

Cervical intraepithelial neoplasia

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Introduction

The cervical epithelium is derived from two embryologically distinct sources. The portio vaginalis of the cervix is covered by non-keratinised stratified squamous epithelium, similar to the vaginal epithelium. Mucus-secreting columnar cells of the same embryological derivation as the uterine endometrium cover the endocervical canal. At birth there is an abrupt junction between the original squamous epithelium and the columnar epithelium of the endocervix, known as the original squamo-columnar junction (OSCJ). In the majority of cases, this junction is on the ectocervix, but may be on the vagina, particularly in diethylstilbestrol (DES) exposed women. At about one year of age, the cervix begins to elongate resulting in the migration of the squamocolumnar junction towards the external os. At the time of menarche or during pregnancy, both the uterus and cervix enlarge. Enlargement of the cervix is accompanied by alterations in its shape, resulting in greater eversion of the endocervical columnar epithelium towards the vagina.¹

Over time, the columnar epithelium exposed on the portio vaginalis of the cervix is remodelled and replaced by metaplastic squamous epithelium. As this occurs, the original squamocolumnar junction (OSCJ) moves towards the endocervical os or endocervical canal, creating a new SCJ. The area between the original and the new SCJs is called the transformation zone (TZ) and is characterised histologically by metaplastic epithelium.

The concept of the TZ is central to the understanding of the pathogenesis of squamous cell cancer of the cervix and its precursors, because virtually all cervical squamous neoplasia originates at the new SCJ and coincides with the distribution of the TZ. In addition, in reproductive life, the TZ is located on the exposed portion of the cervix and is amenable to cytological and histological sampling and colposcopic examination. While the TZ is difficult to visualise with the naked eye, its localisation is greatly enhanced by the application of 5% acetic acid and the use of the colposcope.

Natural history of cervical cancer precursors

It has long been recognised that cervical cancer develops from histologically well-characterised precursors. The first evidence suggesting the existence of precursor lesions for invasive squamous carcinoma of the cervix were observations made in the late 1800s that non-invasive epithelial abnormalities frequently existed adjacent to invasive lesions. Schottlander and Kermauner were the first to propose the term carcinoma-in-situ (CIS) to describe these intraepithelial abnormalities.²

Subsequently a number of case-controlled studies demonstrated that a significant proportion of women with CIS, who were untreated, developed cervical cancer.^{3,4,5} Petersen³ followed 127 women with biopsy-confirmed high-grade pre-invasive lesions of the cervix (called then Epithelial

Hyperplasia with Nuclear Abnormalities) for a minimum of three years. He found that overt cervical cancer developed in 4% of women at the end of one year, 11% at the end of three years, 22% at the end of five years and in 33% at the end of nine years of follow up. Kottmeier found that 25 of 34 women with CIS who were followed for 20 years or more without treatment developed invasive cancer.²

In the 1950s it became apparent that there was another large group of cervical lesions that had some of the characteristics of CIS, but to a lesser degree. Reagan⁶ first introduced the term dysplasia to describe these lesions. Dysplasia referred to abnormalities that included a cytological and histological spectrum of lesions intermediate between CIS and normal epithelium. The WHO (World Health Organization) adopted this terminology as mild, moderate, severe dysplasia and CIS for cytological and histopathological classification of cervical cancer precursors.

The natural history of dysplasia was studied extensively in the 1950s and 1960s. Population based screening programmes of previously unscreened populations showed that women with mild dysplasia were younger than women with moderate dysplasia who in turn were younger than women with severe dysplasia and CIS.⁷ This age distribution suggested that mild dysplasia progressed over years to higher grades of dysplasia and finally to CIS. CIS was considered very high risk for progression to invasive cancer and was aggressively treated with cone biopsy or hysterectomy.

Development of CIN terminology

In the 1960s Richart introduced the CIN classification.⁸ Laboratory-based studies showed that the differences between the different grades of dysplasia were quantitative as well as qualitative. On the basis of these studies, as well as long-term clinical follow up studies, Richart suggested that dysplasia and CIS constituted a histological continuum rather than a series of discrete entities and introduced the term 'Cervical Intraepithelial Neoplasia', known as CIN. In the original CIN terminology CIN1 corresponded to mild dysplasia, CIN2 to moderate dysplasia and CIN3 to severe dysplasia and CIS.

The Bethesda Classification of Cervical Cytology

The Bethesda Classification of Cervical Cytology was introduced in 1988 in order to standardise the 'terminology chaos' that existed because of the many definitions used for cervical cancer precursors. The Bethesda system⁹ combined clinically similar intraepithelial diagnoses into broad categories, specifically low-grade Squamous Intraepithelial Lesions (LSIL), representing the changes of koilocytic cytological atypia and CIN1, and high-grade SIL (HSIL), representing the changes of CIN 2 and 3. LSIL is relatively common and represents the usually benign cytopathological signs of HPV infection. In contrast, HSIL is rare and represents a truly premalignant condition. Although LSIL can be viewed as an epidemiological

exposure or risk factor for cervical cancer, HSIL can be viewed as more closely linked to the cancer outcome. The Bethesda system was revised in 2001¹⁰ and again in 2006¹¹ and readers are advised to access these publications for the full classification.

Aetiology of cervical cancer

For over 100 years it has been recognised that the epidemiology of squamous cancer of the cervix has many of the characteristics of sexually transmitted diseases.¹² Sexual factors such as early age of first intercourse, early age of first pregnancy, multiple partners or a partner who has multiple partners and a history of sexually transmitted diseases are known to increase a woman's risk of developing cervical cancer.^{13,14,15} This strong association with sexually transmitted diseases stimulated the search for an infectious agent as the cause of cervical cancer and its precursors.

In the 1970s Zur Hausen^{16,17} first suggested a role for the papillomaviruses in the pathogenesis of genital tract cancer (he was awarded the Nobel Prize for this work in 2008). There is now a substantial body of epidemiological, clinical and molecular evidence that persistent infection of the cervix with certain types of human papillomavirus (HPV) is the central cause of cervical cancer and its precursors.^{18,19}

Diagnosis and treatment of cervical cancer precursors

Historically, exfoliative cytology has been the most widely used method for detecting cervical cancer precursors. In fact, where applied correctly, mass, organised cytology-based screening programmes have dramatically reduced the incidence of and mortality from cervical cancer.²⁰ In recent years in attempts to circumvent the many pitfalls associated with cytology, alternative screening tests have been subjected to scientific scrutiny and these include HPV DNA testing, Visual Inspection with Acetic Acid (VIA), Visual Inspection with Lugol's Iodine (VILI) and methods that use real-time imaging.

Munoz et al¹⁹ pooled data from 11 case-control studies from nine countries involving 1918 women with histologically confirmed squamous-cell cervical cancer and 1928 control women.

The impetus for new screening technologies in the developed world is driven by the need to increase the positive predictive value of the screening tests and reduce the over-management of low-grade and often transient abnormalities (i.e. to increase specificity). In the developing world, where there are few, if any, successful screening programmes, the impetus is towards coverage and the use of low technology, affordable and reliable screening tests.

Current practice is to refer all women with cytological diagnoses of ASC-H, HSIL, AGUS or any suspicion of cancer for colposcopy. At colposcopy, if the diagnosis of HSIL is confirmed, most centres practice a 'Look and LLETZ' or 'See and Treat' approach i.e. proceed to treatment without prior histological confirmation of the diagnosis. This approach has been adopted to reduce the number of visits required, improve follow up and to simplify management. Bigrigg et al²¹ reported on over 1000 patients managed using this approach with very good outcomes. Denny et al²² showed that the 'negative LLETZ rate' (i.e. no CIN found in the final specimen) was not significantly different in women who underwent 'Look and LLETZ' compared to women who first underwent a punch biopsy.

LLETZ (Large Loop Excision of the Transformation Zone) or LEEP (Loop Electrosurgical Excision Procedure) is performed under local anaesthetic as an outpatient procedure and provides a sample for histological evaluation. LLETZ is a therapeutic procedure and the following criteria should be met prior to treatment:

1. A colposcopy should be performed;

2. There should be no significant disparity between the cytological and colposcopic diagnosis;
3. There should be no suspicion of microinvasion;
4. The upper limit of the lesion should be visible and
5. There should be no glandular abnormality.

The most controversial area in management is how to treat LSIL, as many of these lesions, up to 60%²³ will regress spontaneously, particularly in women less than 30 years of age. It is not clear whether these lesions should be treated or not. It is our practice to only refer women with LSIL for colposcopic assessment after two consecutive LSIL smears taken 6–12 months apart. If the colposcopic diagnosis is LSIL/CIN1, this is confirmed with histological sampling to avoid over-treatment. If the lesion is histologically confirmed, the decision to treat is determined by factors such as age and availability for follow-up. HIV status also plays a role, with a very high recurrence rate post-treatment due to persistent HPV infection in immuno-compromised HIV positive women. Thus HIV positive women with LSIL are only offered treatment if they indicate that they will not return for follow up. Otherwise they are followed with cytology and colposcopy at 2–3 yearly intervals. This approach is based on data from a longitudinal study of cervical disease of 400 HIV-1 positive women in Cape Town, who were followed over a 36 month period. This study found that cytological progression from normal/ASCUS cytology to any grade of SIL occurred in 17% of women, and from LSIL to HSIL in only 4% over a 36 month period.²⁴ No cases of cancer were diagnosed.

The ALTS Trial (ASCUS/LSIL Triage Study) was a large multisite randomised trial that showed that high-risk HPV was found in approximately 80% of women with a cytological diagnosis of LSIL.²⁵ This high rate of HPV positivity undermined the ability of HPV testing to discriminate between clinically non-significant cytological abnormalities and those representing true cervical cancer precursors. The study concluded that HPV testing was not of value in managing women with LSIL and it is recommended that women with LSIL undergo colposcopy instead of HPV testing.

With regard, however, to the management of women with ASCUS cytology, the ALTS trial found that HPV testing detected 96.3% of women with previously undiagnosed CIN3 or cancer, and resulted in the referral of only 56.1% of women for colposcopy.²⁵ This would significantly reduce the number of women requiring colposcopy, particularly in settings where the management strategy is to perform colposcopy on all women with ASCUS cytology.

Visual inspection with acetic acid for detection of cervical cancer precursors

The challenges posed by cytology-based screening programmes, have prompted the search for alternative, technologically more appropriate and more affordable screening methods. Visual inspection with acetic acid, known as VIA, involves examination of the cervix after the application of 3–5% acetic acid, using the naked eye aided by a bright light source. VIA can be performed by mid-level health practitioners such as nurses, at a primary care level and provides the patient with an immediate result, without the necessity of expensive laboratories and the infrastructure required by cytology. The test characteristics of VIA have been evaluated in a number of cross-sectional studies in developing countries, summarised by Sankaranarayanan et al²⁶ and Denny.²⁷ These studies have included nearly 150 000 women and have reported sensitivities of VIA for high-grade intraepithelial lesions which have ranged from 49–96% with specificities between 49 and 98%.

Recently Sanakaranarayanan et al have reported on a cluster-randomised trial performed in 114 clusters in Dindigul district, India.²⁸ Fifty seven

clusters or areas were randomised to one round of VIA by trained nurses, with colposcopy and histological sampling performed prior to treatment with cryotherapy if positive and 57 to a control group. This study was the first to report on a reduction in cervical cancer incidence in the intervention versus the control group (all other VIA studies have used intraepithelial lesions as the outcome). In the intervention group, 274 430 person years, 167 cervical cancer cancers and 83 cervical cancer deaths accrued (crude rate 30.2/100 000 women person-years) compared to 178 781 person years, 158 cases and 92 deaths in the control group (crude rate 51.5/100 000) between 2000 and 2006.

Denny et al²⁹ performed a randomised controlled trial of 6555 women aged 35–65 in Cape Town, South Africa. This trial evaluated three ‘screen and treat’ strategies:

1. Screening with VIA followed by cryotherapy if positive;
2. Screening with HPV DNA testing using Hybrid Capture II followed by cryotherapy if positive;
3. Control group had delayed treatment for six months regardless of the result of the screening tests (VIA and HPV DNA testing).

The prevalence of high grade cervical cancer precursors (defined histologically) was significantly lower in the two ‘screen and treat’ groups 12 months post randomisation compared to the delayed evaluation group. HPV DNA testing followed by cryotherapy was twice as effective in reducing high-grade lesions compared to VIA followed by cryotherapy. The cumulative detection of CIN 2+ in women in the ‘HPV DNA and treat’ group was 1.42%, 2.91% in the ‘VIA and treat’ group, and 5.41% in the delayed treatment group. While minor complaints, such as discharge and bleeding were common after cryotherapy, major complications were rare. While the study was not designed to evaluate the HIV transmission rate in treated versus untreated women, there was no increase in HIV transmission in these two groups.

VIA lends itself to ‘screen and treat’ strategies and has many advantages in low resource settings, particularly the option of a ‘one-stop’ visit to the clinic. Disadvantages of VIA include its relatively low specificity and PPV, resulting in considerable over-treatment. In addition, it is very difficult to provide reliable quality control of VIA which may lead to very different performance characteristics in different settings. However, despite its shortcomings, no other current screening option is economically viable in many poor countries. Currently the World Health Organization is funding ‘VIA and treat’ roll-out studies in six African countries and preliminary reports are very positive, indicating that ‘something is better than nothing’.²⁹ VIA has enabled severely resource-restricted countries to establish some form of screening infrastructure for older women. While the impact on cervical cancer prevention is likely to be modest to small, creating an infrastructure for the health care of older women is a very good start. The study in India²⁸ indicated clearly that a ‘VIA and treat’ programme was associated with a significant reduction in cervical cancer compared to no screening at all.

Table 1: Indications for therapeutic procedure (ablation or excision)

1. Abnormal Pap smear and ability to perform colposcopy
2. Entire extent of the lesion is visible
3. No significant disparity between diagnostic modalities e.g. cytology, colposcopy and histology
4. No suspicion of microinvasive disease
5. No evidence of glandular abnormality

Table II: Indications for diagnostic procedure (cone biopsy)

Maxim: ‘If you cannot see it you cannot treat it’
1. Abnormal Pap smear but no access to colposcopy
2. Upper limit of the lesion is not visible
3. Significant disparity in diagnostic modalities e.g. HSIL Pap, negative colposcopy
4. Suspicion of invasive disease
5. Evidence of significant glandular abnormality (it is not possible to do colposcopy of endocervix)

HPV DNA testing for cervical cancer precursors

It is beyond the scope of this article to review the extensive literature on testing for high-risk types of HPV as a screening tool. Most studies have shown that HPV DNA testing has a much higher sensitivity than cytology for the detection of cervical cancer precursors, but a significantly lower specificity and positive predictive value. The negative predictive value is virtually 100%, which is important in countries with long screening intervals (i.e. 10 years in South Africa). Many different algorithms have been devised for HPV DNA testing but the FDA in the USA has suggested that there is sufficient evidence to use HPV DNA testing for triage of ASCUS cytology and as a complementary test to cytology in women over 30 years. Other algorithms suggest screening with HPV DNA testing and using cytology to evaluate those with positive tests and only if both tests are positive, should women be referred for colposcopy. The utility and costs of these approaches are still being investigated.

Table 3: HPV types

High risk types:	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82
Probable high risk types:	26, 53, 66
Low risk types:	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108

Munoz et al [19] pooled data from 11 case-control studies from nine countries involving 1918 women with histologically confirmed squamous-cell cervical cancer and 1928 control women.

Conclusion

For the past nearly 100 years, our approach to the prevention of cervical cancer has been to detect cervical cancer precursors and to remove them, either with ablation or excision, in order to prevent progression to cancer. Cytology was once hailed as a great public health intervention but in recent times has come under critical appraisal and considered to be a largely unreliable diagnostic tool, unless performed at regular intervals and subjected to stringent quality control. Alternative methods for secondary prevention of cervical cancer have also been evaluated and shown promising results. However, we remain unable to determine which pre-invasive lesions are most likely to develop into cancer, so we continue to screen large numbers of women, to detect presumed precursors of cervical cancer, without any really reliable discriminatory test.

The advent of HPV vaccines will no doubt introduce a dramatic new tool for the primary prevention of cervical cancer, by prophylactically preventing infection with the most common types associated with cancer, types 16 and 18. However, types 16 and 18 are only associated with 70% of cervical cancers and the contribution of the other high-risk types is not yet well quantified. Hence, secondary prevention with screening will most likely need to continue for a long time to come. It is hoped that with time we will develop screening tests that reliably and reproducibly identify those lesions truly destined to develop into cervical cancer, rather than our current assumption of 'all CIN is bad'.

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