CHAPTER 4.

The effect of oncogenic HPV on transformation zone epithelium

All squamous cervical cancer (and probably all cervical adenocarcinoma) is associated with oncogenic HPV, and the absence of oncogenic HPV means that there is virtually zero risk of developing cervical cancer or genuine precancer during the next 5 years or more Oncogenic HPV tests will reveal the presence or absence of viral DNA in a sample taken from the cervical epithelium or adjacent epithelial surfaces. But the presence of oncogenic HPV does not always mean that an active infection is present. The virus may be latent or may even be present at the epithelial surface but not active or productive. Even when an infection is present, this does not, by itself, mean that disease or pre-disease is likely. An infection may be transient and therefore harmless, and this is the usual outcome in young women. An infection may also be highly productive and yet confer no increased risk of cervical precancer. Many LSILs are highly productive and have very high viral load counts but confer minimal risk of progression to high-grade lesions and, thereafter, cancer. Fig. 3.3 illustrates the difference between latent, productive, and transforming infections. A positive oncogenic HPV test does not indicate whether the infection is latent or productive or whether it is the much rarer transforming infection.

Fortunately, an increasing number of molecular biological markers have enabled a better understanding of the pathway that progressive lesions take (Bergeron et al., 2010; Doorbar, 2006; Doorbar et al., 2012). Different measurable viral or cellular products are produced at different stages of oncogenic HPV infection, depending on whether it is a productive infection or a transforming infection.

The secondary biomarkers produced at different biological stages of viral activity are proteins that are viral or cellular gene products, and these proteins are measurable in cervical samples cytologically and/or histologically. In summary, oncogenic HPV tests determine the presence or absence of virus particles, but protein biomarkers determine the virus activity, and it is this activity that reflects the risk of progression to cervical cancer. These markers include HPV proteins, surrogate markers (e.g. p16), and methylation patterns. Perhaps the best way to understand the relative value of cytology, histology, and these biomarkers in the recognition and management of cervical precancer is by looking at cytological, histological, and biomarker images of normality, LSIL, and HSIL.

4.1 Biomarker assessment

4.1.1 Normal viral cellular pathway

In a normal but HPV-infected cell, the viral infection expresses proteins in a defined order and in different layers at different stages of the normal cell life-cycle (Fig. 4.1).

The E6 and E7 viral proteins are expressed in the lower layers of the epithelium, and they reflect an initiation of the cell cycle. The basal cells proliferate, and viral copy numbers increase. The E6 and E7 proteins are expressed at very low levels, but fortunately surrogate markers, such as MCM and Ki-67, are identifiable, and they faithfully reflect the presence of E6 and E7. Also, some true viral proteins are measurable, for example E4. This protein is not part of the virus particle but is present in very high quantities during the normal cell cycle. It is a marker for cell-cycle completion.

4.1.2 LSIL

With an LSIL, there is early deregulation of the cell cycle. There are increased levels of E6 and E7 in the basal and parabasal layers, and therefore MCM is present (Fig. 4.2).

Also, the biomarker p16 begins to appear in the lower epithelial layers, for two reasons: because of E6 and E7 deregulation, but also because p16 is a marker for oncogenic HPV activity, especially activity of HPV type 16. It is not necessary to use the biomarker p16 when there is clear cytological and histological evidence of LSIL, because the morphological criteria for diagnosis are clear and most cytologists and pathologists will agree.

Fig. 4.1. Productive HPV infection; normal viral life-cycle. Expression of E6 and E7 is recognized by the presence of surrogate markers, such as MCM. E4 is present and detectable mostly in the upper layers of the epithelium.

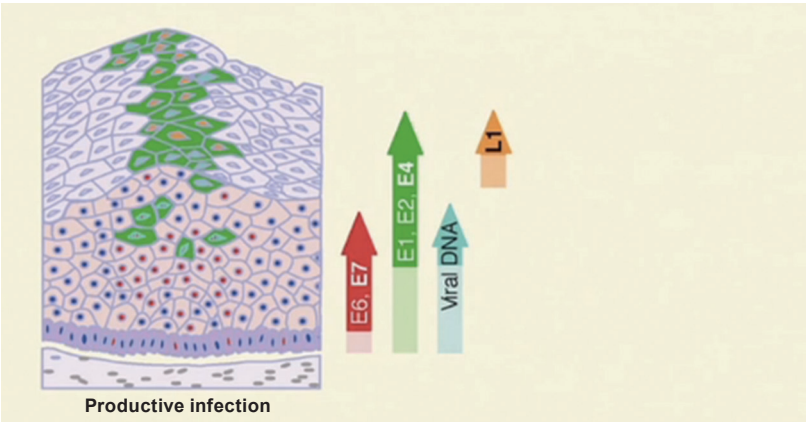
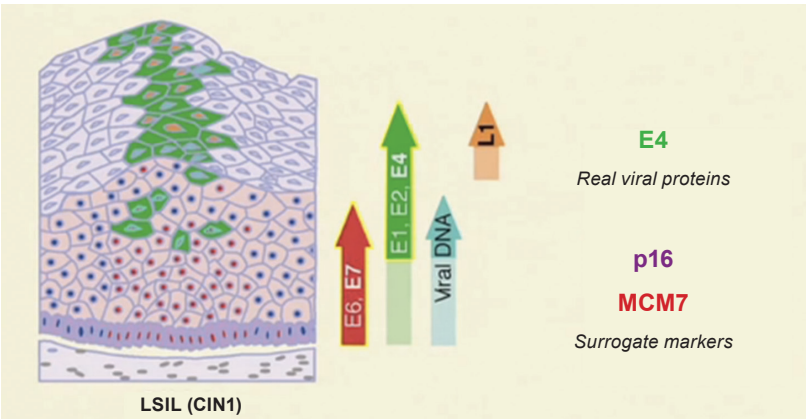


Fig. 4.2. Biomarker expression in a low-grade lesion.



4.1.3 HSIL-CIN3

In a case of HSIL-CIN3, cellular activity is completely disordered and the abnormal basal and parabasal cells get pushed up through the cellular layers. At the same time, E4 (which reflects cell-cycle completion) appears higher and higher up the cellular layers and eventually disappears. p16 will be present and detectable in the histological section (Fig. 4.3). This is because p16 is overexpressed in high-grade lesions, because the normal regulatory inhibition of p16 is diminished. With CIN3, the morphological patterns are again relatively clear and interobserver variation is uncommon, so it is not necessary to use p16 because the morphological changes are clear and almost all pathologists will agree on the grade.

4.1.4 HSIL-CIN2

Biomarkers are particularly helpful in the evaluation of a case reported as being HSIL that histologically appears to be a moderate abnormality of CIN2. The pattern of MCM, p16, and E4 can discriminate between whether the CIN2 is actually just a proliferative infection and is destined to behave like a CIN1 lesion or whether it is associated with a transforming infection and will behave like a CIN3 lesion (Fig. 4.4). Here, both E4 and p16 will discriminate between those lesions that have a transforming infection and are CIN3 and those that do not and may be classified

Fig. 4.3. Biomarker expression in CIN3.

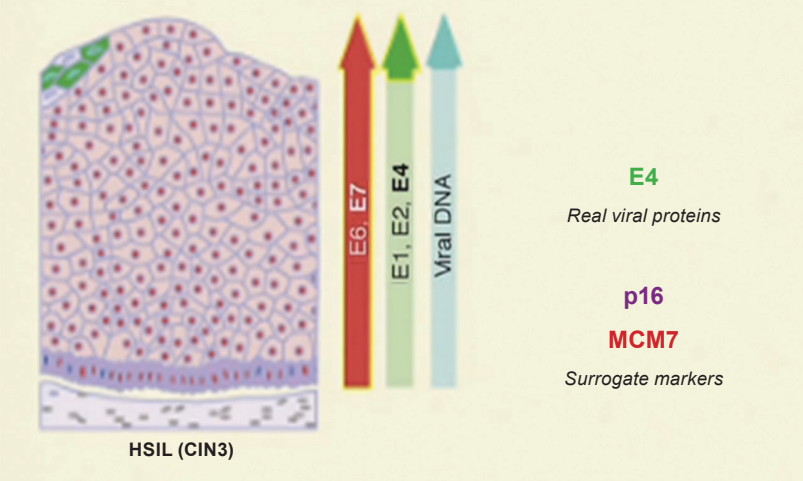
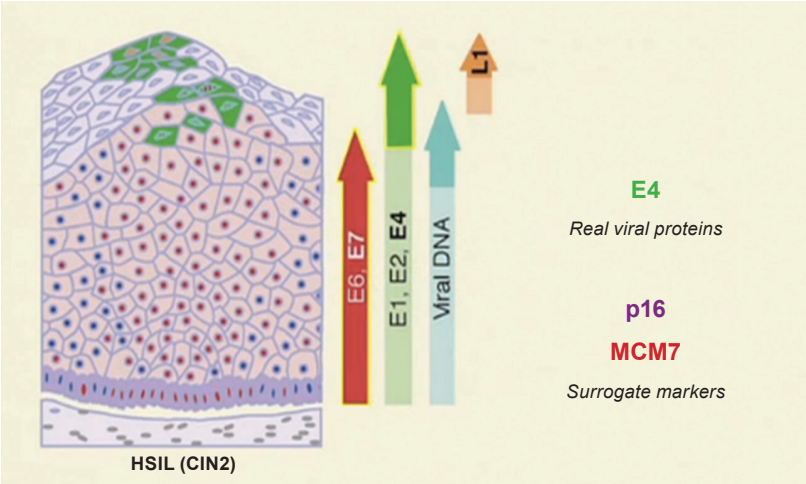


Fig. 4.4. Biomarker expression in an HSIL case that is CIN2.



as CIN1. E4 will be absent and p16 will be strongly present throughout the full thickness of the epithelium in those HSILs that are actually CIN3 but that morphologically may be considered to be CIN2. Fig. 4.5 illustrates the markers of different oncogenic HPV infection states.

4.2 Clinical utility of biomarkers

4.2.1 Biomarkers in histology

In the recent consensus report of the LAST Project (Darragh et al., 2012), the authors read more than 2000 articles dealing with molecular markers and culled this to 72 publications that satisfied pre-specified quality criteria. Of these, there were 53 that dealt with p16, and from these 53 studies consensus recommendations were derived. p16 is the only biomarker that the consensus document considered had sufficient evidence to recommend its routine use in histological specimens in defined cases. These recommendations are summarized in Table 4.1.

Fig. 4.6 shows an example of how p16 can aid in the evaluation of a lesion perhaps considered to be CIN2, which is best interpreted as HSIL. Fig. 4.7 shows an example of a histological section where p16 influenced the diagnosis as LSIL, which might otherwise have been considered HSIL.

4.2.2 Biomarkers in cytology

p16 on its own is not currently considered to be a useful discriminator in cytology. As described above, its value is in histological examination of specific equivocal diagnoses where the degree of staining and where the stain can be seen throughout the cell layers of the epithelium. p16 is expressed in about 40% of low-grade lesion smears in the lower epithelial levels. In cytology, the cellular architecture and site of staining cannot be recognized, and p16 is therefore less valuable.

Ki-67 is a proliferative marker and is expressed in proliferating cells within the nucleus of parabasal cells of normal epithelium. When Ki-67 is overexpressed, it indicates a proliferative cellular state.

The combination of p16 and Ki-67 (known as dual testing) is useful in cytology (Fig. 4.8). The recent international multicentre study of the utility of p16 and Ki-67 in cytology has produced good evidence that this combination will confirm that

Table 4.1. Recommendations for biomarkers in HPV-associated lower anogenital squamous lesions

Recommendation	Comment
1. p16 IHC is *recommended* when the H&E morphological differential diagnosis is between precancer (CIN2 or CIN3) and a mimic of precancer (e.g. processes known to be not related to neoplastic risk, such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting).	Strong and diffuse block-positive p16 results support a categorization of precancerous disease.
2. If the pathologist is entertaining an H&E morphological interpretation of CIN2 (under the old terminology, which is a biologically equivocal lesion falling between the morphological changes of HPV infection [low-grade lesion] and precancer), p16 IHC is *recommended* to help clarify the situation. Strong and diffuse block-positive p16 results support a categorization of precancer. Negative or non-block-positive staining strongly favours an interpretation of low-grade disease or a non-HPV-associated pathology.	
3. p16 is *recommended* for use as an adjudication tool for cases in which there is a professional disagreement in histological specimen interpretation, with the caveat that the differential diagnosis includes a precancerous lesion (CIN2 or CIN3).	
4. WG4 *recommends against* the use of p16 IHC as a routine adjunct to histological assessment of biopsy specimens with morphological interpretations of negative, CIN1, and CIN3.	
a. SPECIAL CIRCUMSTANCE: p16 IHC is recommended as an adjunct to morphological assessment for biopsy specimens interpreted as ≤ CIN1 that are at high risk for missed high-grade disease, which is defined as a prior cytological interpretation of HSIL, ASC-H, ASCUS/HPV-16+, or AGC (NOS).	Any identified p16-positive area must meet H&E morphological criteria for a high-grade lesion to be reinterpreted as such.

AGC (NOS), atypical glandular cells – not otherwise specified; ASC-H, atypical squamous cells, cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; H&E, haematoxylin and eosin; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IHC, immunohistochemistry; WG4, Working Group 4.

Fig. 4.5. High-risk HPV infection and its possible consequences. (a) The detection of HPV DNA in a tissue biopsy may indicate productive (LSIL) or abortive (HSIL) infection, the presence of virus particles at the epithelial surface without infection (e.g. from recent transmission), or a latent or silent infection. Infection requires the entry of HPV virions into the mitotically active epithelial cells of the basal layer, which in stratified epithelium is thought to require a microwound. In the columnar cell layers, infection is thought to be facilitated by the proximity of the target cell to the epithelial surface, which may allow the virus to access a cell type that is unable to support the full productive life-cycle (right). The significance of infection of different cell types remains to be properly assessed. (b) After infection, shown in (a), expression from the viral genome can sometimes be suppressed (e.g. by genome methylation), leading to a "silent" infection in which the viral genomes are retained in the basal layer without apparent disease. Infection may alternatively lead to an ordered pattern of viral gene expression, leading to virus synthesis and release from the upper epithelial layers (productive infection or LSIL [CIN1]) or to deregulated viral gene expression and high-grade neoplasia (HSIL [CIN2/CIN3]). Persistent high-grade disease is associated with an increasing risk of genome integration into the host cell chromosome and progression to cancer. Cells in cycle are indicated by the presence of red nuclei. Cells expressing E4 are shown in green, and those expressing L1 are shown in yellow. The brown shading identifies all the cells (differentiated and undifferentiated) that contain viral genomes. (c) In most cases, HPV infections are resolved as a result of a cell-mediated immune response (left). This may lead to viral clearance or to viral latency and the persistence of viral episomes in the epithelial basal layer without life-cycle completion. Viral gene expression patterns during latency are not well characterized. Persistent deregulated gene expression, as occurs in CIN3 and after viral genome integration, can lead to the accumulation of secondary genetic changes in the infected host cell and development of cancer. This is facilitated by overexpression of the high-risk E6 and E7 proteins. Cells in cycle are shown by red nuclei. Brown shading in the immune latency state indicates cells harbouring viral episomes. In cervical cancer, the viral genome is often integrated, with loss of expression of full-length E1, E2, E4, and E5 and the L1 and L2 capsid proteins, and with deregulated expression of E6 and E7.

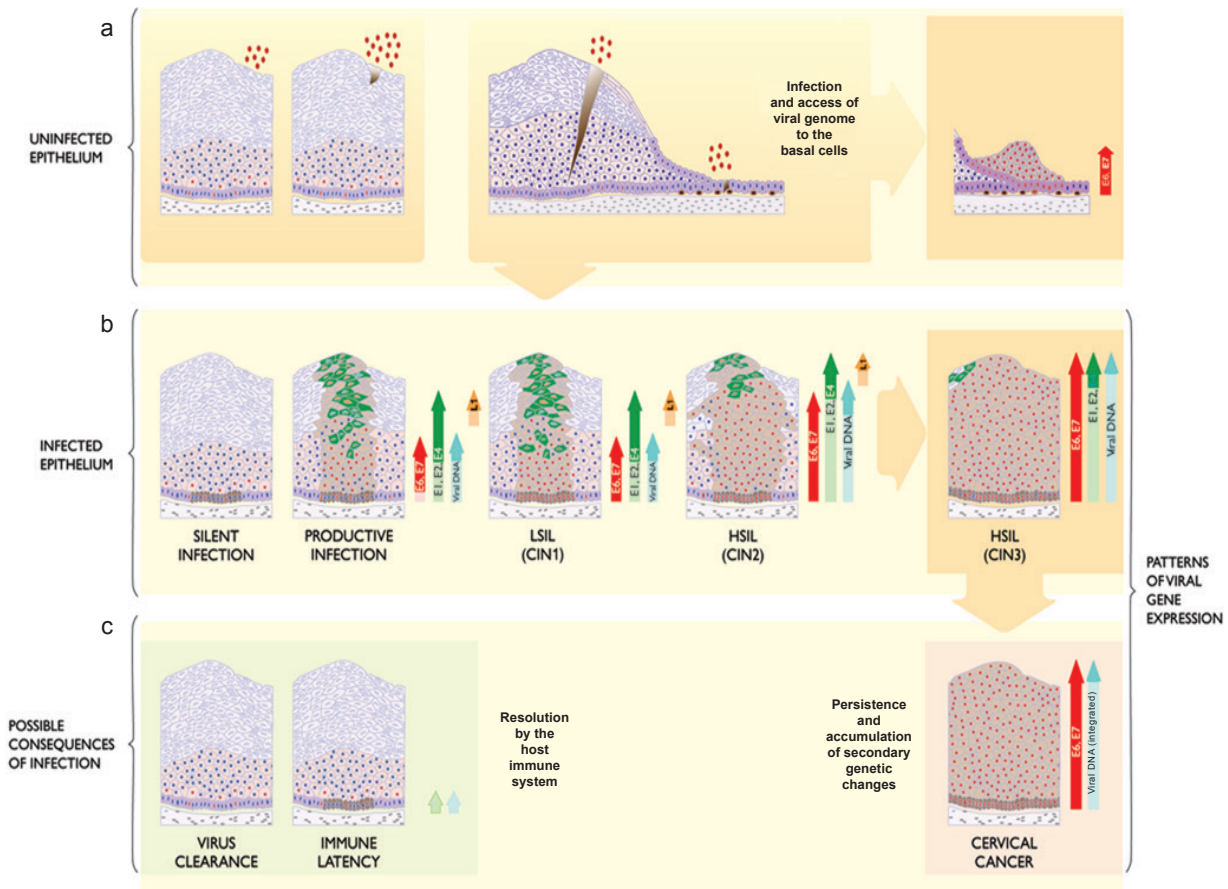


Table 4.2. Utility of biomarkers to qualify cytological and histological interpretation

Test	Performance	Biomarker	How biomarker helps
Cytology	75% sensitivity and 95% specificity	HPV	98% sensitivity but low specificity
		p16/Ki-67	90% sensitivity but better specificity than HPV and similar specificity to cytology
Histology	High kappa for HSIL (CIN3) Poor discrimination of CIN2 lesions	p16	Pushes CIN2 to either true low-grade or true high-grade lesion

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

Fig. 4.6. Cervical biopsy with SIL showing partial maturation; some might question the lesion grade (CIN2?). (a, c) Haematoxylin and eosin morphology at low and medium power with atypical parabasal-like cells extending into the middle third of the epithelium (c). (b, d) Corresponding p16 immunohistochemical stains with diffuse strong staining meeting the definition of strong and diffuse block-positive p16. Therefore, this case is best interpreted as HSIL.

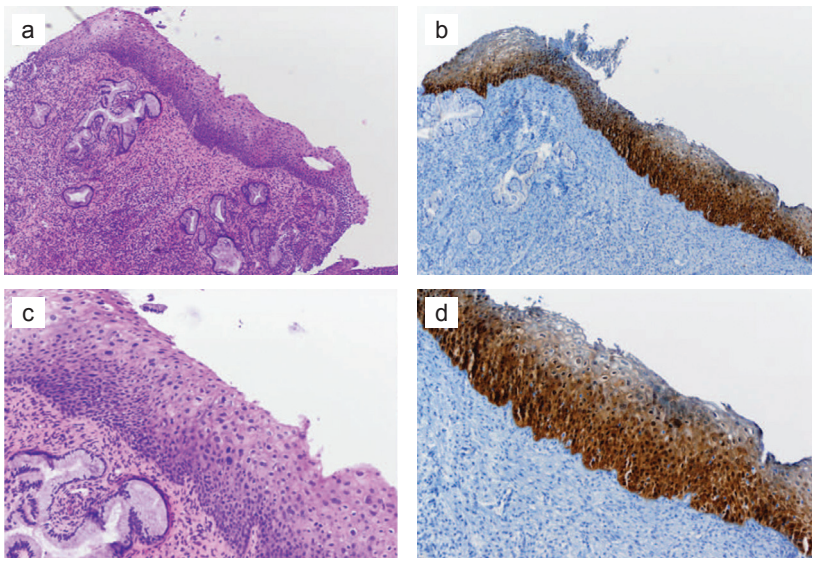
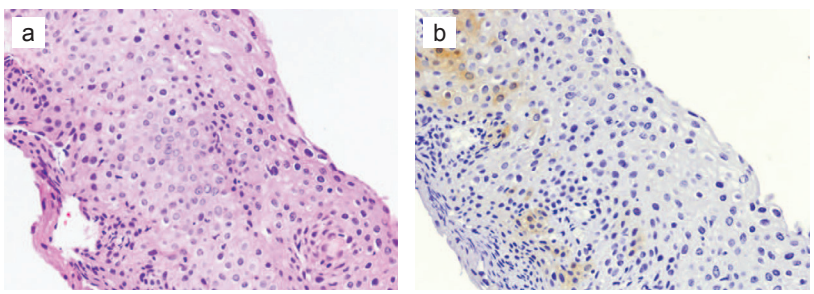


Fig. 4.7. (a) Cervical biopsy (high power, haematoxylin and eosin) with unequivocal SIL that is tangentially cut, raising the differential diagnosis of LSIL versus HSIL. (b) High-power p16 immunohistochemical stain, demonstrating weak, patchy p16 reactivity that starts above the basal layer, a pattern that should be interpreted as negative, which, in this case, supports the final combined interpretation as LSIL.



there is a proliferative cellular state and a transforming infection, thereby indicating the likelihood of genuine cervical precancer, i.e. a high-grade lesion (Ikenberg et al., 2013). Indeed, as Ikenberg and colleagues concluded in their large pan-European multicentre study of dual testing, "The p16/Ki-67 dual-stained cytology combines superior sensitivity and non-inferior [similar] specificity over Pap cytology for detecting CIN2+. It suggests a potential role of dual-stained cytology in screening, especially in younger women where HPV testing has its limitations."

4.3 Summary

The understanding of the biology of HPV and precancerous lesions has improved, and there are now objective biomarkers to identify specific and clinically important stages in the natural history of oncogenic HPV and the host cervical epithelium. As a result, the discipline of molecular biology has entered the arena of management and has improved the functional test characteristics of both cytology and histology (Table 4.2). As well as the information resulting from cytology, histology, colposcopy, and biomarker tests, the individual case characteristics of each patient will be weighed up by the colposcopist in deciding management (Fig. 4.9). There is no doubt that the newer objective biomarker tests are likely to make this decision analysis easier.

Fig. 4.8. p16/Ki-67-positive abnormal basal cells.

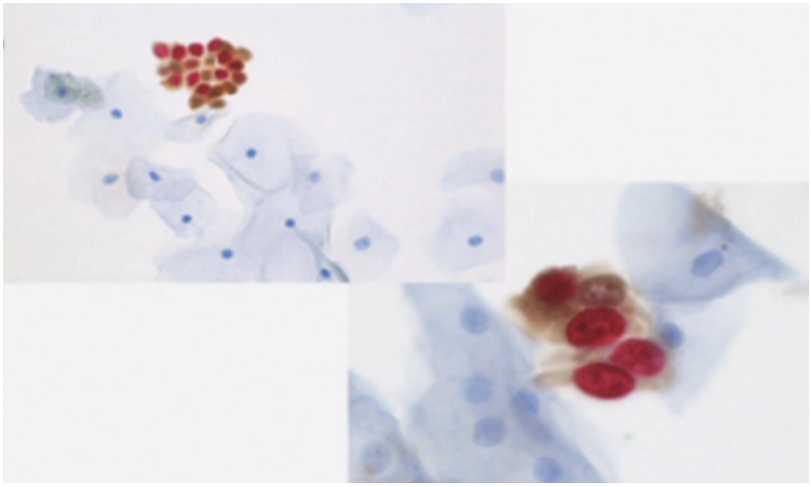
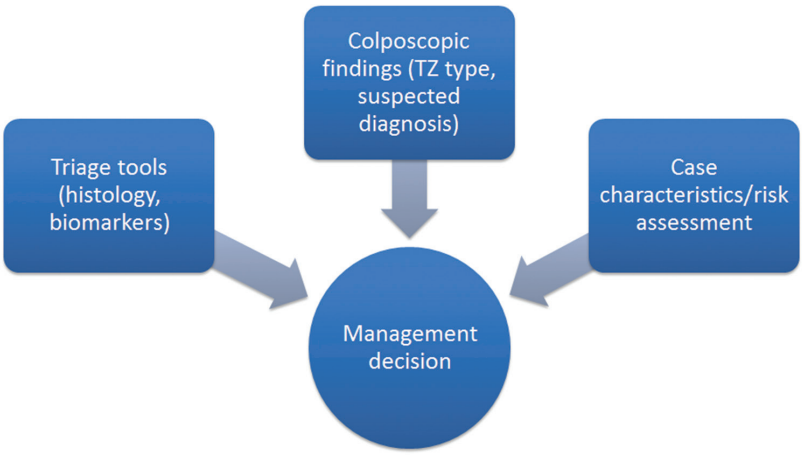


Fig. 4.9. Influences on the colposcopist's management advice. TZ, transformation zone.



Key points

- HPV affects the genital tract of most women early on in their reproductive life and only very seldom causes cervical precancer.
- The negative predictive value of a negative oncogenic HPV test approaches 100% over the subsequent 5 years.
- Secondary biomarkers produced at different biological stages of viral activity are measurable in cervical samples. Some protein biomarkers reflect viral activity rather than viral presence and are helpful in selecting those HPV states that are likely to progress to cancer.