Human Papillomavirus Infection and Cancer Risk in Peri- and Postmenopausal Women

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4.1 Introduction

The most commonly acknowledged complication of human papillomavirus (HPV) infection is invasive cervical cancer (ICC), to which this infection is a *sine qua non* condition. However, the burden of disease associated with HPV is much higher than that of ICC and its precursor lesions (high-grade squamous intraepithelial lesions [HSIL] encompassing cervical intraepithelial neoplasia [CIN] 2 and 3) and not limited to women. Besides benign diseases, even if distressing (genital warts, recurrent respiratory papillomatosis, low-grade squamous intraepithelial neoplasia [LSIL]), HPV infection is regarded as a necessary cause of a variety of preinvasive/invasive lesions of the vulva and vagina, penis, anus/perianus, and oral cavity and oropharynx [1].

The risk of cancer and of squamous intraepithelial lesions is related to the HPV genotype and infection persistence. However, it is still up to debate if a persistent infection implies continuous detection of the virus or can include latent periods—translating into a very relative definition of "transient" and "persistent" infection [2, 3]. HPV is the most prevalent sexually transmitted infection (STI) in the world, with more than 80% of the sexually active women being infected during their lifetime with at least one genotype [4]—a situation that will definitely change in the future, as the effects of HPV vaccination start to be noticed. Several factors must interplay,

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as only a minority of infected people develops disease (HPV genotype, age, smoking, other STIs, vaginal microbiome, immunosuppression, hormonal and genetic factors, etc.).

While HPV infection is more common in younger women, the serious complications tend to occur at later ages (in the peri- or postmenopause). According to that, politics of cervical cancer/precancer screening and treatment tend to take that into account and ponder the risk of being too aggressive (starting screening at young age, treating CIN2 in women younger than 30 years old), with serious impact on obstetrical outcomes (for instance, preterm labor or premature rupture of membranes) against that of missing or not treating significant disease.

As we start anticipating the end of screening, derived from massive vaccination [5], it must not be forgotten that, even in developed countries, there will be extensive cohorts of unvaccinated women for three or four decades more, which cannot be neglected by the euphoria of the anticipated elimination of ICC.

Some older women may consider they are not at risk at this stage, as they already had several screening tests or because they have lost their partners or are in a monogamous relationship.

4.2 HPV Epidemiology

Despite the significant geographical variations, global prevalence of cervical high-risk HPV in young women can be as high as 50% in the early twenties, dropping to about 10–15% after the age of 25–30 [6]. After that, in developed countries, there is a slight decrease until the fifth or sixth decades of life, when a new peak is encountered [7–9]. This peak may be even more pronounced for non-oncogenic (low-risk) HPV genotypes, which may be explained, in part, by its higher tropism for the mature squamous epithelial cells, rather than for the transformation zone (TZ) [10]. In less developed countries, the prevalence tends to be higher, and the second peak is typically absent [11]. One study has clearly shown that the risk of having a positive HPV test, 3 years after a negative one, is similar between women aged 51 years and younger ones [12].

The prevalence of HPV16 and HPV18 seems to be stable after the age of 35 years old, but, at least in some countries, they are responsible for significantly less cervical high-grade lesions than in premenopausal women; less aggressive genotypes seem to play a more important role in older women [13, 14].

While the first peak, in young women, can be attributed to the onset of sexual debut, the second one may be due to new sexual partners (especially after divorce or widowing), "Viagra effect," and hormonal milieu (and consequent associated microbiome changes) or just a consequence of a waning of the immune system (immunosenescence). In the second peak, besides newly acquired infections, there may be a role for reactivation of older, latent infections. Having had two or more sexual partners, after a negative HPV test, in postmenopausal women, was associated with an odds ratio of 3.9 (95% CI = 1.2–12.4) of having a subsequent positive test—highlighting the role of acquisition of new infections for the second peak of prevalence of HPV infection. In

this same study, having had two or more sexual partners in the past was associated with a 1.7-fold (95% CI 1.1–2.5) higher likelihood of having a positive HPV test later in life [10]. In a study by Gravitt et al [9], it was found that women with a higher number of sexual partners (≥5) during their lives were at a greater risk of being HPV positive later in life—thus supporting the hypothesis of reactivations of HPV infections. Women entering now the menopause lived their youth after the sexual revolution and, thus, are likely to have had more sexual partners than the previous generation. This may translate into higher rates of HPV infections and cervical intraepithelial neoplasia than what has been usually encountered in postmenopausal women.

As we have previously discussed, there is now enough evidence that previous infections can be reactivated, thus women cannot discontinue screening, even in the absence of a (new) sexual partner [15]. This concept is important and may be worth explaining to patients, as it can help, in some cases, to overcome suspicion of infidelity or feelings of guilt. On the same way, it can be explained that a negative test is not always synonymous of absence of the virus but rather that the viral load is below the defined risk threshold.

The risk of reactivation of HPV at older ages, associated with a lower performance of diagnostic tests (discussed ahead), has led some authors to theorize that in well-screened populations, this will translate into a proportional increase in the diagnosis of ICC, rather than HSIL [9].

4.3 The Interaction of Aging, Hormones, Vaginal Microbiome, and HPV

In general, 90% of HPV infections clear within a year. There are contradictory data concerning clearance of HPV in older women, but apparently, the time for clearance of new HPV infections does not seem to be increased [8, 10, 16]. On the contrary, regression of cervical intraepithelial neoplasia seems to decrease with the advance of age: a recent meta-analysis has shown that for every 5 years of age, the odds for regression decrease 21%, while those of progression increase. These tendencies were shown to be independent of the grade of the lesion and the presence or not of a high-risk HPV infection ($p < 0.001$) [17]. Adding to the effect of age on the immune system, there is some evidence that the hormonal changes of menopause, namely, estrogen deprivation, can also contribute to the attenuation of the immune response and an increase in the inflammatory markers [18, 19]. Despite this, the CD4 T-lymphocytes population, which has a key role in the regression of intraepithelial lesions, along with CD8+ and CD56+ macrophages, is diminished. The same is true for B lymphocytes (which contributes to explain the lower efficacy of vaccines in older women), as well as for the activity of NK cells [20–22]. It has been demonstrated that women with a lower immunological response to a challenge with HPV16 virus-like particles are at higher risk of developing an HPV infection [15].

Despite the immunosuppression associated with age, the risk of HSIL associated with a newly identified positive HPV test does not seem to be higher in older women [23].

Several studies have associated the use of combined oral contraceptives with increased risk of HSIL and ICC, assumed to be due to transactivation of viral oncogenes [24]. Postmenopausal hormone therapy has not been associated with increased prevalence of HPV nor with increased viral replication, despite a potential role in modulating the immune response [20, 25]. The EPIC cohort study found a decreased risk of ICC in women who ever used menopausal hormone therapy (HT) (HR = 0.5, 95%CI 0.4–0.8); longer duration of its use also had a trend toward lower risk [26]. The interpretation of these findings is not straightforward, as women who took HT are more likely to have attended more routine gynecological appointments and, thus, more often screened and treated for cervical cancer precursors.

The unopposed use of estrogen was associated with a higher risk of CIN3/CIS [26] and HPV infection [27]. There is evidence that estrogen receptors α (ERα) are essential in the initiation of the invasive process. This led to the theory that selective estrogen receptor modulators (SERMs), namely, raloxifene, can play a role in the prevention of the progression of HSIL to invasive cancer and even that it can be used as an adjuvant treatment of ICC [24, 28].

In one observational study, however, the use of intravaginal estrogen cream, isolated or associated with other modalities of treatment, was associated with rates of regression of vaginal intraepithelial neoplasia (VaIN) [29]. It should be emphasized that its use is usually recommended before other treatments, to increase the thickness and elasticity of the vaginal mucosa.

Given the current knowledge, HPV infection or cervical dysplasia should not affect the decision to start or discontinue menopause HT, contrary to what has been previously suggested [30].

In recent years, a staggering amount of information is being gathered showing that vaginal flora or microbiome plays a key role in the acquisition and persistence of HPV infection, as well as on the development and persistence of CIN. We are, however, far from the full understanding of the picture.

Increased vaginal pH, a surrogate for abnormal vaginal flora (AVF) (absent/severely depleted number of lactobacilli—usually bacterial vaginosis [BV] or aerobic vaginitis [AV]) has been associated with a 30% increased risk of HPV infection and abnormal Pap test, especially in women younger than 35 or older than 65 years [31]. Data linking BV and mixed flora (most likely AV) to persistence of HPV infection has led some authors to recommend its screening and treatment, even if asymptomatic [32, 33]. BV is encountered more frequently in women with a Pap test worse than LSIL (20.5% vs. 13.2%, $p = 0.09$); moderate/severe forms of AV are significantly more common in women with these results (16.9% vs. 7.2%, $p = 0.009$) [33].

Given that the prevalence of BV seems to steadily increase after menopause, and persistence/recurrence seems to be very common, this can be another factor posing these women at risk for HPV acquisition, persistence, or reactivation, as well as development and progression of intraepithelial neoplasia [34]. It is, however, indissociable from the complex relation between vaginal microbiome and hormones.

Recently, it has even been shown that 3 months after loop electrosurgical excision procedure (LEEP) for HSIL, there is a decrease in species diversity and that vaginal microbiome shifts from *Prevotella*, *Leptotrichia*, and *Clostridium* to a *L. iners* dominance [35].

4.4 Vaccination

The anti-HPV vaccines have been proven to be highly efficacious in the prevention of the infection, genital warts, abnormal Pap tests, and the development of intraepithelial neoplasia and adenocarcinoma in situ (AIS). Although a significant impact is also expected to be seen in terms of cervical cancer (carcinoma and adenocarcinoma), there are not yet studies with enough duration to prove it [36].

Most studies have focused on the impact of the vaccines in women under the age of 26 years old. The few studies enrolling older women (>25 years old) demonstrated that the efficacy is lower than in younger ones. A recent Cochrane review concluded that in women 24–45 years old, the rate of CIN2+ associated with HPV16/18 and any CIN2 is equivalent between vaccinated and unvaccinated women (RR 0.74 [0.52 to 1.05] and RR 1.04 [0.83 to 1.30], respectively); no conclusions could be drawn for CIN3 or AIS. If women are selected according to HPV16/18 status, those who are DNA negative still benefited from the vaccine (RR 0.30 [0.11 to 0.81]) for CIN2 associated with these specific genotypes [36].

Previous exposure to HPV and a lower immunological response can explain the decrease in vaccine impact. It is still not proven that newly diagnosed HPV infections in older women are indeed associated with a significant increase of relevant disease [10].

The available data does not allow recommending systematic vaccination of postmenopausal women. However, to those who request it, it is reasonable to offer the vaccine, as the existing data are reassuring in terms of safety and the potential benefits seem to last at least for 10 years [37]. Some countries have not licensed the vaccine for women older than 26 (i.e., USA) or 45 years old (i.e., Canada), thus making its use beyond these ages off-label eventhough reasonable [38].

Three doses of vaccine should be administred, as in any individual older than 15 years old.

The vaccines have proven to decrease the risk of vulvar and vaginal HSIL, when given to young girls, an effect that is expected to lead to significant decrease of these diseases in the future. The impact of vaccination of older women in these conditions remains unknown [39]. There is no clinical evidence that HPV vaccination will lower the number of head and neck cancers. However, salivary antibodies have been identified in the majority of people following vaccination [40, 41].

4.5 Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer

Cervical cancer (Fig. 4.1) is the seventh most common cancer among women in developed countries and the second one in developing countries. Among the 527,000 estimated new cases per year, 444,500 occur where the efficacious preventive tools (screening, vaccines, treatment of HSIL) are still lacking. This cancer is responsible for the death of 230,000 women die every year [42]. This, however, is expected to change if massive vaccination of girls is implemented in those countries—even in the absence of well-organized screening programs [43, 44]. Several low-income countries have already shown that high-coverage vaccination is feasible [45].

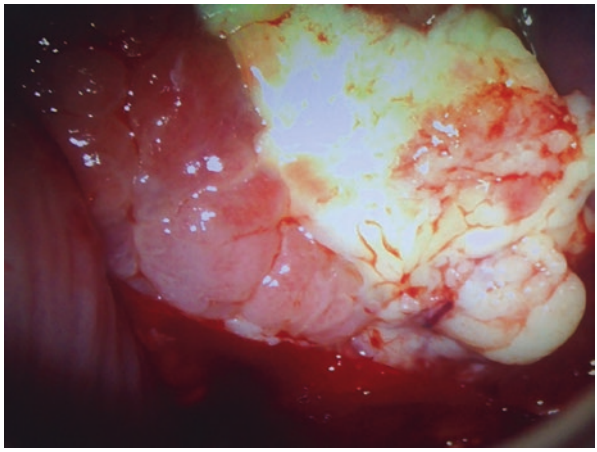

Fig. 4.1 Locally advanced cervical cancer

In countries without an effective screening program, the incidence of cervical cancer has a sudden rise in the perimenopause years; after that, the incidence keeps rising with advancing age. In those countries with effective screening, the distribution is usually bimodal: one peak around 35 years old and a latter one around 65 years old [13, 46] (Table 4.2).

In less developed countries, as the toll of deaths attributable to other infectious diseases reduces (namely, with the available treatments for human immunodeficiency virus [HIV]), the number of cervical cancers in older women is expected to increase.

4.5.1 Particularities of Screening

The aging of western countries' population will lead to a substantial number of screened women for cervical cancer being postmenopausal. On the other hand, with mass anti-HPV vaccination, disease will become less prevalent in younger women, leading to postponing of the age of beginning of the screening programs. In practical terms, during the next years, there will be an apparent shift of the peak of HSIL [47].

Primary high risk (HR)HPV test for screening is widely acknowledged as the best way to perform cervical cancer screening, in women older than 25–30 years old. A few studies have evaluated specifically how Pap test compares to HR-HPV test in postmenopausal women. The increased rate of parabasal cells, with a higher nucleus-cytoplasm ratio, along with more or less severe inflammation, partially explains the increased difficulty in the assessment of the Pap test in hypoestrogenic women. A Swedish study has shown that screening with Pap test alone misses more than half of the high-grade lesions detected using HR-HPV test [14]. On the other hand, as the positive predictive value of the Pap test seems to be lower in older women, some authors consider that triage with HR-HPV tests of ASC-H and even HSIL Pap tests can be a useful approach in this age group [48, 49]. Besides the

added difficulties for Pap test in postmenopausal women (atrophy, inflammation, regression of the transformation zone to the endocervix [type 3 transformation zone] [50, 51]), it must be taken into account that the Pap test is highly operator dependent—in other settings the results could have been dramatically different [52]. A short course of intravaginal estrogens can improve the performance of the Pap test [53].

Postmenopausal women are more likely to have Pap test without representation of the transformation zone and/or the glandular epithelium. While some clinicians feel uncomfortable with these results, studies have shown that as long as it was classified as negative (NILM), there is no increased risk of missing disease. In the case of absence of representation of the transformation zone, it is, however, preferable to perform an HR-HPV test. The result of this test is not at all influenced by the lack of representation of the transformation zone [54–56].

Cuzick et al., in 2013, presented data showing that HPV triage of LSIL Pap test is an effective measure to avoid unnecessary referrals for colposcopy. After the age of 40, only half these tests are HR-HPV positive; HR-HPV negativity successfully predicted the absence of HSIL [55, 57]. Employing triage of LSIL Pap tests in postmenopausal women can safely reduce to half the number of colposcopies, which is highly significant, given the limitations of this exam in this population. Additionally, in case of positivity, a better risk stratification can be made. The cases that test negative should be reassessed at 12 months, and if either one or both of the tests (Pap test or the HR-HPV test) are positive, the patient should be referred for colposcopy [55].

There is no consensus among different societies on the age to discontinue screening. Most societies recommend, for previously well-screened women, without history of HSIL (CIN2/CIN3) or adenocarcinoma *in situ*, to discontinue it at 65 years old [55, 58]. Some women, however, will be uncomfortable with that or will feel that the system is giving up on them, because of their age. In opportunistic (personalized) screening, the benefits of continuing the screening must be considered and discussed with the woman. A Pap test ASC-US, even if the HPV test is negative, does not allow stopping the screening [55]. Interestingly, there are data suggesting that maintaining screening up to the age of 79 years old could lead to a reduction in ICC of 77–79% in the USA [59]. It has also been shown that long-term survival is higher, also in older women, if the diagnosis of ICC is made in the sequence of a screening test, rather than a clinical diagnosis [60]. These issues must be taken into account, especially with the increase in life expectancy.

According to ASCCP and ACOG guidelines, women with a previous diagnosis of HSIL or AIS should continue routine screening for 20 years after the treatment or regression of the lesion, independently of the age at which it occurred and the presence or not of the cervix [55, 58].

4.5.2 Colposcopy and Treatment of Lesions

Colposcopy is a true challenge in postmenopausal women. Lack of estrogens leads to thinning of the vaginal and cervical mucosa and capillary fragility (Figs. 4.2 and 4.3)

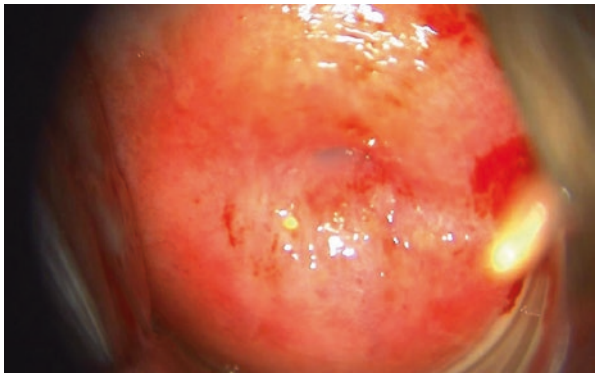

Fig. 4.2 Colposcopy of a postmenopausal woman, before the application of acetic acid. Note the presence of petechiae and easy bleeding upon touch

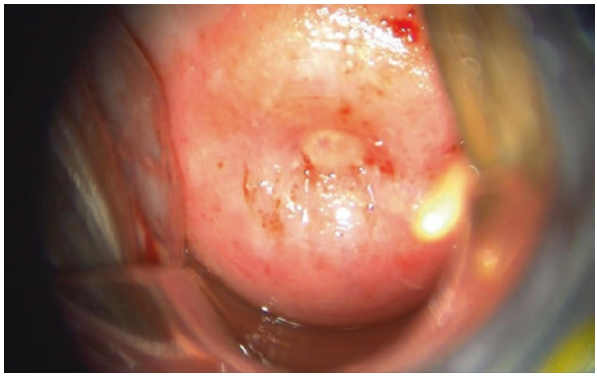

Fig. 4.3 Same woman as in Fig. 4.2, after the application of acetic acid

that can lead to discomfort/pain during sexual intercourse and gynecological examination and easy bleeding upon speculum placement.

As previously referred, it is uncommon to find an active TZ readily visible in the ectocervix in these women; the rate of poor visibility colposcopies is thus higher. Up to 44% of the CIN lesions are endocervical in postmenopausal women—and, in accordance, the rate of positive endocervical curettages is also higher in this age group [61, 62]. There are limited and sometimes contradictory data on the role of misoprostol or vaginal estrogens to reduce the number of inadequate colposcopies in hypoestrogenic women. Short courses (3–6 weeks) of intravaginal estrogens seem to be more efficacious and associated with a lower rate of adverse effects [53, 63–66].

The role of endocervical sampling and the best way to perform it are controversial. There is evidence that the use of endocervical brushes has a similar sensitivity and specificity to that of curettage (Fig. 4.4). The former is associated with less discomfort and lower number of insufficient samples (0–7.6% vs. 0–22%), but, on the other hand, grading of the lesions is more difficult, due to the lack of an organized tissue sample [67–69]. The role of this procedure is debatable, with studies finding it to be positive in the range of 1.4–17.9% in women with a good visibility

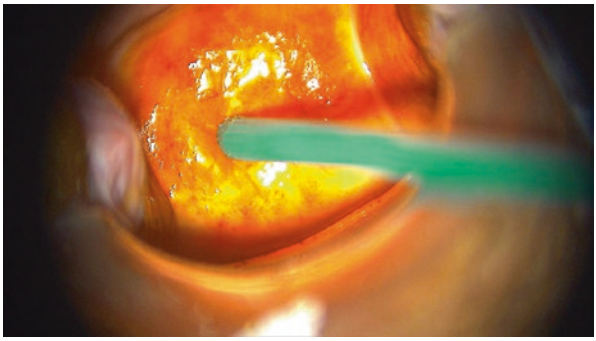

Fig. 4.4 Sampling of the endocervix with a cervical brush, due to nonvisible squamous columnar transition. Notice the diffuse lack of staining with iodine solution

of the TZ and up to 57.3% if visibility is poor. Most studies do not favor its use if the squamous columnar junction is fully visible [69]. It has also been reported that endocervical curettage can miss 45% of cases of HSIL while having a 25% false-positive rate [70]. Its role in the study of glandular abnormalities is less debatable, even though performance may be below the desirable [69].

The lack of an active TZ and the fact that the epithelium is usually thinner (Fig. 4.3) imply that any acetowhite lesion must be valued in this age group. Some lesions are not apparent without previous estrogen treatment. Sometimes, in women taking oral or transdermal HT, that may not be enough for proper vaginal epithelium maturation and topical treatment may be needed for a proper valuation of the lesions.

The use of Lugol's iodine solution (Schiller's test) is of more limited value in postmenopausal women, especially in those without estrogen HT: the lack of glycogen in the vaginal cells leads to a diffuse light brown to yellow coloration (Fig. 4.4) that can be mistaken as a negative iodine response.

Like in premenopausal women, excision of the transition zone (TZ) is the definite treatment for HSIL, significantly decreasing the risk of progression to ICC (estimated in some series to be as high as 40%, for untreated CIN3 [17]). The risk of failure of LEEP is usually inferior to 10% [71]. Ablation is usually not considered as an option in postmenopausal women, given the frequency of inadequate colposcopies and the significant rate of invasive disease only diagnosed with the excisional procedure (up to 4.3%) [72].

Usually, given the characteristics of the TZ, the height of the cone needs to be bigger than in premenopausal women [73]—and also there is no concern with the risk of excessive excision and obstetric outcomes. Even though, the likelihood of positive endocervical margins is higher in postmenopausal women [73, 74].

In case of positive margins, expectant management and a new excision of the TZ are the recommended options. Expectant management implies a compliant patient and, preferably, visible squamous columnar junction (even though this may be a minor issue if HR-HPV test is being used for follow-up and it is negative). A small study reported that after repeated excision of the TZ, the rate of residual disease can be as high as 52.3% [75]. However we can theorize that the rate of positivity probably decreases with time elapsed between the first and second procedure. Some

support the performance of hysterectomy, on the basis that residual disease is common when it is performed in postmenopausal women, following excision of the TZ, independently of the status of margins (67.6%) [76]. This, however, does not seem to correlate with daily practice. It must be kept in mind that long-term follow-up is recommended in women with history of HSIL, even after hysterectomy, because of the increased risk of multicentric HPV lesions [77].

In a systematic review and meta-analysis, Arbyn et al. found that the risk of CIN2+ after an excisional procedure was 0–8% if a follow-up HPV test was negative and 3–7% if margins were free [78]. These figures leave some room for expectant management in the presence of positive margins, especially if the SCJ is totally visible post-procedure.

In hypoestrogenic women, cervical stenosis after excision of the transformation zone is common, thus making the follow-up more complicated. The local use of estrogens may reduce this complication, with some supporting that it should be kept for at least 1 year after the procedure [79, 80]. Except for stenosis, the rate of complications seems to be comparable between pre- and postmenopausal women [73].

As in premenopausal women, hysterectomy should not be performed for isolated treatment/exclusion of HSIL. Besides being a much more complex procedure, with a much higher rate of associated complications, there is the risk of an undiagnosed invasive cancer (better managed with more radical surgery).

Follow-up after treatment of HSIL is better accomplished with HPV test, like in premenopausal women. It is useful to have knowledge of the HPV status prior to the treatment, namely, because of the uncommon cases of HSIL lesions that test negative for HPV—in this setting, a negative test would be a false reassurance. The American Society of Colposcopy and Cervical Pathology (ASCCP) recommends follow-up to be performed using co-testing (HPV and Pap test) at 12 and 24 months. After two consecutive negative tests, they recommend that the woman can be discharged for routine screening [55]. Some studies suggest that one single negative HR-HPV test after treatment, as early as 4–6 months, may be considered a test of cure: the risk of HSIL is similar to that of an HR-HPV-negative woman from the general population [81–83]. Earlier testing, however, may be associated with a higher positivity of tests, not necessarily significant. Older women (>67 years) appear to be at higher risk of subsequent CIN2+, probably deserving a more careful and longer follow-up [81].

A recent paper from the Dutch nationwide registry of histopathology and cytopathology found that the 89.018 women with a previous diagnosis of CIN3 had an increased risk of HR-HPV-associated high-grade lesions and carcinomas of the vulva, vagina, anus, and oropharynx when compared with 89.018 control subjects. In particular, the incidence rate ratios were higher for vulvar cancer, 13.66 (93% CI, 9.69 to 19.25); vulvar HSIL, 86.08 (95% CI, 11.98 to 618.08); and vaginal cancer, 25.65 (95% CI, 10.50 to 62.69).

As long-term follow-up still showed increased risk, new strategies for screening of HPV-related neoplasia, other than cervical ones, must be investigated, validated, and adopted in this increased risk group of patients [84].

4.6 Other Lesions

4.6.1 Vagina

High-grade vaginal intraepithelial neoplasia (VaIN) (VaIN2 and VaIN3) is considered the malignant precursor of vaginal cancer. It is usually asymptomatic and HPV-related, mostly with HPV16 (>50% of the cases) [85]. Although its incidence has been reported to be increasing in the last decades, especially in younger women, it is still a rare lesion—100 times less frequent than CIN [86, 87]. That increase, however, may not be real, but rather just translating better screening methods, more awareness, and higher compliance with screening guidelines (for instance, maintenance of follow-up in hysterectomized women with a diagnosis of HSIL). Screening for VaIN or vaginal cancer should not be kept in hysterectomized women, without a previous history of HSIL.

Vaginoscopy, independently of the presence or absence of the cervix, is more complicated than colposcopy, more time consuming, and associated with more discomfort. Previous treatment with topical estrogens is mandatory before performing vaginoscopy in postmenopausal women. Typical patterns of LSIL or HSIL are difficult to establish, implying that, in doubt, a biopsy is needed. VaIN is located in the upper third of the vagina 80% of the time and usually is multifocal [88]. Vaginoscopy should be performed by experienced colposcopists [89].

The risk of progression of vaginal HSIL to invasion is low (3%), and vigilance rather treatment seems to be a relatively safe option [90]. The use of vaginal imiquimod seems to be an efficacious and relatively safe choice, but further studies are needed before it can be assumed as a first-line option for treatment [91, 92]. However, independently of the chosen treatment modality, recurrence is very common, especially in women with multifocal disease [88].

Primary vaginal cancer is one of the rarest gynecologic malignancies, representing 2% of the total number of it. Its incidence is of around 7:1.000.000 women/year [93, 94]. At least 50% of the vaginal cancers of squamous origin are HPV-related, especially with HPV16; HPV18 seems to play a minor role in this neoplasia. A history of previous cervical cancer and/or pelvic irradiation is also common. Other studies have linked previous hysterectomy (in some series in up to 40% of women) and surgical menopause to this neoplasia [85, 93, 95]. Those with a VaIN history following a hysterectomy tend to be older [85].

4.6.2 Vulva

4.6.2.1 Warts

Genital warts or *condyloma acuminata* are not exclusively found in the vulva, but this location is the most common and is associated with higher levels of psychological suffering [96]. It affects less than 1% of the whole population. It is more prevalent in young people (peaking at the age of 20–24 years old) and rare in postmenopausal women [97–99].

Their presence is frequently asymptomatic but can also manifest by itching and burning or, less frequently, by bleeding. Most frequently, it presents as a cauliflower-like, skin-colored to pink tumor. Lesions are usually multiple and can involve multiple organs; it can measure a few millimeters or, especially in immunosuppressed patients, can measure several centimeters.

The use of acetic acid in the vulva to identify warts is controversial, as it is non-specific of HPV infection. Its use by practitioners who are not aware of this frequently leads to overdiagnosis and overtreatment [100], with potential psychological and physical consequences.

Most of the cases are caused by low oncogenic risk HPV genotypes—HPV6 and HPV11 are responsible for at least 80% of the cases. HPV16 can be involved in up to 10% of cases—which could partly explain the decrease in genital warts noticed in adolescents vaccinated with the bivalent vaccine [101, 102].

Especially in older women, a biopsy may be needed to exclude intraepithelial or invasive disease. Screening for other STIs should be performed, and investigation of immunosuppression should be considered in older women.

4.6.2.2 Vulvar Intraepithelial Neoplasia and Cancer

Vulvar cancer accounts for approximately 3–5% of all gynecological malignancies, with an incidence rate of 2.4 new cases per 100,000 women per year, and represents 0.4% of all new cancers in the USA [103]. The most common histology (almost 90%) is squamous cell carcinoma of the vulva. Other rare histotypes include melanoma, adenocarcinoma, invasive Paget disease, basal cell carcinoma, and sarcoma [104]. Similar incidence rates have been observed in western countries, following a slight preference for white race compared to black and Asian race [105]. Several authors have reported an increase in the overall incidence rate in many countries. Specifically, in Germany, Buttmann-Schweiger et al. observed a doubling in the incidence rate from 1.7 to 3.6 new cases per 100,000 women per year from 1999 to 2011 [106]. In Denmark, a 1.97% per year increase in the incidence rate between 1978 and 2007 has been reported [107]. This trend has been confirmed also in the USA: a 1.0% per year increase in the incidence between 1973 and 2004 [108, 109]. Some authors noticed that this increase has been more pronounced in women below 50 years. Especially in Germany and Denmark, but also in Australia, there was a significant 84% increase in patients below 60, with a substantial stable rate in those older than it; however in the USA, such a pattern was not noticed [106, 107, 109, 110].

These epidemiological data underline the importance of the ISSVD (International Society for the Study of Vulvovaginal Disease) in vulvar disease terminology [111]. Since its foundation in 1970, the ISSVD set the terminology of preneoplastic vulvar lesions as one of its missions, with dedicated committees composed by gynecologists, pathologists, dermatologists, and other specialists. It is clear from the different ISSVD vulvar disease's classifications that not only preneoplastic lesions must be distinguished from nonneoplastic ones, but also that in the former there are different origins (squamous and non-squamous epithelial origin). As a final point, among vulvar squamous intraepithelial lesions (VSIL), ISSVD always underlined the two different precursor lesions of invasive squamous cell carcinoma of the vulva [112].

The most recent ISSVD classification has been published accordingly with these points: VHSIL (vulvar high-grade squamous intraepithelial lesion) is the precursor of HPV-related vulvar squamous cell carcinomas (VSCC), and differentiated VIN (vulvar intraepithelial lesion) is the precursor of non-HPV-related VSCC [113] (Table 4.1). The VSIL classification includes VLSIL (vulvar low-grade squamous intraepithelial lesion), and it clearly underlines that it includes flat condyloma or HPV effect and it is not precancerous and does not need to be treated, unless symptomatic.

The two types of VSCC and its precursors differ in epidemiology, clinical presentation, histopathology, and molecular profile.

HPV-unrelated VSCC (Fig. 4.5) accounts for more than 70% of the cases, while its underdiagnosed precursor, differentiated VIN (DVIN) (Fig. 4.6), represents less than 10% of all diagnosed VSIL [114]. These neoplasms occur at an older age, in patients with a long-lasting history of itching and burning, in a background of chronic dermatologic disease, in most instances lichen sclerosus or lichen planus. HPV-unrelated VSCC and DVIN will not be prevented by HPV vaccination. Only

Table 4.1 2015 ISSVD Terminology of vulvar SILs (from Bornstein et al. [113])

LSIL of the vulva (vulvar LSIL, flat condyloma, or HPV effect)
HSIL of the vulva (vulvar HSIL, VIN usual type)
DVIN

SIL squamous intraepithelial lesion; *LSIL* low-grade SIL; *HPV* human papillomavirus; *HSIL* high-grade SIL; *VIN* vulvar intraepithelial neoplasia; *DVIN* differentiated-type VIN

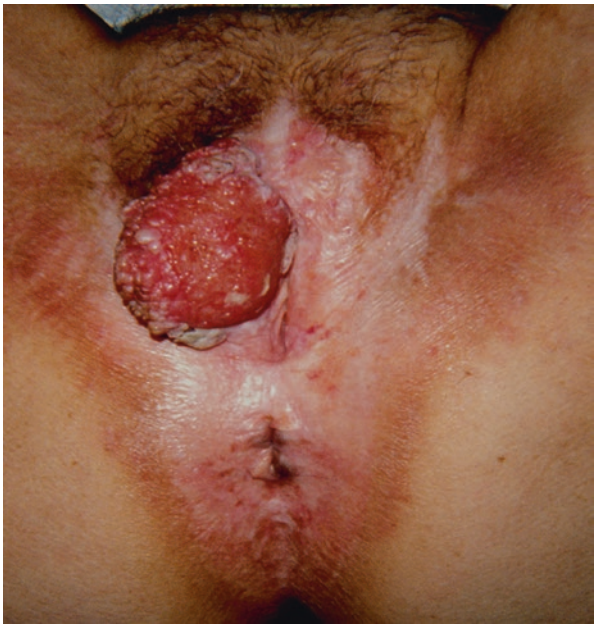

Fig. 4.5 Vulvar cancer. Notice the background of lichen sclerosus

an appropriate examination of the vulva, and biopsy with no delay in suspect cases, can increase DVIN diagnoses, reducing the incidence of invasive cancer.

VHSIL, on the other hand (Fig. 4.7), occurs at younger ages: van de Nieuwenhof et al. found in a series of 1,893 cases of VSIL a median age of 47.8 years (Table 4.2). In this same study, it was described that the incidence almost doubled (from 1.2/100.000 to 2.1/100.000) from 1992 to 2005 [115]. Increasing age in VHSIL

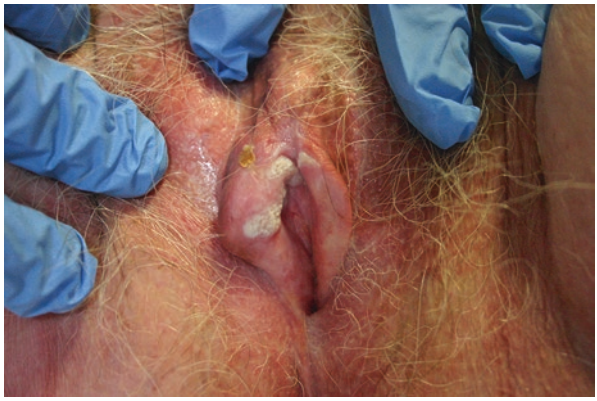

Fig. 4.6 Differentiated VIN, in a background of lichen sclerosus

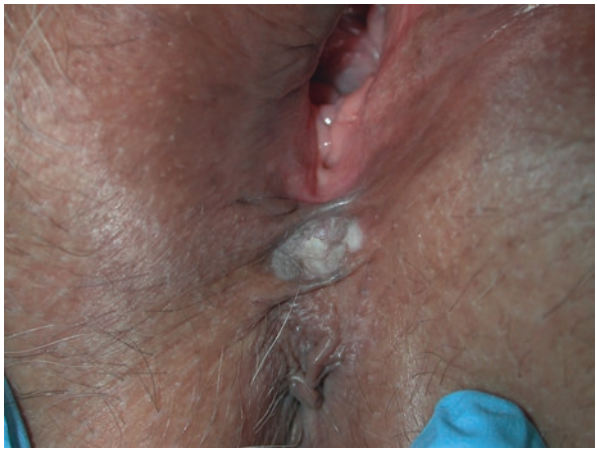

Fig. 4.7 Vulvar high-grade squamous intraepithelial neoplasia

Table 4.2 Age of diagnosis of intraepithelial lesions and invasive cancer

	HSIL (-IN2 or IN3)	Invasive cancer
Cervix [17, 154, 155]	25–41 years old	35–55 years old (>15% in women older than 65 years old)
Vagina [88, 93, 94]	VaIN2—47.2 years old VaIN3—61.8 years old	65–80 years
Vulva [39]	40–44 and >55 years old	55–75 years
Anus [142, 145, 156, 157]	43–49 years old	55–65 years old

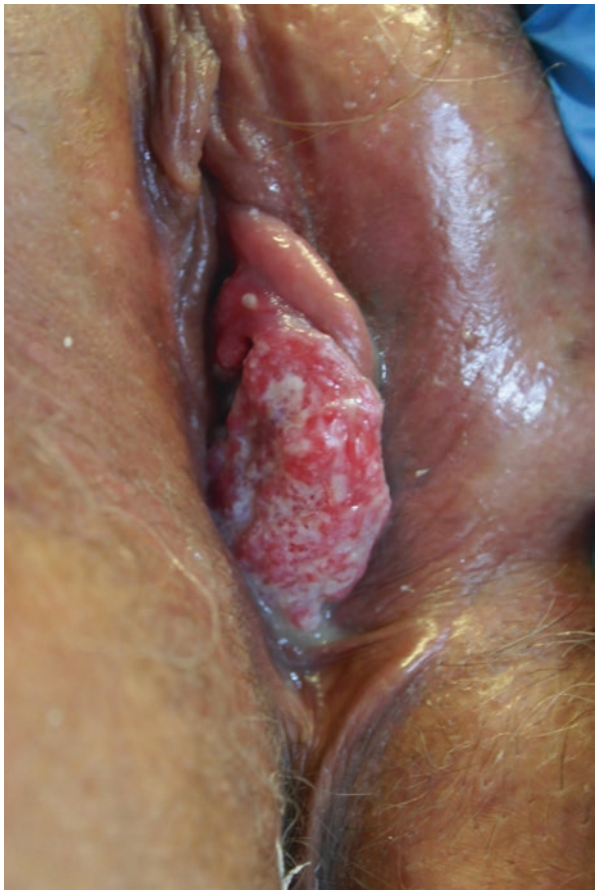

Fig. 4.8 Vulvar squamous cell cancer, HPV-related

patients is associated with higher risk of subsequent diagnosis of VSCC (2.7% if <29 years vs. 8.5% if>75 years) and shorter time of progression (50 months for the <29-year group vs. 25 months for the >75-year group) (Fig. 4.8) [116].

Older age is statistically associated with increased prevalence of stromal invasion in patients undergoing surgical excision after an office biopsy of VHSIL (4.2% in patients younger than 42 years, 10.8% in patients between 43 and 62 years, and 18.3% if older than 62 years) [117].

VHSIL can be located around the introitus, often involving the labia minora and majora. They are usually elevated, sharply defined lesions with normal surrounding skin/mucosa. Lesions can be brownish, white, or red (Fig. 4.5).

HPV16 is the most prevalent HPV type in VHSIL (about three-quarters of all HPV-positive cases), followed by HPV 33 and HPV18 [114] confirming the potential of nine-valent HPV vaccine to eradicate the majority of VHSIL [5].

Multifocality (more than one lesion on the vulva) and multicentricity (involvement of vagina, cervix, and/or anus as well as the vulva) are common and indicate a decreased immune response to HPV infection, with the same HPV type involved in

all the lesions [116]. Younger patients have a higher risk of multifocal lesions (59% in women aged 20–34 and 10% in patients >50 years of age), but older patients have more often intraepithelial lesions at uncommon sites (vagina, anus, and periurethral region) [118].

Treatment of DVIN must be surgical excision, as it has a high potential of progression and it can occur in a very short time. These women must have a close follow-up, as there is a high risk of recurrence [119–121].

Therapy of VHSIL has to take into account (1) characteristics of the lesion (size, configuration, location, multifocality, and multicentricity); (2) characteristics of the patient (age, general condition, symptomatology, associated disease, psychologic issues, work environment, and reliability to follow-up); and (3) available resources and medical skills [122].

Surgery still represents the standard of treatment, with similar results and recurrence rate independent of the technique used (cold knife, LASER, radiofrequency surgery). Many medical treatments have been attempted to avoid surgery in these women. To date, no medications are approved by the Food and Drug Administration (FDA) for this purpose. Several randomized trials have shown imiquimod to be a promising therapy in selected patients [123, 124]. Anyway when, in controlled studies, conservative treatments of VHSIL are chosen, it is of utmost importance to exclude the presence of foci of stromal invasion that exposes patients at risk for metastatic spread to lymph nodes (higher risk if older patients or periclitoral localization) [117].

In the treatment of invasive VSCC that typically occurs in the seventh decade, when comorbidity is common, various conservative and individualized approaches have been proposed to reduce the risk of mutilating en bloc surgery while maintaining oncological radicality and not compromising patients' recurrence-free survival [125–127].

Based on the 1983 definition proposed by the ISSVD, FIGO stage Ia or superficially invasive carcinoma identifies tumors invading the stroma to a depth no greater than 1 mm, with extremely low risk of lymph nodes metastases. These are the only cases where surgical assessment of nodal status can be omitted [128].

For stages higher than Ia, separate incisions for vulva and groins are now considered a standard approach. Radical removal of the tumor can be achieved through a local excision, removing 1–1,5 cm of clinically clear surgical margins in extension and a depth reaching the perineal membrane; the results of this approach are comparable to those of total vulvectomy [129].

Lymph node status is the single most important predictive factor for survival; and nodal recurrence is lethal within 2 years in most patients [130]. The different approaches to inguinofemoral lymphadenectomy must match correct nodal assessment and reduced immediate and long-term morbidity [131].

Sentinel lymph node dissection (SLND) seems to be reliable and safe in early disease [132]. This technique requires specific equipment, a multidisciplinary team (medical physicists and nuclear medicine physicians), and a learning curve for the surgeon. As a consequence, SLND should not be routinely employed by surgeons outside referral centers.

In locally advanced disease [133], new regiments of chemotherapy combined with radio/brachytherapy are promising therapies for this group of patients that usually has a very poor 5-year survival rate [134–137].

There is increasing evidence that HPV-associated VSCC are less aggressive. Recent studies are focusing on p16 overexpression as a prognostic biomarker in VSCC [138]. Other studies showed significant association between p16 expression and improved survival [139, 140].

These promising results have no current impact in the treatment of HPV-associated and HPV-independent VSCC [141], and further cooperative studies are needed to establish HPV status as prognostic factor as in patients with oropharyngeal, anal, and cervical cancers.

4.6.3 Other

4.6.3.1 Anus

Anal cancers are rare (2:100,000 women), but its incidence, and especially that of anal HSIL, has been rising in last decades. Around 90% of anal cancers are HPV-related, having anal HSIL (anal intraepithelial neoplasia [AIN]2-3) as its precursor [142–145]. Anal and cervical cancer share several features, including the existence of a transformation zone, highly susceptible to HPV infection.

While its prevalence is higher in men who have sex with men and immunosuppressed people, the higher number of diagnosis is performed in women—most of them postmenopausal [146]. Women with history of another HPV-related cancer have a three times higher risk of developing an anal cancer [143]. Screening strategies for anal cancer are not yet widespread. In women, the following are likely to benefit from anal cancer screening: HIV patients, organ transplant recipients, past or current history of other intraepithelial neoplasia or cancer (cervical, vaginal, vulvar, or multiple) [142].

Anal cytology seems to be a good option to perform screening in high-risk populations; its low sensitivity precludes its use in low-risk populations. Sampling can be done using a moistened Dacron swabs or cervical brushes. For an optimal specimen, it should be introduced about 4–5 cm into the anal canal and rotated. Classification of the specimens is made according to the Bethesda classification for cervical cytology [142]. The threshold for high-resolution anoscopy is ASC-US.

4.6.3.2 Head and Neck

The list of risk factors for head and neck cavity cancers has included, as most relevant, smoking and alcohol consumption. However, cancers from the oropharynx (tonsils and base of the tongue) do not share these risk factors but rather are HPV-related—especially with HPV16—which is the isolated genotype in 90% of the cases. They have a better prognosis and a better response to chemotherapy and radiotherapy [147, 148]. The relation between HPV and oral cavity cancers in other sites is less clear [149].

While the incidence of other head and neck cancers has been decreasing, in parallel with the decrease in smoking, the ones possibly associated with HPV infection are on the rise. Currently, the latter may represent at least half of the cases [150].

The prevalence of HPV in the mouth ranges between 2 and 8%, with HPV16 being the most prevalent. The infection is less prevalent in women and more frequent in those who engage in oral-genital sex, who are immunosuppressed, or smokers [149]. The reservoir for the infection seems to be the gingival junction [149, 151].

Woon et al., in line with the terminology used for the anus and lower genital tract, have recommended the use of the designation "HPV-associated oral intraepithelial neoplasia" for the dysplastic changes related to HPV and found in the mouth and associated with increased risk for invasive cancer [152].

Currently, there is no recommendation to screen for oral HPV, and no HPV tests have been approved by the FDA for that effect. In a systematic review of studies involving HPV-positive head and neck cancers, the sensitivity of HPV tests was limited (72%, 95% CI 45–89%) [153].

4.7 Conclusion

The burden associated with HPV infection goes far beyond that of cervical cancer. There are lots of suffering—both physical and psychological—associated with this infection. A simple positive HR-HPV test, even in the absence of lesions, can raise several questions and doubts. The practitioner must be prepared to give these answers, finding balance between tranquilizing the patient and, at the same time, not missing relevant disease.

Older women may feel not to be at risk for HPV infections, if they are on a monogamous relationship or have no sexual partner. However, the role of latent and reactivated infections is more and more acknowledge and precludes stopping the screening, even in these patients.

As we anticipate the end of cervical cancer, as a consequence of HPV vaccination, in the next decades we will still have large cohorts of unvaccinated women. Any future programs must take this into consideration.

References

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, Ghissassi FE, et al. A review of human carcinogens—Part B: biological agents. Lancet Oncol. 2009;10(4):321–2.
2. Denis F, Hanz S, Alain S. Clairance, persistance et récidive de l'infection à Papillomavirus. Gynecol Obstet Fertil. 2008;36(4):430–40.
3. Roehr B. Presence of HPV in later life may represent reactivation, not reinfection. BMJ. 2012;345:e8517.
4. Syrjänen K, Hakama M, Saarikoski S, Väyrynen M, Yliskoski M, Syrjänen S, et al. Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. Sex Transm Dis. 1990;17(1):15–9.
5. Mariani L, Preti M, Cristoforoni P, Stigliano CM, Perino A. Overview of the benefits and potential issues of the nonavalent HPV vaccine. Int J Gynaecol Obstet. 2017;136(3):258–65.
6. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(12):1789–99.

7. Bosch FX, Lorincz A, Muñoz N, Meijer CJLM, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55(4):244–65.
8. Brown DR, Weaver B. Human papillomavirus in older women: new infection or reactivation? J Infect Dis. 2013;207(2):211–2.
9. Gravitt PE, Rositch AF, Silver MI, Marks MA, Chang K, Burke AE, et al. A cohort effect of the sexual revolution may be masking an increase in human papillomavirus detection at menopause in the United States. J Infect Dis. 2013;207(2):272–80.
10. Gonzalez P, Hildesheim A, Rodriguez AC, Schiffman M, Porras C, Wacholder S, et al. Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. Cancer Epidemiol Biomarkers Prev. 2010;19(12):3044–54.
11. Franceschi S, Herrero R, Clifford GM, Snijders PJF, Arslan A, Anh PTH, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119(11):2677–84.
12. Grainge MJ, Seth R, Guo L, Neal KR, Coupland C, Vryenhoef P, et al. Cervical human papillomavirus screening among older women. Emerg Infect Dis. 2005;11(11):1680–5.
13. Melkert PW, Hopman E, van den Brule AJ, Risse EK, van Diest PJ, Bleker OP, et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is agedependent. Int J Cancer. 1993;53(6):919–23.
14. Gyllensten U, Gustavsson I, Lindell M, Wilander E. Primary high-risk HPV screening for cervical cancer in post-menopausal women. Gynecol Oncol. 2012;125(2):343–5.
15. Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. J Natl Cancer Inst. 2005;97(8):577–86.
16. Petry KU, Horn J, Luyten A, Mikolajczyk RT. Punch biopsies shorten time to clearance of high-risk human papillomavirus infections of the uterine cervix. BMC Cancer. 2018;18(1):318.
17. Bekos C, Schwameis R, Heinze G, Gärner M, Grimm C, Joura E, et al. Influence of age on histologic outcome of cervical intraepithelial neoplasia during observational management: results from large cohort, systematic review, meta-analysis. Sci Rep. 2018;8(1):6383.
18. Olsen NJ, Kovaks WJ. Gonadal steroids and immunity. Endocr Rev. 1996;17(4):369–84.
19. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. Nat Rev Endocrinol. 2013;9(1):56–62.
20. Gameiro CM, Romão F, Castelo-Branco C. Menopause and aging: changes in the immune system—a review. Maturitas. 2010;67(4):316–20.
21. Stanley MA, Sterling JC. Host responses to infection with human papillomavirus. Curr Probl Dermatol. 2014;45:58–74.
22. Scherer EM, Smith RA, Gallego DF, Carter JJ, Wipf GC, Hoyos M, et al. A single human papillomavirus vaccine dose improves B cell memory in previously infected subjects. EBioMedicine. 2016;10:55–64.
23. Rodriguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. J Natl Cancer Inst. 2010;102(5):315–24.
24. Ramachandran B. Functional association of oestrogen receptors with HPV infection in cervical carcinogenesis. Endocr Relat Cancer. 2017;24(4):R99–108.
25. Ferenczy A, Gelfand M, Franco E, Mansour N. Human papillomavirus infection in postmenopausal women with and without hormone therapy. Obstet Gynecol. 1997;90(1):7–11.
26. Roura E, Travier N, Waterboer T, de Sanjosé S, Bosch FX, Pawlita M, et al. The influence of hormonal factors on the risk of developing cervical cancer and pre-cancer: results from the EPIC cohort. PLoS One. 2016;11(1):e0147029.
27. Boonyanurak P, Panichakul S, Wilawan K. Prevalence of high-risk human papillomavirus infection (HPV) and correlation with postmenopausal hormonal therapy in Thai women aged more than 45 years old. J Med Assoc Thai. 2010;93(1):9–16.
28. Munger K. Are selective estrogen receptor modulators (SERMs) a therapeutic option for HPV-associated cervical lesions and cancers? Am J Pathol. 2014;184(2):358–61.
29. Rhodes HE, Chenevert L, Munsell M. Vaginal intraepithelial neoplasia (VaIN 2/3). J Low Genit Tract Dis. 2014;18(2):115–21.

30. Smith EM, Levy BT, Ritchie JM, Jia J, Wang D, Haugen TH, et al. Is use of hormone replacement therapy associated with increased detection of human papillomavirus and potential risk of HPV-related genital cancers? Eur J Cancer Prev. 2002;11(3):295–305.
31. Clarke MA, Rodriguez AC, Gage JC, Herrero R, Hildesheim A, Wacholder S, et al. A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection. BMC Infect Dis. 2012;12:33.
32. Kero K, Rautava J, Syrjänen K, Grenman S, Syrjänen S. Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. Eur J Clin Microbiol Infect Dis. 2017;36(11):2215–9.
33. Vieira-Baptista P, Lima-Silva J, Pinto C, Saldanha C, Beires J, Martinez-de-Oliveira J, et al. Bacterial vaginosis, aerobic vaginitis, vaginal inflammation and major Pap smear abnormalities. Eur J Clin Microbiol Infect Dis. 2016;35:657–64.
34. Hoffmann JN, You HM, Hedberg EC, Jordan JA, McClintock MK. Prevalence of bacterial vaginosis and Candida among postmenopausal women in the United States. J Gerontol Ser B Psychol Sci Soc Sci. 2014;69(Suppl 2):S205–14.
35. Zhang H, Lu J, Lu Y, Cai Q, Liu H, Xu C. Cervical microbiome is altered in cervical intraepithelial neoplasia after loop electrosurgical excision procedure in China. Sci Rep. 2018;8(1):4923.
36. Arbyn M, Xu L, Simoens C, Martin-Hirsch PP. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. Cochrane Database Syst Rev. 2018;5:CD009069.
37. Schwarz TF, Galaj A, Spaczynski M, Wysocki J, Kaufmann AM, Poncelet S, et al. Ten-year immune persistence and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15-55 years of age. Cancer Med. 2017;6(11):2723–31.
38. Faubion SS, Larkin LC. Immunizations for adult women. Menopause. 2016;23(12):1345–7.
39. Preti M. VIN usual type—from the past to the future. Ecancermedicalscience. 2015;9:531.
40. Parker KH, Kemp TJ, Pan Y, Yang Z, Giuliano AR, Pinto LA. Evaluation of HPV-16 and HPV-18 specific antibody measurements in saliva collected in oral rinses and merocel® sponges. Vaccine. 2018;36(19):2705–11.
41. Pinto LA, Kemp TJ, Torres BN, Isaacs-Soriano K, Ingles D, Abrahamsen M, et al. Quadrivalent human papillomavirus (HPV) vaccine induces HPV-specific antibodies in the Oral cavity: results from the mid-adult male vaccine trial. J Infect Dis. 2016;214(8):1276–83.
42. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-tieulent J, Jemal A. Global cancer statistics, 2012. CA A Cancer J Clin. 2015;65(2):87–108.
43. Garland SM, Giuliano A, Brotherton J, Moscicki AB, Stanley M, Kaufmann AM, et al. IPVS statement moving towards elimination of cervical cancer as a public health problem. Papillomavirus Res. 2018;5:87–8.
44. Shrestha AD, Neupane D, Vedsted P, Kallestrup P. Cervical cancer prevalence, incidence and mortality in low and middle income countries: a systematic review. Asian Pac J Cancer Prev. 2018;19(2):319–24.
45. Franceschi S, Clifford GM, Baussano I. Options for design of real-world impact studies of single-dose vaccine schedules. Vaccine. 2018;36(32 Pt A):4816–22.
46. Harper DM, Vierthaler SL. Next generation cancer protection: the bivalent HPV vaccine for females. ISRN Obstet Gynecol. 2011;2011:1–20.
47. Oakley F, Desouki MM, Pemmaraju M, Gargano JM, Markowitz LE, Steinau M, et al. Trends in high-grade cervical Cancer precursors in the human papillomavirus vaccine era. Am J Prev Med. 2018;55(1):19–25.
48. Halford J, Walker K-A, Duhig J. A review of histological outcomes from peri-menopausal and post-menopausal women with a cytological report of possible high grade abnormality: an alternative management strategy for these women. Pathology. 2010;42(1):23–7.
49. Patton AL, Duncan L, Bloom L, Phaneuf G, Zafar N. Atypical squamous cells, cannot exclude a high-grade intraepithelial lesion and its clinical significance in postmenopausal, pregnant, postpartum, and contraceptiveuse patients. Cancer. 2008;114(6):481–8.

50. Gilani SM, Mazzara PF. Cytohistologic correlation in premenopausal and postmenopausal women. Acta Cytol. 2013;57(6):575–80.
51. Bornstein J, Sideri M, Tatti S, Walker P, Prendiville W, Haefner HK, et al. 2011 terminology of the vulva of the International Federation for Cervical Pathology and Colposcopy. J Low Genit Tract Dis. 2012;16(3):290–5.
52. Cuzick J, Clavel C, Petry K-U, Meijer CJLM, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119(5):1095–101.
53. Piccoli R, Mandato VD, Lavitola G, Acunzo G, Bifulco G, Tommaselli GA, et al. Atypical squamous cells and low squamous intraepithelial lesions in postmenopausal women: implications for management. Eur J Obstet Gynecol Reprod Biol. 2008;140(2):269–74.
54. Vieira-Baptista P, Sousa C, Saldanha C, Fonseca-Moutinho JA. Consenso da ASCCP 2012—o que traz de novo e implicações para o dia-a-dia. Acta Obs Ginecol Port. 2013;7(4):241–8.
55. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121(4):829–46.
56. Zhao C, Austin R. Human papillomavirus DNA detection in ThinPrep Pap test vials is independent of cytologic sampling of the transformation zone. Gynecol Oncol. 2007;107(2):231–5.
57. Cuzick J, Thomas Cox J, Zhang G, Einstein MH, Stoler M, Trupin S, et al. Human papillomavirus testing for triage of women with low-grade squamous intraepithelial lesions. Int J Cancer. 2013;132(4):959–66.
58. Practice Bulletin No. 168. Obstet Gynecol. 2016;128(4):e111–30.
59. Rustagi AS, Kamineni A, Weinmann S, Reed SD, Newcomb P, Weiss NS. Cervical screening and cervical Cancer death among older women: a population-based, case-control study. Am J Epidemiol. 2014;179(9):1107–14.
60. Andrae B, Andersson TM-L, Lambert PC, Kemetli L, Silfverdal L, Strander B, et al. Screening and cervical cancer cure: population based cohort study. BMJ. 2012;344:e900.
61. Gage JC, Duggan MA, Nation JG, Gao S, Castle PE. Detection of cervical cancer and its precursors by endocervical curettage in 13,115 colposcopically guided biopsy examinations. Am J Obstet Gynecol. 2010;203(5):481.e1–9.
62. Boulanger JC, Gondry J, Verhoest P, Capsie C, Najas S. Treatment of CIN after menopause. Eur J Obstet Gynecol Reprod Biol. 2001;95(2):175–80.
63. Richards A, Dalrymple C. Abnormal cervicovaginal cytology, unsatisfactory colposcopy and the use of vaginal estrogen cream: an observational study of clinical outcomes for women in low estrogen states. J Obstet Gynaecol Res. 2015;41(3):440–4.
64. Makkar B, Batra S, Gandhi G, Zutshi V, Goswami D. Vaginal misoprostol versus vaginal estradiol in overcoming unsatisfactory colposcopy. Gynecol Obstet Investig. 2014;77(3):176–9.
65. Aggarwal R, Suneja A, Agarwal N, Mishra K. Role of misoprostol in overcoming an unsatisfactory colposcopy: a randomized double-blind placebo-controlled clinical trial. Gynecol Obstet Investig. 2006;62(2):115–20.
66. Thanapprapasr D, Wilailak S, Ayudhya NIN, Lertkhachonsuk A-A, Likittanasombut P, Chittithaworn S, et al. Can vaginal misoprostol effectively increase rate of a satisfactory colposcopy? A randomized double-blind placebo-controlled trial. Jpn J Clin Oncol. 2010;40(3):203–7.
67. Boardman LA, Meinz H, Steinhoff MM, Heber WW, Blume J. A randomized trial of the sleeved cytobrush and the endocervical curette. Obstet Gynecol. 2003;101(3):426–30.
68. Tate K, Strickland J. A randomized controlled trial to evaluate the use of the endocervical brush after endocervical curettage. Obstet Gynecol. 1997;90(5):715–7.
69. Driggers RW, Zahn CM. To ECC or not to ECC: the question remains. Obstet Gynecol Clin North Am. 2008;35(4):583–97.
70. Irvin W, Flora S, Andersen W, Stoler M, Taylor P, Rice L. Endocervical curettage. Does it contribute to the management of patients with abnormal cervical cytology? J Reprod Med. 2004;49(1):1–7.

71. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. Cochrane Database Syst Rev. 2013;(6):CD001318.
72. Fine BA, Feinstein GI, Sabella V. The pre- and postoperative value of endocervical curettage in the detection of cervical intraepithelial neoplasia and invasive cervical cancer. Gynecol Oncol. 1998;71(1):46–9.
73. Hasegawa K, Torii Y, Kato R, Udagawa Y, Fukasawa I. The problems of cervical conization for postmenopausal patients. Eur J Gynaecol Oncol. 2016;37(3):327–31.
74. Chen Y, Lu H, Wan X, Lv W, Xie X. Factors associated with positive margins in patients with cervical intraepithelial neoplasia grade 3 and postconization management. Int J Gynecol Obstet. 2009;107(2):107–10.
75. Chambo Filho A, Garbeloto E, Guarconi JRA, Partele MP. Positive endocervical margins at conization: repeat conization or colposcopic follow-up? A retrospective study. J Clin Med Res. 2015;7(7):540–4.
76. Binyamin L, Segev M, Peled Y, Krissi H, Sabah G, Raban O, et al. Simple hysterectomy for residual cervical intraepithelial neoplasm. J Low Genit Tract Dis. 2018;22(2):126–8.
77. González Bosquet E, Torres A, Busquets M, Esteva C, Muñoz-Almagro C, Lailla JM. Prognostic factors for the development of vaginal intraepithelial neoplasia. Eur J Gynaecol Oncol. 2008;29(1):43–5.
78. Arbyn M, Redman CWE, Verdoodt F, Kyrgiou M, Tzafetas M, Ghaem-Maghami S, et al. Incomplete excision of cervical precancer as a predictor of treatment failure: a systematic review and meta-analysis. Lancet Oncol. 2017;18(12):1665–79.
79. Tanaka Y, Ueda Y, Kakuda M, Kubota S, Matsuzaki S, Iwamiya T, et al. Predictors for recurrent/persistent high-grade intraepithelial lesions and cervical stenosis after therapeutic conization: a retrospective analysis of 522 cases. Int J Clin Oncol. 2017;22(5):921–6.
80. Penna C, Fambrini M, Fallani MG, Pieralli A, Scarselli G, Marchionni M. Laser CO2 conization in postmenopausal age: risk of cervical stenosis and unsatisfactory follow-up. Gynecol Oncol. 2005;96(3):771–5.
81. Gosvig CF, Huusom LD, Andersen KK, Duun-Henriksen AK, Frederiksen K, Iftner A, et al. Long-term follow-up of the risk for cervical intraepithelial neoplasia grade 2 or worse in HPV-negative women after conization. Int J Cancer. 2015;137(12):2927–33.
82. Ceballos KM, Lee M, Cook DA, Smith LW, Gondara L, Krajden M, et al. Post–loop electrosurgical excision procedure high-risk human papillomavirus testing as a test of cure. J Low Genit Tract Dis. 2017;21(4):284–8.
83. Cubie HA, Canham M, Moore C, Pedraza J, Graham C, Cuschieri K. Evaluation of commercial HPV assays in the context of post-treatment follow-up: Scottish Test of Cure Study (STOCS-H). J Clin Pathol. 2014;67(6):458–63.
84. RMF E, DWE R, IntHout J, WJG M, LFAG M, Bulten J, et al. Long-lasting increased risk of human papillomavirus–related carcinomas and premalignancies after cervical intraepithelial neoplasia grade 3: a population-based cohort study. J Clin Oncol. 2017;35(22):2542–50.
85. Zhang J, Chang X, Qi Y, Zhang Y, Zhang S. A retrospective study of 152 women with vaginal intraepithelial neoplasia. Int J Gynecol Obstet. 2016;133(1):80–3.
86. Sillman FH, Fruchter RG, Chen YS, Camilien L, Sedlis A, McTigue E. Vaginal intraepithelial neoplasia: risk factors for persistence, recurrence, and invasion and its management. Am J Obstet Gynecol. 1997;176(1 Pt 1):93–9.
87. Dodge JA, Eltabbakh GH, Mount SL, Walker RP, Morgan A. Clinical features and risk of recurrence among patients with vaginal intraepithelial neoplasia. Gynecol Oncol. 2001;83(2):363–9.
88. Diakomanolis E, Stefanidis K, Rodolakis A, Haidopoulos D, Sindos M, Chatzipappas I, et al. Vaginal intraepithelial neoplasia: report of 102 cases. Eur J Gynaecol Oncol. 2002;23(5):457–9.
89. Sopracordevole F, Barbero M, Clemente N, Fallani MG, Cattani P, Agarossi A, et al. Colposcopic patterns of vaginal intraepithelial neoplasia. Eur J Cancer Prev. 2018;27(2):152–7.
90. Ratnavelu N, Patel A, Fisher A, Galaal K, Cross P, Naik R. High-grade vaginal intraepithelial neoplasia: can we be selective about who we treat? BJOG. 2013;120(7):887–93.

91. Tainio K, Jakobsson M, Louvanto K, Kalliala I, Paavonen J, Nieminen P, et al. Randomised trial on treatment of vaginal intraepithelial neoplasia-imiquimod, laser vaporisation and expectant management. Int J Cancer. 2016;139(10):2353–8.
92. Sopracordevole F, Barbero M, Clemente N, Fallani MG, Cattani P, Agarossi A, et al. High-grade vaginal intraepithelial neoplasia and risk of progression to vaginal cancer: a multicentre study of the Italian Society of Colposcopy and Cervico-Vaginal Pathology (SICPCV). Eur Rev Med Pharmacol Sci. 2016;20(5):818–24.
93. Sinno AK, Saraiya M, Thompson TD, Hernandez BY, Goodman MT, Steinau M, et al. Human papillomavirus genotype prevalence in invasive vaginal cancer from a registry-based population. Obstet Gynecol. 2014;123(4):817–21.
94. Lilic V, Lilic G, Filipovic S, Visnjic M, Zivadinovic R. Primary carcinoma of the vagina. J BUON. 2010;15(2):241–7.
95. Pride GL, Buchler DA. Carcinoma of vagina 10 or more years following pelvic irradiation therapy. Am J Obstet Gynecol. 1977;127(5):513–7.
96. Dominiak-Felden G, Cohet C, Atrux-Tallau S, Gilet H, Tristram A, Fiander A. Impact of human papillomavirus-related genital diseases on quality of life and psychosocial wellbeing: results of an observational, healthrelated quality of life study in the UK. BMC Public Health. 2013;13(1):1065.
97. Gunter J. Genital and perianal warts: new treatment opportunities for human papillomavirus infection. Am J Obstet Gynecol. 2003;189(3 Suppl):S3–11.
98. Mayeaux EJ, Dunton C. Modern management of external genital warts. J Low Genit Tract Dis. 2008;12(3):185–92.
99. Patel H, Wagner M, Singhal P, Kothari S. Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis. 2013;13:39.
100. van Beurden M, van der Vange N, de Craen AJ, Tjong-A-Hung SP, ten Kate FJ, ter Schegget J, et al. Normal findings in vulvar examination and vulvoscopy. Br J Obstet Gynaecol. 1997;104(3):320–4.
101. Chang L, Ci P, Shi J, Zhai K, Feng X, Colombara D, et al. Distribution of genital wart human papillomavirus genotypes in China: a multi-center study. J Med Virol. 2013;85(10):1765–74.
102. Howell-Jones R, Soldan K, Wetten S, Mesher D, Williams T, Gill ON, et al. Declining genital warts in young women in England associated with HPV 16/18 vaccination: an ecological study. J Infect Dis. 2013;208(9):1397–403.
103. Vulvar cancer—cancer stat facts. https://seer.cancer.gov/statfacts/html/vulva.html.
104. Kurman RJ, American Registry of Pathology, Armed Forces Institute of Pathology (U.S.). Tumors of the cervix, vagina, and vulva. American Registry of Pathology in collaboration with the Armed Forces Institute of Pathology; 2010. p. 431.
105. Saraiya M, Watson M, Wu X, King JB, Chen VW, Smith JS, et al. Incidence of in situ and invasive vulvar cancer in the US, 1998-2003. Cancer. 2008;113(10 Suppl):2865–72.
106. Buttmann-Schweiger N, Klug SJ, Luyten A, Holleczek B, Heitz F, Du Bois A, et al. Incidence patterns and temporal trends of invasive nonmelanotic vulvar tumors in Germany 1999-2011. A population-based cancer registry analysis. PLoS One. 2015;10(5):e0128073.
107. Baandrup L, Varbo A, Munk C, Johansen C, Frisch M, Kjaer SK. In situ and invasive squamous cell carcinoma of the vulva in Denmark 1978-2007-a nationwide population-based study. Gynecol Oncol. 2011;122(1):45–9.
108. Judson PL, Habermann EB, Baxter NN, Durham SB, Virnig BA. Trends in the incidence of invasive and in situ vulvar carcinoma. Obstet Gynecol. 2006;107(5):1018–22.
109. Bodelon C, Madeleine MM, Voigt LF, Weiss NS. Is the incidence of invasive vulvar cancer increasing in the United States? Cancer Causes Control. 2009;20(9):1779–82.
110. Barlow EL, Kang Y-J, Hacker NF, Canfell K. Changing trends in vulvar cancer incidence and mortality rates in Australia since 1982. Int J Gynecol Cancer. 2015;25(9):1683–9.
111. Bornstein J, Bogliatto F, Haefner HK, Stockdale CK, Preti M, Bohl TG, Reutter J, ISSVD Terminology Committee. The 2015 International Society for the Study of Vulvovaginal Disease (ISSVD) terminology of vulvar squamous intraepithelial lesions. Obstet Gynecol. 2016;127(2):264–8.

112. Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. J Reprod Med. 2005;50(11):807–10.
113. Bornstein J, Bogliatto F, Haefner HK, Stockdale CK, Preti M, Bohl TG, et al. The 2015 International Society for the Study of Vulvovaginal Disease (ISSVD) terminology of vulvar squamous intraepithelial lesions. J Low Genit Tract Dis. 2016;20(1):11–4.
114. De Sanjosé S, Alemany L, Ordi J, Tous S, Alejo M, Bigby SM, et al. Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. Eur J Cancer. 2013;49(16):3450–61.
115. van de Nieuwenhof HP, Massuger LFAG, van der Avoort IAM, Bekkers RLM, Casparie M, Abma W, et al. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur J Cancer. 2009;45(5):851–6.
116. de Bie RP, van de Nieuwenhof HP, Bekkers RLM, Melchers WJG, Siebers a G, Bulten J, et al. Patients with usual vulvar intraepithelial neoplasia-related vulvar cancer have an increased risk of cervical abnormalities. Br J Cancer. 2009;101(1):27–31.
117. Preti M, Bucchi L, Ghiringhello B, Privitera S, Frau V, Corvetto E, et al. Risk factors for unrecognized invasive carcinoma in patients with vulvar high-grade squamous intraepithelial lesion at vulvoscopy-directed biopsy. J Gynecol Oncol. 2017;28(4):e27.
118. Bornstein J, Kaufman RH, Adam E, Adler-Storthz K. Multicentric intraepithelial neoplasia involving the vulva. Clinical features and association with human papillomavirus and herpes simplex virus. Cancer. 1988;62(8):1601–4.
119. McAlpine JN, Kim SY, Akbari A, Eshragh S, Reuschenbach M, von Knebel Doeberitz M, et al. HPV-independent differentiated vulvar intraepithelial neoplasia (dVIN) is associated with an aggressive clinical course. Int J Gynecol Pathol. 2017;36(6):507–16.
120. American College of Obstetricians and Gynecologists' Committee on Gynecologic Practice, American Society for Colposcopy and Cervical Pathology (ASCCP). Committee opinion no. 675. Obstet Gynecol. 2016;128(4):e178–82.
121. Kokka F, Singh N, Faruqi A, Gibbon K, Rosenthal AN. Is differentiated vulval intraepithelial neoplasia the precursor lesion of human papillomavirus-negative vulval squamous cell carcinoma? Int J Gynecol Cancer. 2011;21(7):1297–305.
122. Preti M, Scurry J, Marchitelli CE, Micheletti L. Vulvar intraepithelial neoplasia. Best Pract Res Clin Obstet Gynaecol. 2014;28(7):1051–62.
123. Terlou A, Van Seters M, Ewing PC, Aaronson NK, Gundy CM, Heijmans-Antonissen C, et al. Treatment of vulvar intraepithelial neoplasia with topical imiquimod: seven years median follow-up of a randomized clinical trial. Gynecol Oncol. 2011;121(1):157–62.
124. Hurt CN, Jones SEF, Madden T, Fiander A, Nordin AJ, Naik R, et al. Recurrence of vulval intraepithelial neoplasia following treatment with cidofovir or imiquimod: results from a multicentre, randomised, phase II trial (RT3VIN). BJOG. 2018;25(9):1171–7.
125. Dellinger TH, Hakim AA, Lee SJ, Wakabayashi MT, Morgan RJ, Han ES. Surgical management of vulvar cancer. J Natl Compr Cancer Netw. 2017;15(1):121–8.
126. Micheletti L, Preti M. Surgery of the vulva in vulvar cancer. Best Pract Res Clin Obstet Gynaecol. 2014;28(7):1074–87.
127. Baiocchi G, Rocha RM. Vulvar cancer surgery. Curr Opin Obstet Gynecol. 2014;26(1):9–17.
128. Preti M, Rouzier R, Mariani L, Wilkinson EJ. Superficially invasive carcinoma of the vulva: diagnosis and treatment. Clin Obstet Gynecol. 2005;48(4):862–8.
129. Zhou J, Shan G. The prognostic role of FIGO stage in patients with vulvar cancer: a systematic review and meta-analysis. Curr Med Res Opin. 2016;32(6):1121–30.
130. Luchini C, Nottegar A, Solmi M, Sergi G, Manzato E, Capelli P, et al. Prognostic implications of extranodal extension in node-positive squamous cell carcinoma of the vulva: a systematic review and meta-analysis. Surg Oncol. 2016;25(1):60–5.
131. Hudson CN, Shulver H, Lowe DC. The surgery of "inguino-femoral" lymph nodes: is it adequate or excessive? Int J Gynecol Cancer. 2004;14(5):841–5.
132. Te Grootenhuis NC, Van Der Zee AGJ, Van Doorn HC, Van Der Velden J, Vergote I, Zanagnolo V, et al. Sentinel nodes in vulvar cancer: long-term follow-up of the GROningen

INternational study on sentinel nodes in vulvar cancer (GROINSS-V) i. Gynecol Oncol. 2016;140(1):8–14.
133. Aragona AM, Soderini AH, Cuneo NA. Defining the concept of locally advanced squamous cell carcinoma of the vulva: a new perspective based on standardization of criteria and current evidence. J Gynecol Oncol. 2014;25(4):272–8.
134. Kellas-Śleczka S, Białas B, Fijałkowski M, Wojcieszek P, Szlag M, Cholewka A, et al. Interstitial high-dose-rate brachytherapy in locally advanced and recurrent vulvar cancer. J Contemp Brachytherapy. 2016;8(1):32–40.
135. Natesan D, Hong JC, Foote J, Sosa JA, Havrilesky L, Chino J. Primary versus preoperative radiation for locally advanced vulvar cancer. Int J Gynecol Cancer. 2017;27(4):794–804.
136. Gill BS, Bernard ME, Lin JF, Balasubramani GK, Rajagopalan MS, Sukumvanich P, et al. Impact of adjuvant chemotherapy with radiation for node-positive vulvar cancer: a National Cancer Data Base (NCDB) analysis. Gynecol Oncol. 2015;137(3):365–72.
137. Oonk MHM, Planchamp F, Baldwin P, Bidzinski M, Brännström M, Landoni F, et al. European Society of Gynaecological Oncology guidelines for the management of patients with vulvar cancer. Int J Gynecol Cancer. 2017;27(4):832–7.
138. Cao H, Wang S, Zhang Z, Lou J. Prognostic value of overexpressed p16INK4a in vulvar cancer: a meta-analysis. PLoS One. 2016;11(3):e0152459.
139. Tringler B, Grimm C, Dudek G, Zeillinger R, Tempfer C, Speiser P, et al. p16INK4a expression in invasive vulvar squamous cell carcinoma. Appl Immunohistochem Mol Morphol. 2007;15(3):279–83.
140. Dong F, Kojiro S, Borger DR, Growdon WB, Oliva E. Squamous cell carcinoma of the vulva: a subclassification of 97 cases by clinicopathologic, immunohistochemical, and molecular features (p16, p53, and EGFR). Am J Surg Pathol. 2015;39(8):1045–53.
141. Rakislova N, Saco A, Sierra A, del Pino M, Ordi J. Role of human papillomavirus in vulvar cancer. Adv Anat Pathol. 2017;24(4):201–14.
142. Moscicki A-B, Darragh TM, Berry-Lawhorn JM, Roberts JM, Khan MJ, Boardman LA, et al. Screening for anal cancer in women. J Low Genit Tract Dis. 2015;19:S26–41.
143. Acevedo-Fontánez AI, Suárez E, Torres Cintrón CR, Ortiz AP. Risk of anal cancer in women with a human papillomavirus–related gynecological neoplasm. J Low Genit Tract Dis. 2018;22(3):225–30.
144. Hillman RJ, Cuming T, Darragh T, Nathan M, Berry-Lawthorn M, Goldstone S, et al. 2016 IANS International guidelines for practice standards in the detection of anal cancer precursors. J Low Genit Tract Dis. 2016;20(4):283–91.
145. Nielsen A, Munk C, Kjaer SK. Trends in incidence of anal cancer and high-grade anal intraepithelial neoplasia in Denmark, 1978-2008. Int J Cancer. 2012;130(5):1168–73.
146. Key statistics for anal cancer [Internet]. https://www.cancer.org/cancer/anal-cancer/about/what-is-key-statistics.html.
147. Vokes EE, Agrawal N, Seiwert TY. HPV-associated head and neck cancer. J Natl Cancer Inst. 2015;107(12):djv344.
148. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsagué X, Laporte L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol. 2014;15(12):1319–31.
149. Hübbers CU, Akgül B. HPV and cancer of the oral cavity. Virulence. 2015;6(3):244–8.
150. O'Sullivan B, Huang SH, Su J, Garden AS, Sturgis EM, Dahlstrom K, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncol. 2016;17(4):440–51.
151. Ortiz AP, González D, Vivaldi-Oliver J, Castañeda M, Rivera V, Díaz E, et al. Periodontitis and oral human papillomavirus infection among hispanic adults. Papillomavirus Res. 2018;5:128–33.
152. Woo S-B, Cashman EC, Lerman MA. Human papillomavirus-associated oral intraepithelial neoplasia. Mod Pathol. 2013;26(10):1288–97.

153. Gipson BJ, Robbins HA, Fakhry C, D'Souza G. Sensitivity and specificity of oral HPV detection for HPV-positive head and neck cancer. Oral Oncol. 2018;77:52–6.
154. Chan PKS, Chang AR, Yu MY, Li W-H, Chan MYM, Yeung ACM, et al. Age distribution of human papillomavirus infection and cervical neoplasia reflects caveats of cervical screening policies. Int J Cancer. 2010;126(1):297–301.
155. Key statistics for cervical cancer [Internet]. https://www.cancer.org/cancer/cervical-cancer/about/key-statistics.html.
156. Watson AJM, Smith BB, Whitehead MR, Sykes PH, Frizelle FA. Malignant progression of anal intra-epithelial neoplasia. ANZ J Surg. 2006;76(8):715–7.
157. Scholefield JH, Castle MT, Watson NFS. Malignant transformation of high-grade anal intraepithelial neoplasia. Br J Surg. 2005;92(9):1133–6.