

Epidemiology: VIN and vulvar cancer

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This chapter summarizes our current knowledge regarding the epidemiology of vulvar intraepithelial neoplasia (VIN) and vulvar cancer, with an emphasis on recent research into the possible aetiological diversity of different histopathological types of vulvar cancer. Attention is focused on the emerging hypothesis that human papillomavirus (HPV) is related to most VINs and some vulvar cancers, but a proportion of invasive tumours is entirely unrelated. Other points of discussion include the potential role of cigarette smoking, other sexually transmitted viruses, diabetes, obesity and immunosuppression.

HISTOPATHOLOGY

The histopathology of vulvar cancer is reviewed in detail elsewhere in this volume (Chapters 2 and 3). Approximately 80 per cent of invasive vulvar cancers are squamous cell tumours; the remaining histological

types include basal cell carcinoma, Paget's disease, adenocarcinomas, malignant melanoma, sarcoma and other rare neoplasms.¹ More recently, three histological subtypes of invasive squamous vulvar cancer have been described morphologically: (1) keratinizing squamous carcinoma (KSC); (2) basaloid carcinoma (BC); and (3) warty squamous carcinoma (WC). As these last two subtypes have been related to HPV, pathological and epidemiological studies have often combined them into one group (basaloid warty carcinoma or BWC).² Various small studies have found that between 50 per cent and 85 per cent of invasive vulvar cancers are KSC and that 15–50 per cent are BWC.^{3–9} The proportion containing oncogenic types of HPV DNA appears to vary across these histological subtypes. HPV DNA has been detected in 4–39 per cent of KSCs using methods based on the polymerase chain reaction (PCR).^{3,5,7–9} A higher proportion of BWC, between 50 per cent and 86 per cent, have been reported to be HPV DNA positive using PCR

methodology.^{3,5,7-9} Among women with invasive vulvar carcinoma, several studies suggest that women with HPV-positive tumours are younger than those with HPV-DNA-negative tumours.² It has also been reported that VIN 3 and BWC are more likely to be adjacent to cervical cancer and other lower genital tract tumours than KSC.¹⁰

Pathological data suggest that VIN 3 represents the precursor of BWC but not of KSC.² Between 50 per cent and 90 per cent of VIN 3 lesions have been found, in various studies, to contain HPV DNA.¹⁰⁻¹⁵ In contrast to the variable proportion of HPV-DNA-positive vulvar cancers, HPV DNA is found in nearly all cervical intraepithelial neoplasia (CIN) lesions and invasive cervical cancers.¹⁶ As in cervical cancer, HPV 16 is the most frequent type associated with vulvar cancer, but HPV 18, -31 and -45 play a much larger role in the cervix than in the vulva.¹⁶

Clinicopathological studies indicate that KSC may be more common than BWC, and that HPV is associated mainly with BWC and its precursor lesion, VIN 3. Investigation of a large, population-based series of VIN 3 and vulvar cancer is an essential next step in evaluating the relationship between histopathological types of vulvar cancer and HPV infection.

INCIDENCE AND MORTALITY

Invasive vulvar cancer is rare, with incidence rates rarely exceeding 2 per 100 000 women in different parts of the world. Variations between countries in the incidence of invasive vulvar cancer are generally unremarkable, with the possible exception that elevated rates have been reported from Portugal and parts of Brazil.¹⁷ In the USA, the age-adjusted incidence rates of invasive vulvar cancer for 1990-94 among whites and blacks were 1.8 and 1.5, respectively.¹⁸ As a point of reference, the age-adjusted incidence rates of invasive cervical cancer for 1990-94 among whites and blacks were 7.7 and 12.2, respectively.¹⁸ Age-specific incidence rates of vulvar cancer and cervical cancer also vary dramatically. The incidence of squamous carcinoma of the cervix increases rapidly between the ages of 20 and 40 and then plateaus, whereas the incidence of vulvar cancer is low at younger ages and rises sharply only after the age of 50.²

Sturgeon and colleagues¹ examined the US incidence trends for VIN 3 and invasive squamous vulvar cancer between the period 1973-77 and 1985-87.

Invasive vulvar cancer rates among white women were remarkably stable during this period, with annual age-adjusted rates of 1.3 per 100 000 and 1.2 per 100 000 in 1973-77 and 1985-87, respectively. Consistent with clinical observations that vulvar cancer is a disease of older women, almost 80 per cent of the invasive squamous cases were diagnosed among women aged 55 years and older.

In contrast to the pattern for invasive cancer, the incidence of VIN 3 nearly doubled from 1.1 to 2.1 per 100 000 among white women during this same period, surpassing the rate for invasive squamous cell cancer. The largest proportional increase occurred among women younger than 35 years, for whom the rate almost tripled. The peak rate of VIN 3 shifted over this time period from women aged 35-54 years to those aged 35 years or younger.

Changes in sexual behaviour in the USA over the last several decades have translated into increases in the prevalence of HPV infection. Thus, changes in sexual behaviour may explain the increase in VIN 3 over time. Increasing detection and reporting, related partly to a better appreciation of the link between HPV and lower genital tract neoplasia of multiple sites, could also explain some of the rise. The discordant incidence trends for VIN 3 and invasive vulvar carcinoma are consistent with the hypothesis that almost all cases of VIN 3 are aetiologically linked to HPV, whereas significant numbers of invasive carcinomas are related to other factors. Alternatively, it is possible that the cohort of women affected by changes in sexual behaviour or other putative factors is not yet old enough to develop invasive vulvar cancer, or that early diagnosis and treatment of VIN 3 has blunted the anticipated increase in invasive vulvar cancer.

The age-adjusted mortality of vulvar cancer among both blacks and whites for 1990-94 was 0.3 per 100 000 women in the USA.¹⁸ The mortality rate from this cancer declined by about 17.8 per cent between 1973 and 1994. The 5-year relative survival rate for vulvar cancer is approximately 75 per cent.

METHODOLOGICAL CONSIDERATIONS IN EVALUATING EPIDEMIOLOGICAL DATA

As a result of the rarity of vulvar cancer, only a few, relatively small, case-control studies have been performed.¹⁹⁻²³ Moreover, the validity of these studies has been limited by several methodological problems. Interview participation rates for vulvar cancer cases

were between 60 per cent and 74 per cent in several investigations^{19,23} and participation rates were even lower for the serological component of these studies.^{9,24} Furthermore, one study did not investigate the potential aetiological role of sexually transmitted diseases²⁰ and two other investigations ascertained a history of sexually transmitted viruses by interview only.^{21,22}

Many large epidemiological studies of cervical cancer and its precursor have used cytological samples collected in special buffers or frozen tissue for HPV-DNA testing. Epidemiological studies of vulvar cancer, by contrast, have relied on the detection of serum levels of antibodies to assess HPV exposure in cases and controls, a technique that is believed to be less sensitive and specific than detection of cellular HPV DNA. HPV-DNA testing in epidemiological studies of vulvar cancer has largely been carried out on formalin-fixed, paraffin-embedded tumour tissue, which is a technique that is historically less sensitive than testing fresh specimens.

As epidemiological studies of vulvar cancers have been limited by their small numbers and incomplete evaluation of HPV infection, investigators may have been unable to control adequately for potential confounding factors. Another limitation of published studies is that only one small study investigated the possibility that histologically distinct forms of squamous carcinoma may be aetiologically distinct.¹³ The failure to analyse risk factors for different histological types of vulvar cancer separately may have led to an underestimation of the relevant risk factors for specific tumour types. Thus, the risk factors that have emerged from these studies (see below) remain to be confirmed in larger studies involving detailed histological information and epidemiological risk factor assessment.

BEHAVIOURAL CORRELATES OF SEXUALLY TRANSMITTED INFECTIONS

Several epidemiological studies have examined vulvar cancer risk and correlates of sexually transmitted infections, including number of sexual partners and age at first intercourse. Mabuchi and colleagues,²¹ in a US case-control study consisting of 149 vulvar cancers of unspecified type, and a similar number of hospitalized controls, found no increased risk among women who had one or more sexual partners compared with women who had no partners. However, the effect on risk of increasing number of sexual partners was not

evaluated. In an Italian case-control study involving 73 invasive cases and 572 hospitalized controls,²² no association was observed between number of sexual partners and vulvar cancer (relative risk or RR = 1.2, 95 per cent confidence interval or 95%CI = 0.4-4.1, for three or more partners versus one or no partners).

In two other larger case-control studies conducted in the USA, a positive association was observed between number of sexual partners and vulvar cancer.^{19,23} A study of 209 vulvar cancer cases (96 VIN 3, 113 invasive) and 348 community controls reported that multiple sexual partners was a more convincing risk factor for VIN 3 than invasive vulvar cancer. For example, the RR associated with five to nine partners compared with no to one partner was 5.1 (95%CI = 1.7-14.8) for VIN 3 and 1.5 (95%CI = 0.6-3.9) for invasive cancer. Adjustment in these analyses was made for age, number of sexual partners, smoking, previous abnormal Papanicolaou smear and a history of genital warts. In a subsequent analysis involving a small subset of the subjects from this same study (48 cases of SC, 21 cases of BWC, 54 VIN 3 cases and 87 matched controls),¹³ the age-adjusted RRs associated with two or more sexual partners were 2.2 (95%CI = 0.7-7.4) for KSC and 2.9 (95%CI = 1.0-8.4) for VIN 3. By contrast, the age-adjusted RR for BWC was 8.1 (95%CI = 1.7-37.9).

In another US case-control study of 180 VIN 3 cases, 53 invasive cases and 459 population-based controls,²³ women with 15 or more sexual partners had RRs of approximately sixfold and eightfold for invasive carcinoma and VIN 3, respectively. Adjustment in these analyses was made for age, education and age at first intercourse. In several studies,^{19,23} early age at intercourse has not been related to vulvar cancer risk, after adjustment for number of sexual partners.

GENITAL WARTS

In an analysis of cancer registry data from Washington State between 1974 and 1981, it was noted that 16.6 per cent of women with squamous vulvar cancer had coexisting condyloma, compared with none of the women with non-squamous vulvar cancer.²⁵ These data, along with clinical observations that women with vulvar squamous tumours may have one or more condylomata,²⁶⁻³⁰ led to the suggestion that genital warts are involved in the development of vulvar cancer.

In two subsequent case-control studies,^{19,23} a self-reported history of genital warts was associated with substantially increased risks of vulvar cancer. One study reported RRs of 15.8 (95%CI = 8.4–29.8) for VIN 3 and 17.3 (95%CI = 6.3–47.2) for invasive disease.²³ In these analyses, adjustment was made for age, smoking, number of sexual partners and education. Comparable RRs in the study by Brinton and colleagues were 18.5 (95%CI = 5.5–62.5) and 14.6 (95%CI = 1.7–125.6).¹⁹ Adjustment was made in this study for age, cigarette smoking, number of sexual partners and a previous abnormal Papanicolaou smear. In a reanalysis involving a subset of cases from this study,¹³ a self-reported history of genital warts was reported by 21 per cent of the VIN 3 cases, 25 per cent of the BWC cases and none of the KSC cases. This observation supports the hypothesis that KSC is less clearly linked to a sexually transmitted agent such as HPV than BWC.

The interpretation of these data is puzzling because genital warts are usually caused by HPV 6 and HPV 11, HPV types that are not generally believed to be oncogenic.³¹ Although the association between vulvar cancer risk and genital warts has been observed even for warts occurring 10 or more years before the diagnosis of vulvar cancer,^{19,23} it is possible that some genital warts are an exophytic preinvasive phase of BWC. Other possible explanations are that women diagnosed with condyloma may tend to have infections with multiple HPV types or that a history of condyloma serves as a marker of poor host response.¹⁹

HUMAN PAPILOMAVIRUS

Human papillomavirus is now known to be the major causal factor involved in the development of cervical cancer. As vulvar and cervical cancer often occur synchronously or asynchronously in the same patient,^{32–36} it is often proposed that these two tumours share a common aetiology. Two epidemiological studies have examined the association between presence of serum antibodies to HPV 16 virus-like particles and risk of vulvar cancer.^{9,24} In one study,²⁴ a much stronger association between HPV 16 seropositivity and disease was observed for VIN 3 (RR = 13.4; 95%CI = 3.9–46.5) than for invasive disease (RR = 2.9; 95%CI = 0.9–8.7). Adjustments in these analyses were made for herpes simplex virus (HSV), *Chlamydia trachomatis*, age, number of sexual partners, education, cigarette smok-

ing and oral contraceptive use. Further analyses suggested that the association was stronger for BWC (RR = 3.8; 95%CI = 0.8–18.9) than for KSC (RR = 1.6; 95%CI = 0.4–7.4), but age was the only adjustment factor considered in these analyses.

In contrast, Madeleine and colleagues⁹ reported that HPV 16 seropositivity was associated with similarly elevated risks for VIN 3 (RR = 3.6; 95%CI = 2.6–4.8) and invasive disease (RR = 2.8; 95%CI = 1.7–4.7). Adjustment was made in this study for age, education, smoking and body mass index (BMI). Furthermore, HPV 16 seropositivity was also associated with HPV-DNA-positive (RR = 4.5; 95%CI = 3.0–6.8) and -negative tumours (RR = 2.9; 95%CI = 1.6–5.0). The authors speculated that those seropositive case subjects whose tumours are HPV negative could be revealing a response to HPV infection that is no longer necessary to maintain the tumour, an infection that is unrelated to tumour development or methodological problems in the PCR or serological assays.

Human papillomavirus infection almost certainly plays a role in the aetiology of vulvar cancer, the molecular basis of which is explored further in Chapter 4. On present epidemiological evidence, it is unclear whether there is a subgroup of vulvar cancers that are non-HPV related.

OTHER SEXUALLY TRANSMITTED INFECTIOUS AGENTS

Although some studies have found associations between cervical cancer risk and serological markers of sexually transmitted infections, such as HSV 2 and *Chlamydia trachomatis* infections, it is difficult to rule out the possibility of confounding by HPV status.³⁷ Few epidemiological data are available to address the question of the role of specific infectious agents other than HPV in the development of vulvar cancer. In one case-control study,²⁴ HSV 2 seropositivity was associated with an increase in vulvar cancer risk, after adjustment for HPV 16 serology, *Chlamydia trachomatis*, cigarette smoking and oral contraceptive use (RR = 3.2; 95%CI = 1.0–10.0). In another study,⁹ HSV 2 seropositivity was weakly associated with VIN 3 (RR = 1.9; 95%CI = 1.4–2.6) and invasive vulvar cancer (RR = 1.5; 95%CI = 0.9–2.6) after adjustment for age, HPV 16 serology, smoking and BMI.

Sherman and colleagues²⁵ found a modest association between a self-reported history of an infection

with *Trichomonas vaginalis* and risk of VIN 3 (RR = 1.5), but an inverse association with invasive disease, after adjustment for age, number of partners, smoking, education, a history of genital warts and gonorrhoea. In the study by Hildesheim and colleagues,²⁴ *Chlamydia trachomatis* seroprevalence was associated with a 1.5-fold increase in vulvar cancer risk, decreasing to 1.4 (95%CI = 0.8–2.8), after adjustment for HPV 16 and other confounding factors. The interpretation of this finding is not clear because *Chlamydia* does not infect vulvar tissue. It is therefore likely that the modest association between *Chlamydia* and vulvar cancer risk represents confounding by HPV or other sexually transmitted infections.

There is some anecdotal evidence that syphilis is associated with vulvar cancer, especially in areas with high prevalence rates of this condition.^{38,39} Several epidemiological studies have reported that cases are more likely than controls to have a self-reported history of syphilis or gonorrhoea,^{21,23} but it is difficult to interpret these studies because of the rarity of these conditions and the potential for confounding by HPV status.

In summary, studies demonstrate inconsistent associations between sexually transmitted diseases other than HPV and vulvar cancer. It is unclear whether the associations observed between sexually transmitted diseases and vulvar cancer reflect incomplete control for HPV infection, a role for other sexually transmitted diseases as cofactors for progression of HPV infection to cancer or a separate aetiological role for some infectious diseases.

CIGARETTE SMOKING

Studies of cervical cancer have usually observed RRs of about 2 among cigarette smokers, but questions remain about whether these associations reflect confounding by HPV infection.³⁷ In the few studies that have examined the effects of smoking, controlling for HPV, no residual effect of smoking was observed.^{40,41} A number of investigations that have not accounted for HPV status also reported a positive association between cigarette smoking, especially current use, and vulvar cancer.^{19–21,42} Relative risks associated with current smoking in these studies have ranged from 1.5 to 4.8. Most of these studies reported a stronger association between cigarette smoking and VIN 3 compared with invasive disease.^{19,20,42}

The relationship between cigarette smoking and vulvar cancer has been investigated in only two studies

that included information on HPV infection. In an analysis of a subset of data from the original study by Hildesheim and colleagues,²⁴ the RR associated with early age at initiation of smoking remained elevated (RR = 1.7; 95%CI = 0.7–3.8), after adjustment for age, HPV 16 antibody serology, HSV, *Chlamydia trachomatis*, number of sexual partners, education and oral contraceptive use. In a reanalysis involving a larger study population than in the original investigation by Madeleine and colleagues,⁹ current smoking was associated with VIN 3 (RR = 6.4; 95%CI = 4.4–9.3) and invasive disease (RR = 3.0; 95%CI = 1.7–5.3), after adjustment for age, education and HPV 16 antibody serology. Among current smokers, intensity and number of years smoked further increased the risk of disease.

It has been proposed that the immunosuppressive effects of cigarette smoke could enhance the persistence of HPV infection and, in turn, increase the risk of HPV-related tumours.⁴³ Some indirect support for this hypothesis is derived from the observation that cigarette smoking seems to be more strongly linked with VIN 3 as opposed to invasive vulvar cancers. Furthermore, two studies have reported that the effect of HPV antibody seropositivity on vulvar cancer risk is greater among cigarette smokers than among non-smokers.^{9,24} As an interesting corollary, several studies have found a substantial degree of effect modification between genital warts and smoking.^{19,23} For example, compared with non-smokers with no genital warts, smokers with genital warts had a 51-fold greater risk of developing vulvar cancer, after adjustment for age, number of sexual partners, cigarette smoking and a history of an abnormal Papanicolaou smear.⁹

Data on the role of cigarette smoking in non-HPV-related vulvar cancers are limited and conflicting. Trimble and colleagues¹³ reported that ever smoking was associated with age-adjusted RRs of 4.9 (95%CI = 1.7–14.3) and 12.3 (95%CI = 1.5–101) for VIN 3 and BWC, respectively. By contrast, the age-adjusted RR for KSC, the histological type presumed to be unrelated to HPV, was 0.26 (95%CI = 0.1–0.8). Madeleine and colleagues⁹ found that cigarette smoking was somewhat more strongly associated with HPV-DNA-positive than with HPV-DNA-negative tumours, but it still appeared to be an important risk factor for both tumour types, suggesting that cigarette smoking may play a role in HPV-related and non-HPV-related tumours.

OBESITY

Several case-control studies have examined the relationship between obesity and vulvar cancer risk. In a study restricted to invasive vulvar cancer, an age-adjusted RR of 2.3 (95%CI = 1.1–4.5) was observed among women with a body mass index (BMI, kg/m²) of 30 or more compared with women with one of less than 25.²² In another study,⁴⁴ an elevated risk of invasive disease (RR = 2.9; 95%CI = 1.5–5.8) but not VIN 3 (RR = 1.0; 95%CI = 0.7–1.5) was observed among women in the highest versus those in the lowest category of BMI. These analyses were adjusted for age, number of sexual partners, smoking, education and history of genital warts. Newcomb and colleagues²⁰ also found no association between weight at age 30 and risk of VIN 3 but heavier women, after adjustment for height, had a slightly higher risk of invasive cancer. Brinton and colleagues¹⁹ reported that obesity was unrelated to risk of vulvar cancer (RR = 1.2 for BMI ≥ 25 versus < 21). A lack of association between weight at age 20 and 40 and vulvar cancer risk was also reported in another case-control study.²¹ Risk estimates from the last two studies relate to a mixture of VIN 3 and invasive vulvar cancer cases. Overall, the data suggest, but do not conclusively demonstrate, that obesity may be a risk factor for invasive carcinoma but not for VIN 3. This pattern of findings has the potential to reveal an aetiological clue for non-HPV-related vulvar cancers. It is possible that chronic vulvar dermatitis involving the genital skinfolds of overweight women may be important. Other possible factors that may be involved include hyperinsulinaemia or insulin-like growth factors, which have been related to breast cancer in some studies.⁴⁵

REPRODUCTIVE FACTORS

Although multiparity has been linked with cervical cancer risk,^{40,41} there is little evidence that various reproductive factors are involved in the aetiology of vulvar cancer. In one study, multiparity was associated with an increased risk mainly of VIN 3,²⁰ but this finding was not replicated in another study.⁴⁴ Other studies have reported either an inverse association²¹ or no association between parity and vulvar cancer.^{19,22,23,44} Other reproductive variables, including age at first live birth, age at menarche and age at menopause, have

also not been convincingly linked with vulvar cancer risk.^{19–22,44}

EXOGENOUS HORMONES

Some experimental evidence suggests that hormones may enhance viral wart infections⁴⁶ and mediate the malignant transformation of HPV-infected cells.⁴⁷ There is some evidence that prolonged use of oral contraceptives may increase the risk of cervical cancer.³⁷ Newcomb and colleagues²⁰ reported a fourfold increase in risk associated with ever-use of oral contraceptives for VIN 3. These analyses were adjusted only for age, education and obesity. Sherman and colleagues⁴⁴ reported a slight increase in risk of VIN 3 associated with 5 or more years of oral contraceptive use (RR = 1.3), but an inverse association with invasive disease. These analyses were adjusted for age, number of sexual partners, smoking, education and a history of genital warts. Brinton and colleagues¹⁹ also found a slight increase in risk of vulvar cancer with increasing years of use, rising to 1.3 among women who used oral contraceptives for 10 or more years. These analyses were adjusted for age, number of sexual partners, previous abnormal Papanicolaou smear, history of genital warts and current cigarette smoking. Menopausal oestrogens, with or without concomitant progestins, have not been associated with vulvar cancer risk, but few studies included women with extensive usage.^{19,20}

MEDICAL HISTORY

Women with VIN 3 and invasive vulvar cancer are substantially more likely to have a history of anogenital tumours than women in the general population.⁴⁸ A number of other disorders have been linked with vulvar cancer clinically, but most of these associations have not been confirmed in epidemiological investigations. The association most deserving of additional attention is a possible link between diabetes and vulvar cancer risk. O'Mara and colleagues⁴⁹ reported a positive association between diabetes mellitus and a combined category of cancers of the vulva and vagina, after adjustment for age and obesity. The greatest risk was observed among women diagnosed with diabetes before the age of 29 years. In the case-control study by Newcomb and colleagues,²⁰ a history of diabetes was

associated with an almost eightfold increase of invasive vulvar cancer, after controlling for age, education and obesity. By contrast, a history of diabetes was unrelated to risk of VIN 3. A history of diabetes was associated with a non-significant 1.3-fold increase in risk in the study by Brinton and colleagues.¹⁹ This analysis was adjusted for age, education, number of sexual partners, oral contraceptive use and a prior abnormal Papanicolaou smear. In a study conducted in Israel, Voliovitch and colleagues⁵⁰ reported that the frequency of vulvar cancer patients with a history of diabetes was higher than in the general Jewish population of the same age range. Although Mabuchi and colleagues²¹ observed no association between a history of diabetes and vulvar cancer risk, a limitation of this study is the use of hospitalized controls. Further exploration is needed on the possible role of chronic candidiasis with inflammation and the potential role of different types of diabetes in risk.

Most epidemiological studies have not found associations with hypertension,^{19,21} gallbladder disease¹⁹ or thyroid disease.^{19,20} Newcomb and colleagues²⁰ reported a small non-significant association between hypertension and invasive disease but no association with VIN 3. In a retrospective cohort study of 3000 patients who had undergone cosmetic augmentation mammoplasty,⁵¹ five vulvar cancers were observed where only one was expected. This finding may reflect various characteristics of the population compared with the general population, including number of sexual partners and cigarette smoking.

PERSONAL HYGIENE

At present there is no evidence that poor personal hygiene plays a role in the development of vulvar cancer. It has been reported that vulvar cancer is uncommon among orthodox Moslem women who wash after the acts of micturition and elimination.⁵² However, the case-control study by Brinton and colleagues showed no relationship between risk and various hygiene factors, including number of times bathed per week, use of vaginal douches, use of vaginal deodorants and tampon use.¹⁹

DIET

Decreased consumption of fruit and vegetables has been linked with increased risk of various epithelial tumours, including cancer of the cervix.⁵³ Two studies

have examined the relationship between dietary factors and risk of vulvar cancer. In the study by Sturgeon and colleagues,⁵⁴ the risk decreased with increasing consumption of dark yellow-orange vegetables, but the risk was unrelated to intake of dark-green vegetables, citrus fruits, legumes, and vitamins A and C and folate. Risk increased modestly with decreased intake of dark yellow-orange vegetables. Analyses were adjusted for age, cigarette smoking and number of sexual partners. Parazzini and colleagues⁵⁵ also found that the risk of invasive vulvar cancer was inversely related to green vegetable and carrot consumption, after adjustment for age, education and BMI.

Coffee drinkers were at increased risk of vulvar cancer in one study.¹⁹ In another study,⁵⁴ however, the effect of coffee was modest and there were irregular changes in risk with increased frequency of coffee intake, after adjustment for age, cigarette smoking and number of sexual partners. Parazzini and colleagues⁵⁵ also found no association between coffee and vulvar cancer risk, after adjustment for age, education and BMI. Neither alcohol nor intake of specific types of alcoholic beverages has been shown to be associated with vulvar cancer risk.^{54,55}

OCCUPATIONAL HISTORY AND CHEMICAL EXPOSURES

An increased risk of vulvar cancer has been found among maids and servants in private households and women employed in laundry, cleaning and other garment services.²¹ It is possible, however, that these associations reflect confounding by sexual behaviour. There are also several case reports linking vulvar cancer to oil-saturated waste in cotton-mill workers⁵⁶ and to arsenic compounds on a tobacco farm.⁵⁷ Hennekens and colleagues⁵⁸ reported that the risk of developing vaginal/vulvar cancer was elevated among nurses in the USA who used permanent hair dyes, but adjustment was made only for age and cigarette smoking.

IMMUNOSUPPRESSION

Consistent with the presumption that immune impairment is involved in the acquisition or maintenance of HPV infection, numerous case-control and cohort studies have suggested a higher incidence of

CIN among transplant recipients than in the general population.³¹ Data for vulvar cancer are much more sparse, but two cohort studies of organ transplant recipients have reported surprisingly high risks of vulvar cancer (observed to expected ratio or O:E = 56) or vulvar and vaginal cancer combined (O:E = 31).^{59,60} By contrast, only four- to ninefold increases in cervical cancer risk were observed in these two studies. In another study, women with systemic lupus erythematosus, an autoimmune disease of unknown aetiology, were more likely to develop vulvar/vaginal cancer.⁶¹ There are also various reports in the literature of VIN and invasive vulvar cancer among HIV-infected individuals, but the potential for confounding by sexual behaviour precludes any causal association with immune impairment.^{62,63}

As HPV infection is very common in sexually active populations and major immunosuppressive states have been linked to increased risk of vulvar cancer, it is likely that host immune response to HPV is an important predictor of the risk of development of vulvar cancer. The importance of the host immune response has been demonstrated in several recent studies of cervical cancer.^{64,65}

IONIZING RADIATION

Several studies have been performed to determine whether there is an increased risk of developing a second cancer in the genital area after pelvic irradiation. In two large multinational studies, each involving more than 150 000 cervical cancer patients, there was no evidence that radiotherapy increased the risk of vulvar cancer.^{66,67} A subsequent study by Kleinerman and colleagues,⁶⁸ involving approximately 86 000 patients with cervical cancer, also found that vulvar cancer risk was similarly increased in irradiated (O:E = 4.4) and non-irradiated women (O:E = 3.5). However, the observation that risk tended to increase with increasing years since radiotherapy led the authors to speculate that radiotherapy may play a role in the development of vulvar cancer.

SCREENING

In several studies,^{19,22} a history of prior Papanicolaou smears has been associated with a decreased risk of invasive vulvar cancer. As women participating in Pap

smear programmes are likely to undergo physical pelvic examinations, it is possible that the protective effect is the result of the detection and treatment of cancer precursors.

SECOND PRIMARIES

Women with vulvar cancer have been shown to be at elevated risk of developing second primaries related to cigarette smoking, including cancers of the lung, oesophagus, buccal cavity and pharynx, and nasal cavity and larynx. Sturgeon and colleagues⁶⁹ reported that women with VIN 3 were 2.8 times more likely to develop smoking-related cancers than women in the general population. Women with invasive vulvar carcinoma had a 1.6-fold increased risk of developing smoking-related tumours. These data are consistent with epidemiological studies that suggest that cigarette smoking may be involved in vulvar cancer, particularly VIN 3.

Women with VIN 3 and invasive vulvar cancer have been found to be at increased risk of cancers of the cervix, vagina and anus.⁶⁹ This observation is consistent with the role of HPV infection in the development of several anogenital tumours. Sturgeon and colleagues⁶⁹ also found that women with VIN 3 and invasive vulvar cancer were also at increased risk of developing non-Hodgkin's lymphoma. As the women in whom non-Hodgkin's disease developed were elderly in this study, it seems unlikely that they were infected with HIV.

EPIDEMIOLOGICAL CORRELATES OF *p53* GENE MUTATION AND EXPRESSION

Most clinicopathological studies have demonstrated that *p53* abnormalities are uncommon in VIN. Kagie and colleagues⁷⁰ reported that *p53* was immunohistochemically detectable in 13–18 per cent of VIN lesions of varying grade associated with carcinomas compared with 13 per cent of tissues removed from normal women. Similarly, Kohlberger and colleagues⁷¹ did not detect *p53* with immunohistochemistry in 28 VIN lesions not associated with carcinoma. HPV DNA was detected in 92.8 per cent of these cases. Kurvinen and colleagues⁷² also did not identify *p53* mutations in exons 5 to 9 among eight cases of VIN or vulvar cancer tested with a single-strand conformation polymor-

phism analysis and DNA sequencing. In contrast, Milde-Langosch and colleagues⁸ identified *p53* mutations and/or abnormal expression in VIN associated with carcinomas harbouring *p53* mutations. In summary, most data suggest that *p53* mutations are not related to VIN, a lesion that is linked to HPV infection.

Analysis of invasive carcinomas has also demonstrated that *p53* abnormalities are uncommon in HPV-related carcinomas. Lee and colleagues⁷³ detected a *p53* mutation in only 1 of 12 HPV-associated carcinomas, compared with four (44.4 per cent) of nine tumours that tested negative for HPV. One tumour contained HPV DNA and showed a *p53* mutation. Milde-Langosch and colleagues⁸ reported that 52.5 per cent of vulvar carcinomas were associated with a *p53* mutation, compared with 7.8 per cent of cervical cancers. In this study, HPV was detected in 80.4 per cent of cervical cancers and only 27.5 per cent of vulvar cancers; however, *p53* mutations were not detected more commonly in HPV-negative as opposed to HPV-positive vulvar tumours. The authors noted that *p53* immunohistochemistry results and gene mutation analyses were not closely correlated. In contrast, Pilotti and colleagues⁷⁴ reported that HPV-related carcinomas contained wild-type *p53* as assessed with immunohistochemistry and molecular techniques, whereas 75 per cent of HPV-negative tumours showed *p53* mutations or expression.

In summary, data from several studies indicate that HPV-related VIN and cancers usually contain wild-type *p53*, whereas lesions unrelated to HPV are associated with *p53* abnormalities in a variable, but relatively small, percentage of cases. These data suggest two different aetiologies of vulvar carcinoma that share a common endpoint: inactivation of *p53* function. In HPV-related tumours, this is most probably accomplished through binding to HPV-E6 protein, with degradation via a ubiquitin-dependent pathway. In tumours unrelated to HPV, mutation leading to loss of *p53* function seems to be frequently involved. The molecular basis for vulvar oncogenesis is explored in more detail in Chapter 4.

Recently, Storey and colleagues⁷⁵ reported that a polymorphism in codon 72 of the *p53* gene renders the protein product more susceptible to degradation by the HPV oncogene E6, and confers an increased risk of cervical cancer compared with other *p53* alleles. If confirmed in large epidemiological studies of cervical cancer, this finding would suggest that the risk of HPV-related vulvar cancer might also involve a genetic component.

SQUAMOUS HYPERPLASIA

Kim and colleagues⁷⁶ studied the clonality of four invasive carcinomas associated with *p53* mutations and multiple adjacent areas of normal epithelium and squamous hyperplasia. The authors found that all three informative cancers were monoclonal with the assay used, whereas the adjacent normal and hyperplastic tissues were polyclonal and did not contain detectable *p53* mutations. The authors concluded that either *p53* mutation is a late event in vulvar carcinogenesis or squamous hyperplasia is not a precursor of these tumours. In contrast, Lin and colleagues⁷⁷ demonstrated that loss of heterozygosity was detectable in both squamous hyperplasia and atypical squamous hyperplasia (differentiated VIN), and that the pattern in the latter overlapped with an associated invasive carcinoma. Another study from the same laboratory⁷⁸ demonstrated monoclonality in six of eight evaluable hyperplasias, one of which was associated with lichen sclerosis. These authors suggested that hyperplasias deserve consideration as possible cancer precursors.

The relationship between lichen sclerosis and vulvar carcinoma has been a subject of controversy, but most studies suggest that the neoplastic potential of lichen sclerosis is minimal. The relationship between lichen sclerosis and other 'benign' maturation disorders and possible carcinogenesis is explored in detail in Chapter 6.

In summary, VIN is the likely precursor of HPV-related vulvar cancers, but the precursor(s) of vulvar carcinomas that are unrelated to HPV is unknown. Data concerning the neoplastic potential of atypical squamous hyperplasia are sparse and not derived from population-based studies. The association between lichen sclerosis and carcinoma is tenuous. Furthermore, the diagnostic reproducibility of non-neoplastic vulvar disease has not been established.

FUTURE DIRECTIONS

Human papillomavirus is a major risk factor for vulvar cancer but there is compelling evidence that some forms of vulvar cancer are non-HPV related. Pathologists have proposed a dualistic model of carcinogenesis based on histopathological classifications of vulvar cancer. In this model, BWCs are HPV-related tumours that arise from VIN 3. As in the cervix, it is postulated

that the E6 and E7 HPV oncoproteins contribute to the development of these tumours by degrading the key cell cycle regulatory proteins p53 and retinoblastoma gene product. By contrast, KSCs are hypothesized to be unrelated to HPV. In these non-HPV-related tumours, p53 mutation may play a role. Using large population-based studies, further research will be required to confirm this hypothesis. It is acknowledged that the rarity of vulvar cancer, and methodological difficulties related to the measurement of exposure to HPV infection, complicate the epidemiological study of this tumour. Additional research should also consider the possible role of cigarette smoking, other sexually transmitted infections, diabetes, obesity, exogenous hormones and immune response.

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