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Unresolved issues in the management of human papillomavirus-associated mucosal high-grade pre-cancers

Charles JN. Lacey

York Biomedical Research Institute, Hull York Medical School, University of York, UK

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ABSTRACT

This article reviews human papillomavirus-associated mucosal high-grade pre-cancers and their management. It examines pre-cancer classification systems, the natural history of HPV-associated pre-cancers, the various types of management and treatment for HPV pre-cancers, the various mucosal site-specific considerations, and then some of the unresolved issues. Different conclusions are reached for each of the relevant mucosal sites, which are cervix, vagina, vulva, anus, penis and oro-pharynx, and indeed there are differing volumes of evidence relating to each of these sites, and thus differing degrees of certainty/uncertainty in the recommendations.

1. Introduction

HPV 16 was described in association with cervical cancer in 1983 [1]. HPV was subsequently shown to cause cancers at other mucosal sites including the vulva, vagina, anus, penis and oro-pharyngeal mucosa. Lesions caused by HPV which are precursors to the development of invasive squamous cell cancer both temporally and aetiologically have been described at all these mucosal sites, and the management of these precursor lesions is the topic of this review.

HPV VLP vaccines were first licenced in 2006 and the first introduction of a national vaccination programme was by Australia in 2007. There is now irrefutable evidence from a number of countries that population-based vaccination programmes cause very large decreases in the incidence of cervical cancer and cervical intra-epithelial neoplasia grade 3 (CIN3), especially when vaccination is administered to pre-teen adolescents [2]. Thus, in the future, provided that HPV vaccination is widely implemented across the world, we can look forward to decreasing incidence and prevalence rates of human papillomavirus-associated cancers and high-grade pre-cancers within most societies.

Nevertheless, there is still significant debate and gaps in the evidence base for the optimal management of human papillomavirus-associated high-grade pre-cancers at these mucosal sites. This article will therefore review in turn pre-cancer classification systems, the natural history of HPV-associated pre-cancers, the various types of management and treatment for HPV pre-cancers, site-specific considerations, and then some of the unresolved issues.

2. Pre-cancer classification systems

The concept of lesions that are precursors to cervical cancer goes back to the 19th century. Williams, in 1886, observed that the epithelium adjacent to invasive carcinomas was frequently abnormal [3]. A number of authors subsequently noted that such tissue contained cellular changes resembling that of invasive malignancy but which were confined to the epithelium, and the concept of carcinoma in situ (CIS) was proposed [4]. Schiller (1933) was the first author to provide evidence that carcinoma-in-situ was intimately linked to the genesis of invasive disease [5], and the view that CIS was a genuinely pre-malignant lesion gained acceptance based on retrospective analysis of histological material from women with invasive cancers [6]. The proof of the association was provided by a series of studies that demonstrated that a significant proportion of women with CIS who were not treated developed invasive cervical carcinoma. Kottmeier (1961) reported on 34 women with CIS who were followed for 20 years or more and of whom 25 (74%) developed invasive cancer [7]. McIndoe et al. (1984) described 131 women with CIS who after various forms of cone and wedge biopsy continued to demonstrate abnormal cytology but did not receive further pre-cancer treatment. Of these women 29 (22%) developed invasive cervical cancer and Kaplan-Meier analysis suggested that 35% of these lesions would have progressed to invasive disease over 20 years [8].

In addition to CIS pathologists noted a wide range of related but less florid abnormalities of the cervical squamous epithelium, which were originally referred to by a variety of terms including anaplasia,

Abbreviations: HPV, Human papillomavirus.

E-mail address: charles.lacey@hyms.ac.uk.

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dysplasia, atypia, basal cell hyperactivity, etc. The first authors to suggest a classification system for histological abnormalities were Reagan et al. (1955) who suggested the terminology atypical hyperplasia of slight, moderate or marked degree [9]. Later that year Galvin et al. (1955) reported on a larger series of women with prospective follow-up [10]. They present clear criteria and illustrations of "basal cell hyperactivity" of "stages I, II and III". The adjective "intra-epithelial" had been used in a good number of papers in the 1940s and 1950s, linked with "tumours" or "carcinomas" but it was Richart in 1967 that first introduced the term, cervical intra-epithelial neoplasia (CIN) [11]. The main propositions of his paper were that mild, moderate and severe dysplasia were a continuum, that severe dysplasia was indistinguishable from carcinoma-in-situ and that all these histological diagnoses would be better described as CIN. It was only in 1969 that Richart first referred to a grading of CIN, and quoting the original work of Galvin et al. Thus the histological categories of CIN I, CIN II, and CIN III became established (also referred to as CIN1, CIN2, and CIN3), representing degrees of intraepithelial neoplasia involving either the lower one-third of the epithelium, the lower two-thirds of the epithelium, or full-thickness change respectively. This classification was also subsequently extended to vulval, vaginal, anal, and penile intraepithelial neoplasia, referred to as VIN, VAIN, AIN, and PIN respectively. Then in 1990, the Bethesda systems for reporting histology and cytology were published, and this proposed a classification comprised of low-grade squamous intra-epithelial lesions, (LSIL) and high-grade squamous intra-epithelial lesions (HSIL) [12].

3. The natural history of HPV-associated pre-cancer

The Bethesda two-grade low/high classification system was accepted enthusiastically by many clinicians and pathologists as it was seen to increase diagnostic certainty and give clear signposting to clinical management (i.e. LSIL – observe, HSIL – treat). In addition, it was initially felt to align with the then recent description and understanding of low risk (LR) and high risk (HR) papillomaviruses.

But from the 1990s onward there was increasing recognition of the dynamic nature of HR papillomavirus infection. Koutsky et al. (1992) studied 241 women attending an STD clinic (mean age 26 years) who had negative cervical cytology at entry over a mean of 2.1 years [13]. The risk of progression to CIN 2/3 over 24 months was 28% among HPV + ve women and was highest among women with HPV 16/18 (RR 11.0, 95% CI 4.6–26.0, compared to HPV –ve women). Nobbenhuis et al. (1999) studied 353 women (mean age 32 years) referred to a colposcopy clinic with mild, moderate or severe dyskaryosis over a mean of 33 months [14]. Women were monitored every 3–4 months; serial colpophotographs were taken and evaluated after each visit by a panel of 3 expert colposcopists. The primary endpoint was a colposcopic impression of CIN3 covering 3 or more cervical quadrants or a cytology smear suggesting microinvasive carcinoma. So as not to interfere with the natural history, women were not biopsied until the primary endpoint was reached. The cumulative 3-year incidence of CIN 3 was 28% and all these women had persistent HR HPV infection.

Thus, a relatively clear picture of the natural history of cervical infection with HPV 16 and other HR HPVs emerged. HR HPV infections are frequent in young sexually active females, and usually transitory, often resolving within 1 year. However, a minority of such HR HPV infections can persist for long periods of time or can quite rapidly (within 24 months) progress to CIN 2/3. Such CIN 3 disease can then persist for many years, and there is usually a relatively long period of persistent CIN3 before invasive cervical cancer can occur. So, prospective studies in this field are difficult and hard to perform, but what is known about progression or regression of established CIN3?

Studies were performed some years ago using mathematical modelling of population-based cervical screening data from Sweden and British Columbia [15,16]. Natural history models were constructed and the observed population data were used to explore the dynamics of the model including progression and regression rates. Results described in the two studies were remarkably consistent and showed that the average length of time for CIN 2/3 to progress to invasive cancer was ~12.5 years. The best fit of the observed data was achieved when different regression rates for CIN2/3 at different ages were assumed, specifically when under age 34 years, ~85% of new lesions regress spontaneously, and when over age 34 years only 40% of new lesions regress. Nobbenhuis et al. [17] reported on the regression rates in their prospective study described above. HR HPV clearance preceded cytological regression by ~3 months. Clearance rates of HR HPV of 25–50% after 12 months were observed across all grades of cytology. Hopfl et al. [18] studied 48 women with CIN over a mean of 27 months using a variety of immunological assays. These included an intradermal skin test using inoculation of overlapping peptides from HPV 16 E7 to look for delayed-type hypersensitivity (DTH, i.e. T cell responses) as well as antibody responses to HPV 6/11/16/18/31 VLPs and to HPV 16/18 E6/E7 proteins. DTH responses to HPV 16 E7 were strongly associated with regression (present in 73% of regressors vs. 7% of progressors, $p = 0.0001$) whereas antibody responses were non-discriminatory.

CIN3 lesions that persist and progress usually gradually increase their physical size within the transformation zone (TZ). Probably the first description in English of the general positive correlation of lesion size with histological abnormality was by Burghardt [19]. He showed that there were significant differences in total lesion area between low grade, high grade and early invasive lesions. Singer and Jenkins further explored the relationship between cervical cytology, lesion size, CIN and microinvasive cancer. They analysed 84 cone biopsies containing preinvasive disease and 39 cone biopsies containing microinvasive carcinoma [20,21]. There was a highly significant correlation between the initial grade of cervical cytology and both the area of the lesion, and the total area of CIN3. The analysis of the cone biopsies revealed that the mean area of CIN3 within the microinvasive lesions was seven times greater than the mean area of CIN3 within cones derived from women whose cytology showed severe dyskaryosis and 100-fold greater than the area of CIN3 in lesions from women with mild dyskaryosis. All microinvasive lesions had concomitant CIN3.

When CIN3 lesions persist over time, as well as gradual increase in size, there is the gradual accumulation of a variety of genetic and cellular abnormalities that can eventually result in invasive carcinoma. These include integration of the viral genome into the cellular genome with consistent disruption of the E2 gene and over-expression of E6/E7 [22]. This study also showed that increases in the levels of cell surface molecules associated with adherence and invasiveness, as well as E6/E7 transcripts, coupled with changes in the cellular localization of the Notch protein, defined the transition from CIN3 to invasive lesions. More recent studies have emphasised the presence of epigenetic as well genetic mechanisms such as methylation of both host and HPV genes, viral integration at chromosomal locations known to be associated with tumour phenotypes, recurrent losses of heterozygosity at a variety of chromosome regions suggesting alteration of putative tumour suppressor genes, and telomerase activation as all being involved in the progression of HPV-induced high-grade neoplasia [23].

Although most work in this area has focussed on the cervix, there has been a recent study showing similar dynamic changes in AIN2/3. The SPANC study in Australia enrolled gay and bisexual men, both HIV negative and HIV positive (~36%), and studied the natural history of anal high-grade intraepithelial neoplasia with no therapeutic intervention, using cytology, high-resolution anoscopy (HRA), histology, and other assessments [24]. A cytological diagnosis of HSIL (cHSIL) was used as the primary reference analysis, and over 3 years follow-up an incidence rate of 11.3/100 person years (PY) (95% CI 9.5–13.5) of cHSIL was observed. It was also seen that 22% of cHSIL cleared, with predictors being age < 45 years (Hazard Ratio (HaR) 1.52, 95% CI 1.08–2.16), AIN2 rather than AIN3 (HaR 1.79, 1.29–2.49), smaller lesions (HaR 1.62, 1.11–2.36) and no persistent HPV16 (HaR 1.72, 1.23–2.41).

4. HPV pre-cancer therapy – surgery

Large loop excision of the transformation zone (LLETZ) as a treatment for CIN2/3 was first described in 1989 [25] and subsequently became the most widely used treatment for uncomplicated cervical HSIL. Long-term cure rates of 95–99% are usually reported [26], although there are increased risks after LLETZ of premature birth (excision versus no treatment, overall 11.2% vs 5.5%, Relative Risk (RR) 1.87, 95% CI 1.64–2.12), and associated maternal/neonatal morbidity and mortality [27]. LLETZ can be regarded as the most successful surgical technique for HPV-associated pre-cancer, although there are many other variations in surgical technique used both at the cervix and at other mucosal sites. LLETZ displaced most of the previously used 'destructive surgical techniques' used at the cervix such as laser therapy, cryotherapy and thermal ablation, as the provision of an excised surgical specimen for further histological analysis provides significant advantages for clinical management.

The ANCHOR study is a recently reported randomised clinical trial in 4459 HIV positive subjects > 35 yrs of age comparing treatment versus no treatment for anal HSIL, with the primary outcome being progression to anal cancer [28]. All subjects were monitored with anal cytology, HRA and biopsy of HSIL at 6-monthly intervals. The treatment arm could receive any outpatient-based therapy, such as ablation, excision, topical fluorouracil (an antimetabolite) or imiquimod (an immunostimulant, see next section), although 91% of treated subjects received some form of surgical therapy (84% hyfrecation) as their initial treatment. Over a median follow up of 26 months a 57% reduction ($p = 0.03$) in progression to anal cancer in the treatment arm versus observation was seen with rates of 173 & $402/10^6$ PY respectively.

5. HPV pre-cancer therapy – immunomodulators, therapeutic vaccines and other anti-HPV agents

There are many effective, safe and tolerable therapeutic agents for many viral infectious diseases and it has always been hoped that a range of suitable agents could be developed for HPV pre-cancer lesions. However, there remains a dearth of such anti-HPV agents, and this paucity of options continues to pose challenges in clinical management.

Imiquimod is an immuno-stimulant that activates the human toll-like receptors 7 & 8 to drive production of type 1 interferons, with associated activation of monocytes, macrophages and plasmacytoid dendritic cells, followed by a subsequent immunological effector cascade [29]. Imiquimod 5% is available worldwide as a topical preparation and is an established therapy for ano-genital warts (AGW). The marketed formulation of imiquimod for AGW is a cream packaged in individual treatment sachets, which is used three times a week. It sometimes produces local side effects, which rarely can be severe, and thus dosing modifications are fairly common in clinical practice. Imiquimod has also shown degrees of effectiveness in CIN, VAIN, VIN, AIN and PIN, but specific delivery techniques or formulation adjustments are needed for intra-vaginal application for CIN and VAIN. The latest developments in the use of imiquimod are summarised in the site-specific sections below, 3.1–3.5.

Attempts to develop therapeutic HPV vaccines have taken place over the last ~100 years [30]. These were given impetus in the molecular era of the 1990s via the studies of Campo and Jarrett in the bovine papillomavirus model [31] but a variety of both LR and HR HPV therapeutic vaccines in human studies have at present failed to show clear-cut effectiveness. This is still a very active area with 67 studies of HPV therapeutic vaccines listed on ClinicalTrials.gov as of 28 July 2022, with 17 actively recruiting. However, many of these approaches are now focussing on the use of such vaccines as adjuvant therapy for HPV-associated invasive oro-pharyngeal and cervical cancer. There are a number of recent review articles on this topic, so this review will not attempt to cover all investigational products, but only give some examples of products in on-going development in human studies for pre-cancer.

VGX-3100 is a DNA vaccine administered by intra-muscular injection and electroporation and consists of two DNA plasmids encoding synthetic consensus E6 and E7 genes of HPV-16 and HPV-18 [32]. The developers, Inovio, have current research programmes in cervical HSIL, vulvar HSIL, and anal HSIL, and presented results from all those programmes at the International Papillomavirus Conference (IPVC) 2021 (Abstract Book https://indd.adobe.com/view/11ae126a-741b-4c93-b038-1a499b9fcd9a, abstracts pages 233, 164, 235).

Vaccitech, a spin-out company from the University of Oxford, UK, have developed a prime-boost regime using the replication-defective vectors ChAdOx1 (a chimpanzee adenovirus) and MVA (modified vaccinia Ankara, a poxvirus) both incorporating the synthetic gene 5GHPV3. This gene contains consensus sequences from the six early proteins E1, E2, E4, E5, E6 and E7, derived from HPVs 16, 18, 31, 52 and 58 [33]. Results from a phase 1/2 study were presented at IPVC 2021 (Abstract Book https://indd.adobe.com/view/11ae126a-741b-4c93-b038-1a499b9fcd9a, abstract pages 618–9).

The development of specific inhibitors of the HPV life cycle is a much under-researched and under-resourced area; one could say the Cinderella of the HPV world. Cidofovir (a licensed nucleoside analogue) has been used in variety of HPV-related diseases but is only available as an intravenous infusion, and so topical formulations need to be produced by a manufacturing pharmacy under sterile conditions utilising a vial containing 375 mg of anhydrous cidofovir. While this is feasible for a clinical trial, it is much less applicable to individual patient management. A Cidofovir related prodrug with an enhanced intra-cellular half-life was tested in HPV-18 raft cultures to examine its effect on HPV replication, and the prodrug was confirmed to have enhanced uptake and activity [34]. One such prodrug, a compound ABI-2280 with pan-HPV genotype activity, formulated as vaginal tablets/pessaries is in a phase 1 trial sponsored by Antiva Biosciences https://www.australianclinicaltrials.gov.au/anzctr/trial/ACTRN12621001540808.

A recent *in vitro* study has demonstrated that EGFR/MEK/ERK signalling directly regulates HR HPV oncogene expression [35], and that the protein kinase inhibitors erlotinib and trametinib significantly inhibited E6/E7 transcription. A phase 2 trial of erlotinib as an oral agent in invasive vulval carcinoma has actually been reported showing an overall clinical benefit of 67.5% [36], although there was a ~25% incidence of grade 3/4 toxicities, as is often seen with such anti-neoplastic agents. However, there is now a plethora of such licenced agents and in terms of HPV-associated mucosal pre-cancer the development of topical formulations would seem a logical step.

6. HPV pre-cancer therapy – watch and wait

As referred to above we know that many cases of CIN 2/3 and AIN 2/3 will spontaneously regress, but also that a small minority of cases will progress to invasive cancers. This poses dilemmas for both clinicians and patients. Some simple prognostic indicators have been identified for regression vs progression – age (younger vs older), size of lesion (small vs large), histology (CIN or AIN 2 vs 3) but nevertheless there is a strong tendency for many clinicians and patients to favour immediate treatment rather than observation over time.

Ideally, we would have sensitive and specific markers based on non-invasive tests that accurately predicted outcomes. A recent prospective study of women with CIN2/3 (CIN2 n = 80, CIN3 n = 34) replaced surgical excision with a wait-and-see policy [37]. Women were eligible if the entire TZ was visible and with colposcopic disease only present on <50% of the cervix. Colposcopy and HPV testing was performed every 6 months, as well as a PCR methylation test on exfoliated cells, the FAM19A4/miR124-2 assay (Qiagen, Germany). Two thirds of women (65.8%) did not receive any surgical treatment. Women with a negative FAM19A4/miR124-2 result on the baseline sample showed more clinical regression (74.7%) than women with a positive methylation result (51.4%, $p = 0.013$). Regression in women with a negative

FAM19A4/miR124-2 methylation test was highest when cytology was reported as atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion (88.4%) or HPV16 was negative (85.1%). The authors conclude that methylation, in combination with cytology or HPV genotyping can be used to support a wait-and-see policy in women with CIN2/3. The results are in keeping with a previous wait-and-see prospective cohort of women with CIN2 where a research methylation assay, the S5 classifier was used [38].

7. HPV pre-cancer sites – cervix

High-grade pre-cancer of the cervix nearly always occurs within the cervical TZ, except for glandular abnormalities (which are not discussed in this review). The topography of the cervical TZ makes it eminently removable by surgical techniques such as LLETZ with high cure rates (see above 2.1) [26] although subsequent obstetric risks are increased (as referred to above) [27]. In keeping with current practice US [39] and UK [40] management guidelines focus on surgical treatment without mention of any conservative/watch and wait strategies.

Two recent clinical trials compared imiquimod with LLETZ for the treatment of CIN 2/3. A non-randomised study allowed women to choose imiquimod therapy (6.25 mg administered intra-vaginally by applicator x3/week up to 16 weeks) or LLETZ [41]. At 6-month follow up histological regression (<CIN 2/3) was seen in 60% vs 95% and HR HPV clearance in 69% vs 67% respectively. However, 21% of women using imiquimod discontinued treatment due to side-effects, with 69% reporting one or more severe side-effects compared to 29% ($p < 0.01$) in the LLETZ group. Another RCT compared the treatment of CIN 2/3 with imiquimod applied vaginally as purpose-manufactured vaginal tablets/pessaries (6.25 mg) used in an escalating dose x3/week for 16 weeks, versus LLETZ [42]. At 6-month follow up histological regression (<CIN 2/3) was seen in 63% vs 84% and HR HPV clearance in 43% vs 64% respectively. No patients discontinued treatment with imiquimod but approximately one third developed grade 3 side effects.

Interest has also focussed on the use of HPV VLP vaccines as adjunctive therapy post-LLETZ (or surgical treatment) to decrease recurrence rates. Two systematic reviews have recently been published [43,44] both suggesting a decrease in CIN 2/3 recurrence rates post-vaccine but stressing that RCTs are necessary to confirm or refute this finding. Two such studies are underway, the NOVEL trial in the UK https://www.clinicaltrialsregister.eu/ctr-search/trial/2018-004662-33/GB and the VACCIN study in the Netherlands https://www.erasmusmc.nl/en/research/projects/vaccin-study. The possible mechanism of action of the vaccine in this context remains unclear. It is possible that vaccination results in blocking of rounds of re-infection from other vaginal HPV foci, and also a hypothesis of induction of anti-L1 T cell responses effective specifically within the transformation zone has been proposed [45]. An RCT testing such vaccine therapy pre-LLETZ in HIV + ve women did not show benefit [46]. One caveat is that the incidence of recurrence of high-grade disease after LLETZ is low in quality assured screening programmes, and that cost effectiveness analyses of such use of HPV vaccines using RCT data would be important in determining the utility of the strategy [44].

8. HPV pre-cancer sites – vagina

Vaginal intra-epithelial neoplasia (VAIN) is far less common than CIN but is often found concomitantly with the presence of CIN or VIN. VAIN1 is a benign condition and is often associated with condylomatous disease. Assessment of VAIN requires colposcopy and gynaecological oncology expertise and laser ablation of VAIN 2/3 is regarded as the standard treatment, although recurrent disease is not uncommon. Topical intra-vaginal imiquimod can be used successfully for the treatment of VAIN 2/3, and a recent systematic review suggested a complete response rate of 0.76 (95% CI, 0.59–0.87) [47]. Indeed an RCT of imiquimod vs laser therapy vs wait-and-see over 16 weeks has been reported in 30 patients (25 VAIN2, 5 VAIN3) [48]. No subject experienced progression during follow-up, and histological regression (to ≤ VAIN 1) was observed in 80% ($n = 8/10$) of patients in the imiquimod arm, 100% ($n = 10/10$) of the laser arm ($p = 0.474$) and 67% ($n = 6/9$) of the expectant management arm ($p = 0.628$).

9. HPV pre-cancer sites – vulva

Women undergoing surgical treatment for vulval cancer and neoplasia frequently develop significant psychological distress, sexual dysfunction and relationship difficulties [49]. Nowadays consensus guidelines for the management of HPV-associated VIN 2/3 not only detail surgical approaches but also acknowledge the substantial post-surgical morbidity and give equal weight to medical therapy [50]. VIN is often a multi-focal disease of the vulva, and many women will want to avoid surgery as treatment in the first instance. An RCT of imiquimod vs surgery for VIN 2/3 was recently published which also reviews previous RCTs and meta-analyses of imiquimod for this indication [51]. Imiquimod was advised to be used only to the lesions on an escalating dosage for up to 6 months. 110 women were randomised, with a mean age of 52 yrs, with 22% having multifocal disease. 80% of the imiquimod treated subjects had a complete clinical response compared to 79% after one surgical treatment. Invasive disease was found in five patients at primary or secondary surgery, but not in patients with per-protocol imiquimod treatment. There was no significant difference in HPV clearance, adverse events, and treatment satisfaction between study groups. The authors conclude that imiquimod is a safe, effective, and well accepted alternative to surgery for women with VIN 2/3 and can be considered as first-line treatment.

10. HPV pre-cancer sites – anus

The results of the ANCHOR study [28] can be seen as a seminal moment in AIN research. Such an RCT with an observation arm involving ~4500 subjects will never be repeated, and that the population were HIV infected, i.e. more immunosuppressed, gives confidence in the results, and that they will likely be as applicable to non-immunosuppressed subjects. However, we are still left with many questions including (i) cost-effectiveness (ii) uncertainty as to the best treatment (any treatment could be used in the active arm) and (iii) whether the invasive cancer rate in the treatment arm (173/100,000 PY) can be improved upon with optimal therapy [52]. Some of these questions may be answered in the post-randomization phase of this trial, as all enrolled participants are being offered treatment for HSIL or follow-up https://clinicaltrials.gov/ct2/show/NCT02135419.

The anal canal shows a number of similarities with the cervix, in that it is a squamo-columnar junction with a transformation zone including stem cells, which is particularly susceptible to HR HPV mediated carcinogenesis. However, in therapeutic terms it differs markedly from the cervix, in that the entire cervical TZ can be excised, say by LLETZ as discussed above, whereas surgical techniques at the anus are limited by the anatomy. There are variations on the precise surgical technology used (e.g. hyfrecation, laser, infra-red coagulation) but until recently this was limited to individual lesional removal. Such approaches are known to be subject to high metachronous (new lesion development) recurrence rates. However, recently a technique used for ablation of Barret's oesophagus, radio-frequency ablation (RFA), has been adapted to deliver RFA to the entire anal canal with a specific probe. Recent data suggests this technique has improved efficacy over targeted ablation although adverse events are common [53].

11. HPV pre-cancer sites – penis

PIN is a relatively rare condition and a wide variety of treatments have been used [54]. Circumcision is always recommended for uncircumcised men and is usually associated with complete remission [54].

Combinations of treatments can be used, as is frequently used for ano-genital warts, and a small case series using cryotherapy and imiquimod reported 100% complete remission [55].

12. HPV pre-cancer sites – oro-pharyngeal

We are at an early stage of our understanding of oro-pharyngeal pre-cancerous lesions. The majority of HPV-related oro-pharyngeal carcinomas (OPCs) are caused by HPV16 and it is known that antibodies to the HPV16 E6 protein are a sensitive (>90%) and specific (>99%) marker for detecting OPC [55]. Prospective cohorts in the US and population-based data were used to estimate the absolute risk of HPV16-E6 antibody positivity for the subsequent development of OPC, and demonstrated 10-year risks of 17%–27% for males and 4%–6% for females aged 50–60 years [56]. Evaluations of structured clinical assessments of the upper oro-pharyngeal airway have also been undertaken and shown to be able to detect early pre-cancerous and cancerous lesions [57].

13. Conclusions and unresolved issues

CIN2/3 – LLETZ is a widely accepted treatment with high cure rates. There is, however, subsequent obstetric morbidity with the technique, but risk factors for such morbidity (increasing cone depth, repeat treatment) have been identified. Many women are interested in conservative approaches to management, but imiquimod treatment has significant morbidity and does not show sufficient efficacy to challenge LLETZ. A more promising alternative to surgery is selection of lower risk CIN 2/3 cases (colposcopically and histologically) and monitoring with methylation assays, HPV genotyping and follow-up. More comparative studies of different methylation assays in this clinical scenario are needed.

VAIN2/3 – Although laser is the accepted treatment modality imiquimod shows high response rates (~80%) and can be regarded as an acceptable alternative.

VIN2/3 – Psychological and psychosexual morbidity is frequent following surgery, and the latest data suggest that imiquimod can be regarded as a first-line treatment.

AIN2/3 – Knowledge is this area is evolving rapidly. However, currently there is limited capacity for HRA, the definitive diagnostic technique, and a high 'number-needed-to-treat' with a screen-all policy. Clearly, a proportion of cases will resolve spontaneously and more studies of non-invasive biomarkers of regression/progression are needed. Some form of surgical ablation can now be recommended for higher risk, or persistent cases and on-going trials should yield new data about optimal treatments and techniques.

PIN2/3 – Circumcision should be recommended in uncircumcised men. The combination of ablation and imiquimod seems highly effective.

High-grade oro-pharyngeal pre-cancer – Further research including studies of specialist assessment of high-definition imaging of the upper airways in high-risk subjects (>50 yrs of age, HPV16-E6 seropositive) are indicated.

HPV therapeutics – there is still a significant unmet need for HPV therapeutic vaccines and anti-HPV therapeutics. Research in these areas should be promoted.

Author statement

Charles Lacey: Conceptualization, Methodology, Resources, Writing – Original Draft, Writing – Review and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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