervical cancer expected in these populations. Thus not only are the large majority of infections in young women transient, but even the majority of those that appear persistent are of no concern. However, in older women, with documented evidence of persistent HPV infection with high-risk types, there is an appreciable probability that CIN 3 will develop.

There is, however, at least one other missing piece of information concerning the natural history of high-risk HPV infections. It is important to know what causes persistence of infection with oncogenic HPV viruses, and what causes precursors to progress and not regress. In this context integration of the virus into the cellular genome, high viral load, diminished immunity of the host by HIV infection and additional genetic alterations such as LOH at 3p, 4, 5p, 10p and activation of hTertmRNA, which codes for the catalytic subunit

of telomerase, with increased levels of telomerase are likely to be important.

An ideal test would indicate that an oncogenic HPV has already enhanced genetic instability and rendered infected cells susceptible to transformation, thereby facilitating the development of cancer. In this respect, it should have the ability to detect those progressive cytological abnormalities that are caused by high-risk HPV infections and to discriminate them from transient low-grade lesions and those that only mimic morphological criteria of the onset of dysplasia or harbour HPV as an independent, but simultaneous event. Such a test should have greater true biological sensitivity and specificity than cytology and could possibly solve two problems inherent to conventional cytology. It could clarify how to consider the ASCUS and LSIL cytological abnormalities which, as already pointed out, represent mostly transient infections or in the case of ASCUS mainly diagnostic uncertainty. The other problem that contributes to low sensitivity of conventional cytology is overlooking and/or misinterpreting abnormal cells, a problem that also ideally should be overcome by a test that fulfils the criteria specified above and avoids sampling errors.

Figure 27 attempts to encapsulate our current understanding of the natural history of preclinical abnormalities of the cervix diagrammatically. It is important to recognize that although precise numbers cannot be given, as one moves from left to right across this figure, the probability of progression becomes higher, and as one moves in the opposite direction, the probability of regression increases.

Considerations for screening programmes

The association of HPV and cervical cancer is unique in that a restricted number of HPV types have been identified as necessary for the development of cervical cancer worldwide. The implication is that in the absence of the viral infection (persistence is probably a requirement), cervical cancer is not expected to develop. Therefore, preventive strategies, either screening or vaccination, that target putative non-HPV-related cancers are no longer scientifically justified. The estimated risk linked to any of the high-risk HPV types for which sufficient evidence is available is, in statistical terms, equivalent to the risk of the most common ones, HPV16 and HPV18. Consequently, it is justified to use as screening tests cocktails of proven high-risk types. The role of the less common types in cervical cancer is not yet fully defined.

Some aspects of the epidemiological findings are relevant to screening recommendations:

(a) Age to initiate screening. Figure 18 shows a typical profile of age-specific HPV prevalence and of the incidence of cervical cancer in countries with established screening practices. The HPV prevalence is a function of the age at initiation of sexual activity of women and of the population-specific patterns of sexual exchange. Recommendations on the age to initiate HPV screening should aim to maximize detection of early cervical cancer cases while avoiding the bulk of transient HPV infections. It is thus important to carefully define country-specific HPV prevalence graphs as well as the age-specific incidence of cervical cancer and, if possible, of its closest precursors.

(b) Age to terminate screening. Most mortality from cervical cancer occurs in women aged 50 years and above. It is thus of interest to consider HPV screening along with cytology in women at the age of exit of their screening protocols, benefiting from the very strong negative predictive value of double negative results of these two screening tests.

(c) Rescue of non-participants of screening programmes. Deaths from cervical cancer in developed countries occur largely among women who escaped screening. There may be opportunities to offer some protection to these women linked to other health-related contacts, taking advantage of the longer protection associated with a normal cytological test result combined with HPV-negativity.

(d) Women exposed to HIV are at higher risk of neoplastic progression. Cervical cancer screening in women exposed to HIV may be enhanced by addition of HPV testing.

(e) Other co-exposures of potential relevance. Case–control studies have shown that at least three co-factors are likely to modulate the HPV-related carcinogenic process, namely high parity, long-term use of oral contraceptives and smoking. Although of little relevance in population-based screening programmes, individual preventive protocols at gynaecological clinics may take into account the presence of some of these exposures when making recommendations for HPV testing.