CHAPTER 3.

Squamous intraepithelial lesions: cytology–histology correlation

This chapter discusses the natural history of cervical precancer, HPV and oncogenesis, cytology nomenclature, and the cytological and histological recognition of cervical precancer.

3.1 Current understanding of the natural history of cervical precancer

Cervical cancer has a long precursor stage. The cervix is accessible and sheds exfoliated cells easily, and cytological examination of these cells reveals precancerous changes that are easily eradicated. The essential causative agent of cervical cancer is the presence of high-risk HPV, which is easily detectable. Cervical cancer is a completely preventable disease. This is quite apart from the availability of an effective vaccination. The disease should not exist.

3.2 Historical context

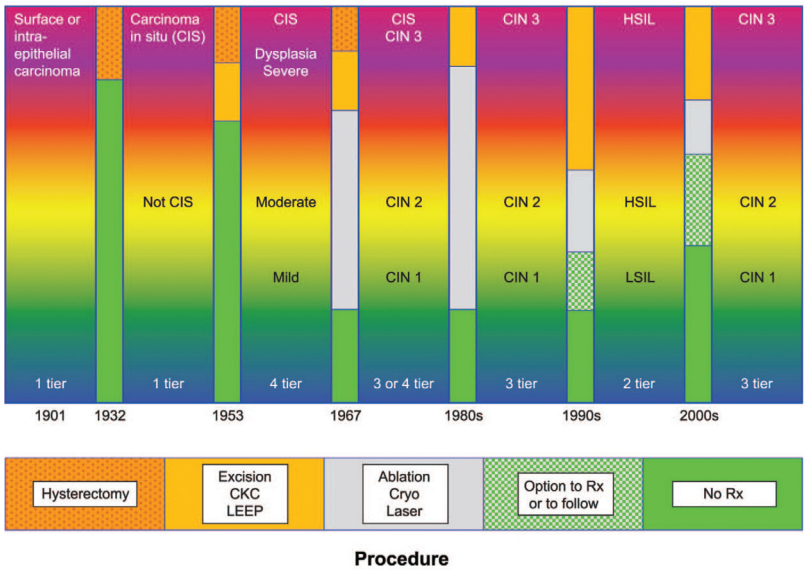
The precursor phase of the natural history of cervical cancer is characterized by cellular changes within the epithelial lining of the cervix; in other words, the abnormality is entirely intraepithelial. John Williams first described intraepithelial cellular changes in tissue adjacent to invasive cancer more than 125 years ago (Williams, 1888). During the early decades of the 20th century, the concept of intraepithelial dysplasia gained acceptance (Cullen, 1900; Rubin, 1910). It implied cancerous-looking cells confined to the epithelium above the basement membrane and led to the term “carcinoma in situ” (Broders, 1932), which was defined as full-thickness cellular changes that looked morphologically similar to undifferentiated invasive carcinomatous cells but were confined to the epithelium. The term “dysplasia” was coined about 20 years later by Reagan and Hicks (1953), and dysplasia was categorized as being mild, moderate, or severe depending on the proportion of the epithelial layers involved in the dysplastic process. Carcinoma in situ was considered to have a greater degree of abnormality and to be the final precancerous state. The term “koilocyte” (halo or vacuolated cytoplasm or empty space cytoplasm) was coined by Koss and Durfee (1956). Meisels and Fortin (1976) first recognized these cells as being infected with HPV.

Richart (1968) introduced the concept of a continuum and subdivided the spectrum of abnormality into three categories, called CIN grades 1 (mild dysplasia), 2 (moderate dysplasia), and 3 (severe dysplasia). In this classification, carcinoma

in situ was combined with severe dysplasia. The cytological classification was similar in that mild, moderate, and severe dyskaryosis were suggestive (but not diagnostic) of CIN1, 2, and 3, respectively. The relative ease of treatment afforded by outpatient therapy, which had begun to replace hysterectomy and cold-knife conization in the 1970s and 1980s, lowered the threshold for treatment of cervical lesions. In an attempt to simplify the classification and because it had become clear that minor-grade lesions did not often progress to cancer, Richart (1990) proposed a two-tier classification system. High-grade lesions were thought to be much more likely to be genuinely precancerous. Low-grade lesions were considered to be transient and rarely precancerous. Many low-grade lesions were associated with koilocytosis and recognized as being HPV-related. However, this classification system was not universally used. Also, moderate abnormalities, some of which were undoubtedly low-grade in nature, were included in the high-grade category and perhaps treated too readily. The different classifications are represented in the diagram in Fig. 3.1, with treatment patterns included below. The traditional "screen, diagnose, and treat" pathway (Fig. 3.2) worked reasonably well when the threshold for referral to colposcopy was set high.

The concept of a continuum persisted until relatively recently. A greater understanding of the biology of oncogenic HPV and its different effects in squamous epithelium of the lower genital tract has led to a different concept. It now seems clear that there are two different types of HPV infection. The first type is an innocent and transient infection, which may produce mild or low-grade lesions that are recognizable cytologically, colposcopically, or histologically.

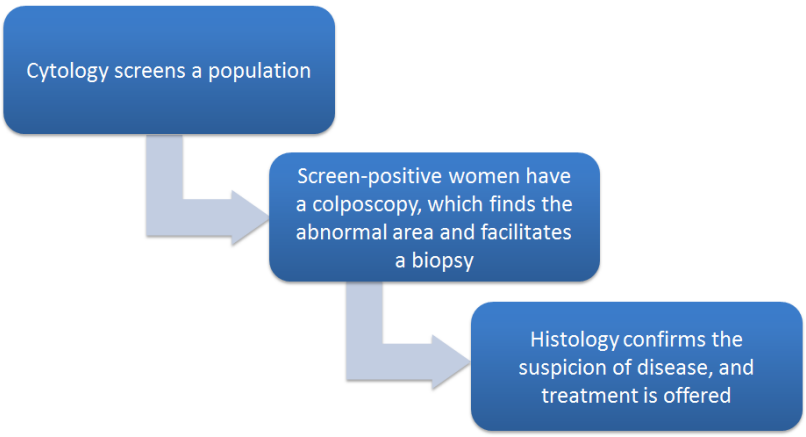
Fig. 3.1. Changing terminology and treatment trends for cervical precancer over the past century. CKC, cold-knife conization; Cryo, cryotherapy; LEEP, loop electrosurgical excision procedure; Rx, treatment.



These lesions have limited, if any, precancerous potential for progression to cancer. This type of infection is called a productive infection. The key step in the pathogenesis of HPV-linked cancers is the activation of the viral oncogenes E6 and E7 in the basal and parabasal cells of the infected epithelium (Bergeron et al., 2015; Doorbar et al., 2012; Duensing and Münger, 2004). If these viral genes are expressed in basal or parabasal cells, they trigger chromosomal instability and major numerical and structural alterations of the host cell chromosomes. This leads to uneven distribution of the overall DNA content (aneuploidy) and is reflected by shifts of the nuclear staining pattern (the staining intensity). This type of infection is more readily recognized cytologically, colposcopically, and histologically and is called a transforming infection (see Chapter 4).

Sometimes moderate dyskaryosis (at cytology) or moderate

Fig. 3.2. Traditional process of screening test, colposcopic assessment, histological diagnosis, and treatment.



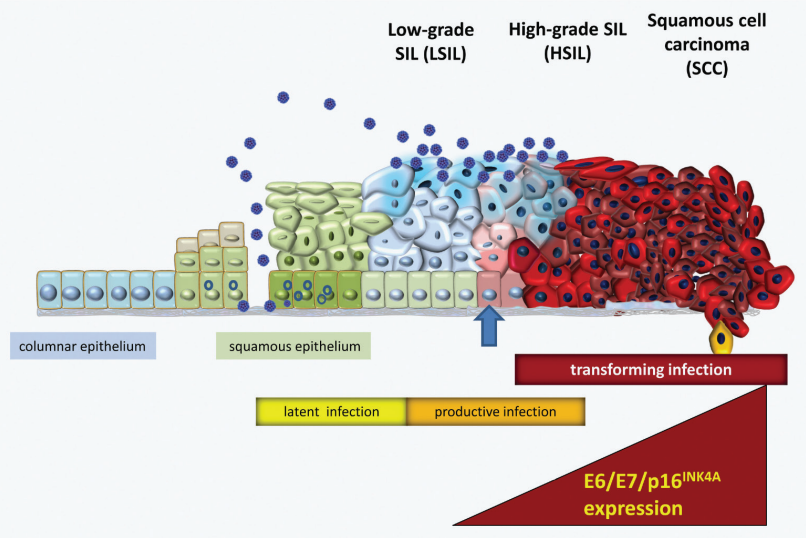
dysplasia (at histology) may contain both types of infection, and these are difficult to distinguish using cytology or histology. Fortunately, developments in molecular biology have led to specific biomarkers of cell biology that can discriminate between these types where doubt exists (see Chapter 4).

3.3 HPV and the genesis of cervical cancer

Several different risk factors have been implicated for cervical cancer and precancer. These include smoking, early age at first intercourse, nutritional deficiency, chlamydial infection, multiple sexual partners, multiple pregnancies, and long-term use of oral contraceptives (Bosch et al., 1995; Franco et al., 1999; IARC, 2007, Schiffman et al., 1996; Walboomers et al., 1999). However, the fundamental and essential causative agent is the persistence of oncogenic HPV in the epithelium of the TZ and/or adjacent glandular epithelium. The relationship between oncogenic HPV and cervical precancer appears, at first, paradoxical. Cervical cancer is always associated with oncogenic HPV, but oncogenic HPV is a normal and usually transient infection that most healthy sexually active women will encounter in early reproductive life. The current thinking is that the oncogenic HPV gains entry to the cervical epithelium at the new SCJ, possibly associated with minor abrasions, and that this allows the virus to access reserve cells underneath the single layer of columnar epithelium (Fig. 3.3).

Most women will be infected with oncogenic HPV, and the great majority will clear the infection without any residual harm or increased risk of cervical cancer. In a small percentage of women, the infection persists, and in a small proportion of those, it becomes integrated into the epithelial cell nuclei and changes from a latent to a transforming infection. It is in those cases that the risk of progression is high. It is not known what distinguishes those cases in which the virus becomes integrated and transforming from those in which the infection is transient and harmless. The relationship between oncogenic HPV infection and the risk of progression or clearance is discussed in Chapter 4. The crucial step is that of the HPV infection becoming a transforming infection.

Fig. 3.3. Different HPV infection stages.



3.4 Cytology nomenclature

To this day, there are several different cytological classifications for cervical precancer. The German classification is used in Germany, Austria, and some countries in eastern Europe. The United Kingdom has its own classification, as do Australia and New Zealand. Perhaps the most widely used classification is the Bethesda terminology system, first introduced in 1988 by the United States National Cancer Institute (Solomon, 1989). It embraced the concept of a two-tier gradation and has undergone several revisions over the past 25 years. These revisions reflect the changing understanding of risk associated with different cytological and histological reporting and a greater understanding of the role of oncogenic HPV. The United Kingdom classification now reflects the Bethesda two-tier classification. To help clinicians manage their patients with different grades of abnormality, the ASCCP developed a series of clinical guidelines linked to the Bethesda classification (Wright et al., 2003). In the United Kingdom, the NHS Cervical Screening Programme (NHS, 2010) produced an evidence-based guidelines document, which linked management to the degree of cytological abnormality and other relevant case characteristics (e.g. HPV test result, age, and smoking history). It has recently been updated (NHS, 2016). Fig. 3.1 attempts to relate some of the previous cytology nomenclatures to the current Bethesda classification, which is probably the most widely used system today.

There has always been an interdisciplinary dependency in management of cervical precancer. Traditionally, this has been using

cytology to screen, using colposcopy to assess and direct biopsy, and using histology to confirm the diagnosis (Fig. 3.2). In this idealized scenario, the cytology screening test identified cases that may or may not have genuine precancer, colposcopy was able to recognize or rule out the lesion, and a colposcopically directed biopsy facilitated definitive histological proof of disease before treatment was advised. But all three of these disciplines are subjective in nature. Until recently, histology was considered the gold standard and HSIL was considered the threshold at which treatment was necessary. It is now clear that morphological assessment at histology is also less than perfect, in particular the determination of disease severity when morphological or histopathological examination reports HSIL-CIN2. A paper from the Lower Anogenital Squamous Terminology (LAST) Project (Darragh et al., 2012) finally confirmed the relative subjectivity of histopathology, especially in the middle grade of CIN2. The WHO 2014 histology terminology (Kurman et al., 2014) proposed a two-tier classification, HSIL and LSIL, with the help of biomarkers to differentiate the difficult or equivocal cases.

3.5 Cytological and histological recognition of cervical precancer

3.5.1 Normal cervical epithelium

Cytological examination of exfoliated cells from the normal ectocervical squamous epithelium will reveal mostly superficial cells; the nuclei are small, are not hyperchromatic, and have normal density and shape with normal chromatin patterns. Crucially, the nuclear–cytoplasmic ratio is low, and mitotic figures are only occasionally seen in the basal layers (Fig. 3.4a).

Histological examination of a tissue biopsy of normal squamous epithelium will reveal normally stratified epithelium with regular maturation and few mitotic figures in the basal layers. As with cytology, there will be normal nuclear–cytoplasmic ratios and the nuclei will be morphologically normal (Fig. 3.4b and Fig. 2.2).

3.5.2 LSIL (HPV infection; CIN1; mild dyskaryosis)

3.5.2.1 Cytology

The cytological recognition of abnormality is based on the finding of nuclear enlargement and variation in the size and shape of abnormal cells. An increased intensity of staining with irregular chromatin patterns is another common feature of abnormality. These abnormalities in the superficial and intermediate cells are koilocytosis, typical of a productive infection (LSIL). Abnormal nuclei and other cell changes in parabasal and basal cells are typical of a transforming infection (HSIL). In the case of an LSIL, as in Fig. 3.5a, there is a productive viral infection, and cytology will reveal enlarged nuclei with vacuolated cytoplasm in superficial and intermediate cells.

3.5.2.2 Histology

Histological determination of abnormality is essentially recognition of abnormal cellular proliferation. It is based on the morphological assessment of cells in the epithelium, the architecture of the cellular layers, and the degree of maturation and cellular differentiation. The relative proportion of the epithelium that is involved with abnormality, the degree of maturation, and the persistence of mitotic figures throughout the epithelium are the usual parameters used to grade the abnormality. Histological examination of LSIL will reveal

Fig. 3.4. (a) Normal cytology preparation; intermediate cells are indicated with arrows. (b) Normal histological section of squamous epithelium.

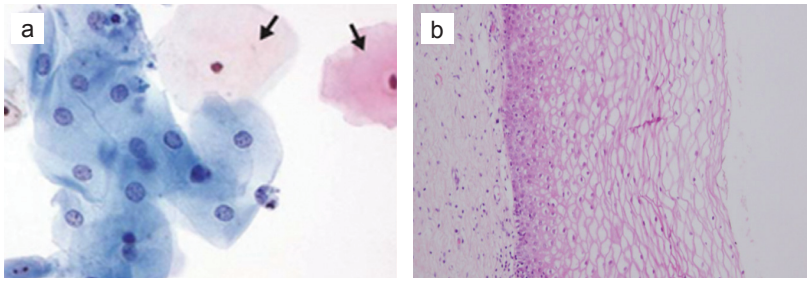
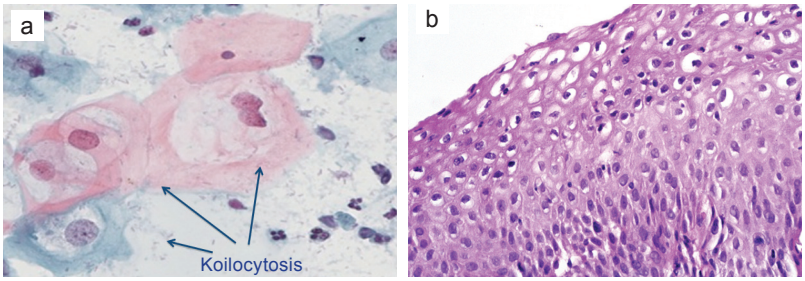


Fig. 3.5. (a) Cytology slide of LSIL. (b) Histological section of LSIL.



koilocytosis in the superficial layers and even part of the intermediate layer, but the undifferentiated cells will be limited to the lower third of the epithelium (Fig. 3.5b).

3.5.3 HSIL (CIN2, CIN3; moderate dyskaryosis, severe dyskaryosis)

3.5.3.1 Cytology

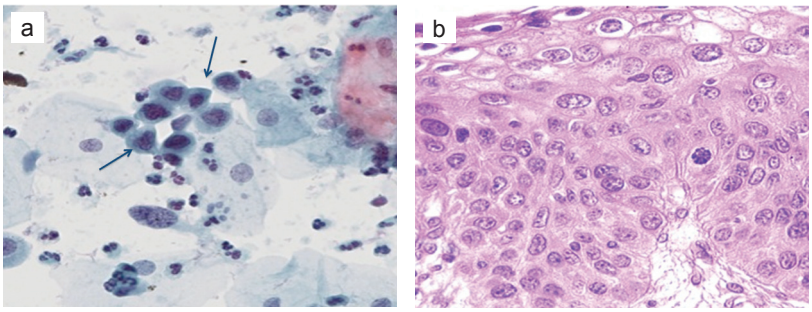
With a severely abnormal CIN3 lesion, cytology will report the diagnosis of HSIL. Cytology, by itself, cannot distinguish between CIN2 and CIN3. The changes seen at cytology will usually include a definite increase in the nuclear–cytoplasmic ratio as well as abnormal nuclear size and density and altered chromatin patterns of basal or parabasal cells (Fig. 3.6a).

3.5.3.2 Histology

At histological examination of a clear case of CIN3, the great majority of pathologists will agree, because the morphological cellular and architecture changes in the epithelium are relatively unequivocal and are disordered throughout all cellular layers (Fig. 3.6b). Cytological examination of an HSIL cannot be as precise, and a cytologist reporting HSIL will probably describe basal cells that have risen to the intermediate or superficial layers, which are abnormal with enlarged nuclei and reduced cytoplasm, as in Fig. 3.6b.

However, the histological diagnosis is not robust in the middle grade, and the category of CIN2 or HSIL-IN2 contains some cases where the virus is transforming and the risk of progression is real and some cases where the virus is proliferative and not transforming and the risk of progression to cancer is very small. Morphological examination of tissue biopsies from CIN2 cases is not reliable, and pathologists will often not agree. Some will call the case CIN3, and some will call it CIN1. In this situation, molecular biology tests can resolve the disparity. To appreciate how molecular biology tests can help, it is necessary to understand a little about the biology of oncogenic HPV and its effect on squamous epithelium (see Chapter 4).

Fig. 3.6. (a) Cytology slide of HSIL. The arrows indicate abnormal squamous basal cells. (b) Histological section of HSIL-CIN3. Cellular abnormality prevails throughout the full thickness of the epithelium. There is an increased nuclear–cytoplasmic ratio, anisocytosis, and a loss of nuclear polarity. Several mitoses are present throughout the upper two thirds of the epithelium.



Key points

- Oncogenic (or high-risk) HPV is an extremely common infection in healthy sexually active women of reproductive age.
- Cervical cancer is a very rare outcome of oncogenic HPV infection but does not occur in its absence. Up to 80% of women will harbour oncogenic HPV during their reproductive life, but only 1 in 10 000 or fewer will develop cervical cancer.
- A positive high-risk HPV test does not imply cancer, precancer, or even an active infection.
- Cytological, colposcopic, and histological recognition of cervical cancer precursor states are all imperfect, because of their innate subjectivity.