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Series Editors: Naveena Singh · W. Glenn McCluggage

C. Simon Herrington *Editor*

Pathology of the Cervix

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Pathology of the Cervix

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Preface

It is a great privilege to edit this volume on cervical pathology as part of the series of books on gynecological pathology. The structure of the book is similar to other volumes in the series, beginning with a discussion of normal structure, in this case focusing particularly on the cervical transformation zone and the putative cells of origin of neoplastic cervical lesions. Cervical disease is dominated by the effects of human papillomavirus (HPV) infection, and the content of the book reflects this, with both a specific chapter on HPV and discussion of HPV and its relationship to cervical lesions in several of the other chapters. Chapters on cervical screening and the management of cervical cancer are followed by sequential treatment of benign, preinvasive, and invasive squamous and glandular lesions of the cervix, both HPV- and non-HPV-related. The final chapters focus on mesenchymal and mixed tumors, and other neoplasms including neuroendocrine tumors. Appendices outlining specimen handling, tumor staging, and the use of frozen section diagnosis are also included.

I am extremely grateful to the chapter contributors for their excellent contributions. Thanks go also to the publishers, who have seen the project through to completion. Finally, I would like to thank the series editors for asking me to edit this volume, which I hope will be of value to those who read it.

C. Simon Herrington

Edinburgh, UK

Preface to the Series

Over the last few decades, the study of cervical cancer has elucidated not only mechanisms of oncogenesis but also the fantastic potential of an effective cancer prevention strategy. Given this knowledge, it is lamentable that this preventable cancer continues to afflict more than half a million women worldwide annually. More than three-quarters of all cases occur in low-resource nations, and in wealthier nations, there are striking differences between privileged and underprivileged areas. The differences in incidence are mirrored in mortality statistics, with far higher proportions of fatal cases in women who have never been screened.

It is important therefore to continuously strive towards improving outcomes, starting with diagnostic accuracy. While the human papillomavirus (HPV) accounts for the vast majority of cervical cancers, recent years have also thrown light on the biology of the less frequent but generally more aggressive HPV-independent cancers which are mainly of glandular type. Advances in diagnostic adjuncts including biomarkers and efforts to standardize terminology and classification have the potential to improve diagnostic reproducibility. Disease outcomes have improved with newer treatment strategies designed to reduce the adverse effects of treatment and to promote fertility-conserving options, while clinical trials continue to address unanswered questions.

The British Association of Gynaecological Pathologists (BAGP) was formed with the objectives to promote the health of women by the study of the pathology of gynecological diseases, to advance the knowledge and practice of gynecological pathology, and to improve the accuracy of pathological diagnosis. The BAGP fulfills these objectives through educational meetings, courses, collaborative projects, surveys, and posting of educational material on its website (www.thebagp.org). Endorsement of this textbook series is part of its goal to promote accuracy and precision in diagnostic gynecological pathology.

It is with pleasure and pride that we present this third volume of the series. Gynecological pathology forms a major part of the workload of most histopathology laboratories. The female genital tract is complex, and the main intention behind producing this series is to provide detailed information on specific areas in a compact and affordable format. We hope that this timely update will be of interest to trainee and consultant pathologists worldwide.

Naveena Singh
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1. Development of the Uterine Cervix and Its Implications for the Pathogenesis of Cervical Cancer

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Abstract
Normal development of the uterine cervix has been widely studied and the origin of both the columnar and squamous epithelia, as well as the molecular basis of their differentiation, has been established. The process of early carcinogenesis in the uterine cervix has also been described extensively, in particular with respect to the role of human papillomavirus (HPV) infection. However, questions remain about the progenitor cell(s) that play(s) a role in normal (embryonic and fetal) development, as well as in the oncogenic processes that take place in the transformation zone of the uterine cervix. This chapter describes the development of the human lower female reproductive tract, in particular the cervical squamocolumnar junction, and its implications for the pathogenesis of cervical cancer.

Keywords Stem cells – Reserve cells – Cervical carcinogenesis – HPV target cells – Fetal uterine cervical development

Introduction

This chapter describes the development of the human lower female reproductive tract,

in particular the squamocolumnar junction, and the implications for the pathogenesis of cervical cancer. Normal development of the uterine cervix has been studied by several research groups, who have described features of the origin of both the columnar and squamous epithelia, as well as the molecular basis of their differentiation [1–5]. The process of early carcinogenesis in the uterine cervix has been described extensively, in particular with respect to the role of human papillomavirus (HPV) infection [6–13]. However, questions with respect to the progenitor cell(s) that play(s) a role in normal (embryonic and fetal) development, as well as in the oncogenic processes that take place in the transformation zone of the uterine cervix, have been addressed only scarcely [4, 14, 15].

Therefore, the different processes that occur close to the squamocolumnar junction during human embryonic development, adolescence, and adulthood are discussed. The metaplastic process and potential progenitor cells in the transformation zone, which play a pivotal role during the early stages of carcinogenesis, are described, and the cells targeted by HPV are characterized. Our conclusions are mainly based on immunophenotypic analysis of the different tissue and cell types in normal tissues and in precancerous lesions, using well-known biomarkers.

Development of the Lower Female Reproductive Tract

The majority of the mammalian female reproductive tract develops from the Müllerian ducts, which form as invaginations of coelomic epithelium into the urogenital ridge mesenchyme, through which they grow caudally [16]. During early development in females, the caudal tip of the Müllerian duct, which is covered with columnar epithelium, reaches the urogenital sinus and fuses with the sinovaginal bulb, a solid squamous epithelial cord on the dorsal wall of the urogenital sinus (Müllerian tubercle) (see Fig. 1.1a) [17].

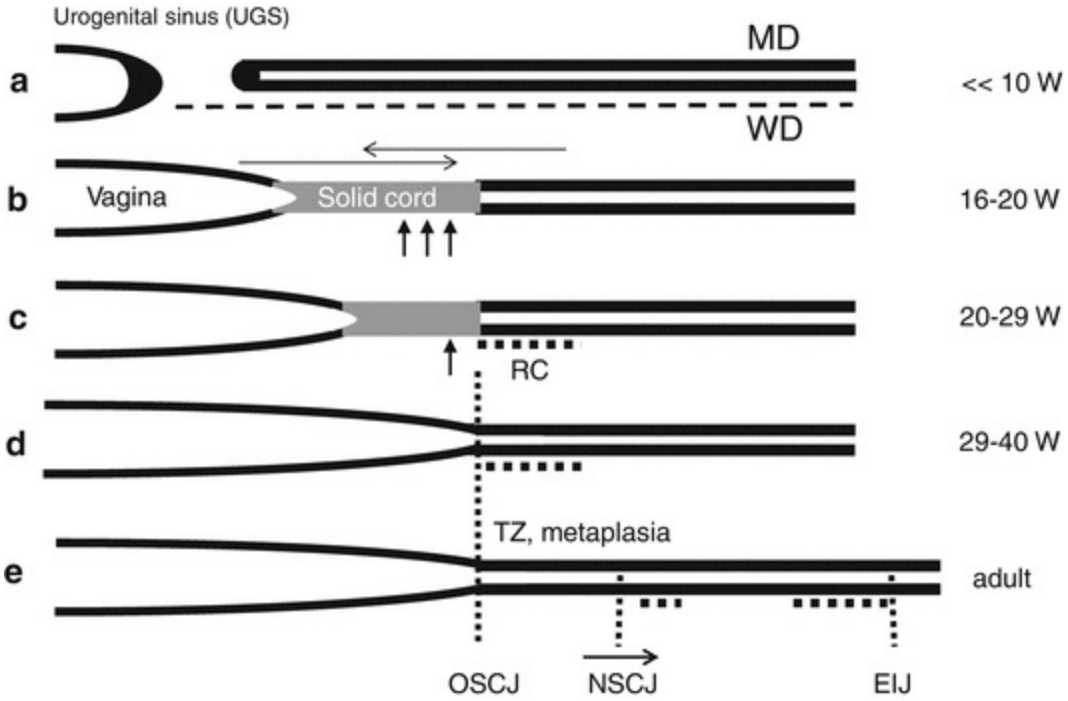

Fig. 1.1 Embryonic and fetal development of the human lower female reproductive tract. During early embryonic development, the Müllerian duct epithelium (*MD*), under guidance of the Wolffian duct (*WD*), reaches the urogenital sinus (*UGS*) (**a**). Expansion of the vaginal plate results in a solid cord in which the Müllerian vaginal epithelium is responsible for the formation of two-thirds of the vagina (**b**). *Horizontal arrows* indicate clonal expansion in caudal and cranial directions. During further development the solid cord opens and a primitive squamocolumnar junction is formed, the later original squamocolumnar junction (*OSCJ*) (**c**, **d**). *Vertical arrows* indicate the mesenchymal signals that influence vaginal epithelialization. After 20 weeks of gestation, reserve cells (*RC*) are formed that are present throughout adult life, extending up to the endocervical-isthmus junction (*EIJ*). (**e**) Reprogramming of these RCs into metaplastic squamous epithelium in the transformation zone (*TZ*) results in a new squamocolumnar junction (*NSCJ*)

At that point the discussion of development touches a long-standing controversy [17, 18]. Questions about the differences between the epithelium covering the vagina and lining the uterus, about the origin of the vagina and on how the columnar epithelium in the Müllerian vagina is converted into stratified squamous epithelium, have not been completely resolved. Several theories have been proposed [5] , but the most widely accepted theory hypothesizes that the upper two-thirds of the Müllerian vagina originate from the caudal part of the Müllerian duct, while the lower part of the vagina develops from the urogenital sinus. It was demonstrated that the Wolffian duct does not contribute cells to the growing Müllerian duct, but plays an important role in guiding the growth of the Müllerian duct tip in the direction of the urogenital sinus [19, 20]. The union of the Müllerian duct epithelium and the urogenital sinus forms a flat epithelial cord called the vaginal plate.

In a cell lineage tracing experiment in mice, it was recently shown that the entire epithelium of the adult mouse vagina is derived solely from Müllerian duct epithelium [3]. The urogenital sinus was shown to play a critical role in the development of the vagina only by providing the path for caudal growth of the Müllerian duct. In mouse

embryonic development, the urogenital sinus remains solid, and the Müllerian vagina is a tube that, even at birth, is lined primarily by columnar epithelium. In the human embryo, this solid cord can be recognized up to week 29 of gestation (see Figs. 1.1b, c and 1.2c). During normal fetal development, the upper part of the vaginal epithelium develops via transformation of the columnar Müllerian duct epithelium through a multilayered cuboidal phenotype into squamous epithelium [21, 22]. The transcription factor p63 plays a key role in this transformation process [23, 24], and a small number of cells positive for p63 are present in the Müllerian vagina at the junction with the sinovaginal bulb. The epithelium in the lower vagina already has a squamous phenotype by the time that the squamous plate is canalized and the lumen is formed.

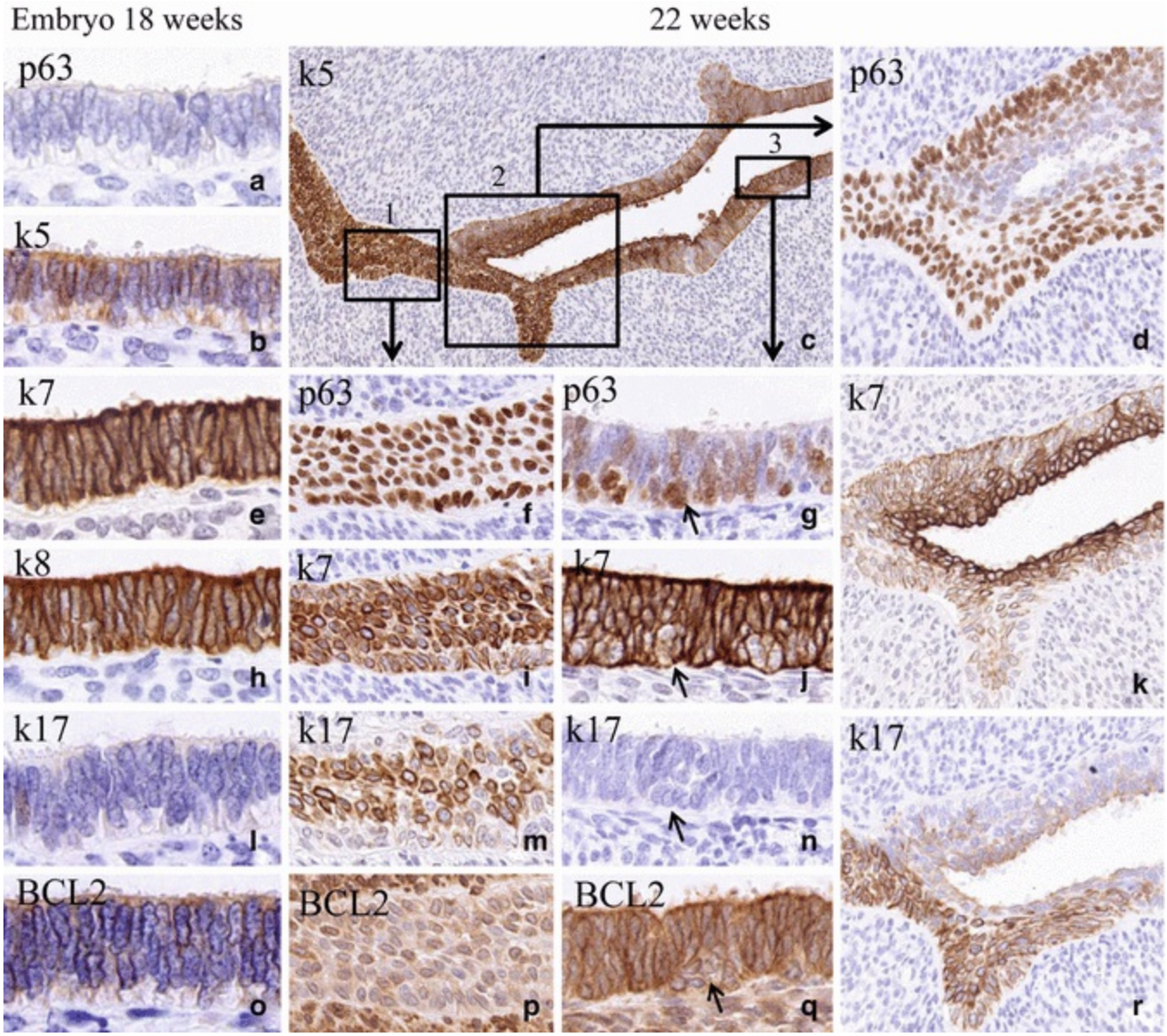

Fig. 1.2 Expression of p63; (cyto)keratins k5, k7, k8, and k17; and BCL2 in human fetal uterine cervix at weeks 18 and 22 of gestation. K7 and k8 are strongly expressed in the Müllerian duct epithelium at week 18, while k5 and BCL2 are expressed at a lower level (**a**, **b**, **e**, **h**, **l**, **o**). At week 22, the expression of biomarkers in the area in the solid cord

(*box 1* in **c**) is shown in (**f**, **i**, **m**, **p**); the primitive squamocolumnar junction (*box 2* in **c**) is shown in (**d**, **k**, **r**); and the Müllerian duct epithelium (*box 3* in **c**) is shown in (**g**, **j**, **n**, **q**). Note the positivity for p63, k7, and k17 in the solid cord, the appearance of p63 positivity in reserve cells in the Müllerian duct epithelium close to the squamocolumnar junction (**g**; *arrows*), and the phenotypic switch for k7 and k17 in the squamocolumnar junction

During fetal life a complex molecular program is followed by which the columnar epithelium is transformed into the squamous epithelium of the vagina, while the columnar Müllerian epithelium is anatomically separated from this vaginal epithelialization. Mesenchymal signals influence epithelial cell fate during this squamous transformation of Müllerian vaginal epithelium, as does expression of p63. In utero exposure to the synthetic estrogen diethylstilbestrol (DES) has shown that permanent p63 expression strongly influences the developmental fate of Müllerian duct epithelium. DES obstructs differentiation of the vaginal Müllerian epithelium, which remains simple columnar in type, causing vaginal adenosis [2, 23–25]. During normal fetal development, the vaginal Müllerian epithelium develops from a multilayered cuboidal phenotype into a stratified squamous epithelium.

The squamocolumnar junction, where columnar and squamous epithelia merge, can be recognized in the human embryo from week 20–24 onward (see Figs. 1.1c–e and 1.2c) and remains present throughout life. Cranial to this junction, the columnar epithelium can also be transformed into squamous epithelium, and the reserve cells (RC) beneath the columnar epithelium can be reprogrammed into squamous epithelium. A new squamocolumnar junction is formed (NSCJ) via a process of squamous metaplasia (see Fig. 1.1e). The area between the old and new squamocolumnar junctions is defined as the transformation zone (TZ). The reserve cells can already be detected in fetal life from week 24 onward and are present throughout life. These progenitor cells are found up to the junction between the endocervical canal and the uterine isthmus. Understanding the properties of the different types of cells in the TZ, including the potential progenitor cells of the squamous and columnar epithelia, is important for understanding the origin and behavior of 90% of all (pre)malignant uterine cervical lesions, which are localized to this dynamic area [11–13].

Immunophenotypic Characterization of the Developing Human Fetal Squamocolumnar Junction

Molecular markers can be applied to study the complex embryonic transition of the Müllerian duct epithelium into the squamous epithelium of the vagina and ectocervix on the one hand and into the columnar epithelium of the endocervix on the other [1, 17, 22, 23]. Also, the stage at which an early or primitive squamocolumnar junction is formed can be studied by applying such markers by immunophenotyping. (Cyto)keratins are differentiation markers for the different types of epithelial cells in the cervix and have

been applied to epithelial neoplasms to determine cell lineage and differentiation and for differential diagnosis [26–30]. For example, antibodies directed to keratin 5 (k5) and k17 can be used to detect squamous differentiation, while k7 and k8 are characteristic of glandular differentiation. The protein p63, a p53 homologue and a transcription factor, is required for normal development and epithelial cell differentiation in the vagina and endocervix [2, 23]. As a marker, p63 is used for recognition of basal squamous cells and other types of progenitor cells in epithelial tissues. BCL2 is a marker for cell survival and an indicator of protection against apoptosis and can be used as such to recognize stem cells [31].

Two studies complement each other in describing the spatiotemporal distribution of these relevant markers in the developing lower uterine tract, including the developing cervix and vagina [1, 22]. In *weeks 14–15*, the squamous markers p63 and k17 are found in the developing lower and upper vagina (vaginal anlage), while k8 is negative [22]. BCL2 was shown to be strongly positive in basal cells of this epithelium. The columnar epithelium of the cervical segment showed scattered BCL2-positive cells and was described to be negative for k8 at this stage. The human Müllerian duct epithelium between *weeks 16 and 18* of gestation is strongly positive for k7 and k8, typical of primitive columnar epithelium. k17 and p63 cannot be detected immunohistochemically in this period, while k5 and BCL2 are weakly positive (see Fig. 1.2, left panels) [1]. Fritsch et al. [22] have shown that the upper vaginal Müllerian epithelium is negative or only weakly positive for k8 at this early stage, while being positive for k17 and p63, both indicators of squamous differentiation. In *weeks 19–25* a primitive squamocolumnar junction can be recognized as a transition between the uterine cavity and the solid cord epithelium. This transition becomes recognizable on the basis of the immunostaining results with the aforementioned antibodies (see Fig. 1.2; middle panels). A solid cord-like structure with a stratified appearance merges with the columnar Müllerian epithelium. In the solid cord the typical markers for programming of squamous differentiation, i.e., k5, k17, and p63, are strongly positive, with additional reactivity for k7 and k8. The basal cell compartment in this solid cord epithelium is weakly positive for k7, k8, and k17. The Müllerian columnar epithelium is strongly positive for k5, k7, k8, and BCL2, but negative for k17. At this stage basal cells can be recognized in the Müllerian duct epithelium as p63-positive cells. These cells were identified as reserve cells (RC) by Martens et al. [1]. It should be noted that Fritsch et al. [22] detected such p63-positive basal cells already in weeks 14–15 of gestation. While these progenitor cells become k17 positive at later stages, they are still negative for this marker at week 22. BCL2 was strongly expressed in the mesenchymal stroma of the Müllerian vagina and Müllerian duct epithelium. A gradient in staining intensity of several markers can be recognized at the site where the solid cord and the Müllerian columnar duct epithelium merge. k5 and p63 expression decreases in the cranial direction, while k7 and k8 expression decreases in the caudal direction (see Fig. 1.2,

right panels). With increasing gestational age, the length of the solid cord reduces, the vaginal epithelium moves cranially, and, around week 24–25, a clear-cut squamocolumnar junction is detectable by conventional histology and is located in the endocervical canal.

It has been shown that during the first semester of embryonic development, the differentiation processes described above are initialized by cellular stimuli from the urogenital sinus or surrounding mesenchymal tissue. Strong BCL2 positivity is detected in the mesenchymal tissue surrounding the solid cord, while the surrounding tissue of the Müllerian duct is weakly BCL2 positive or even negative. This supports the view that in the human situation, the Müllerian vagina and Müllerian duct epithelium are under different stromal stimuli [22, 23, 32, 33].

In the period between *weeks 29–40* of gestation, the differentiation markers clearly demarcate the squamous and glandular epithelia, and the squamocolumnar junction has been firmly established. Also the immunomarker profile for the reserve cells becomes gradually comparable to the newborn and adult expression pattern. In these basally located cells of the developing glandular endocervical epithelium, the expression of p63 and k5 becomes clearly visible, while the beginning of expression of k17 and BCL2 can be seen as weak staining in the fetal reserve cells. As illustrated in Fig. 1.3, immunostaining results in a *32-week*-old embryo show that the squamocolumnar junction is clearly demarcated by k7 and k8 positivity in the columnar epithelium and k5 and p63 positivity in the squamous epithelium.

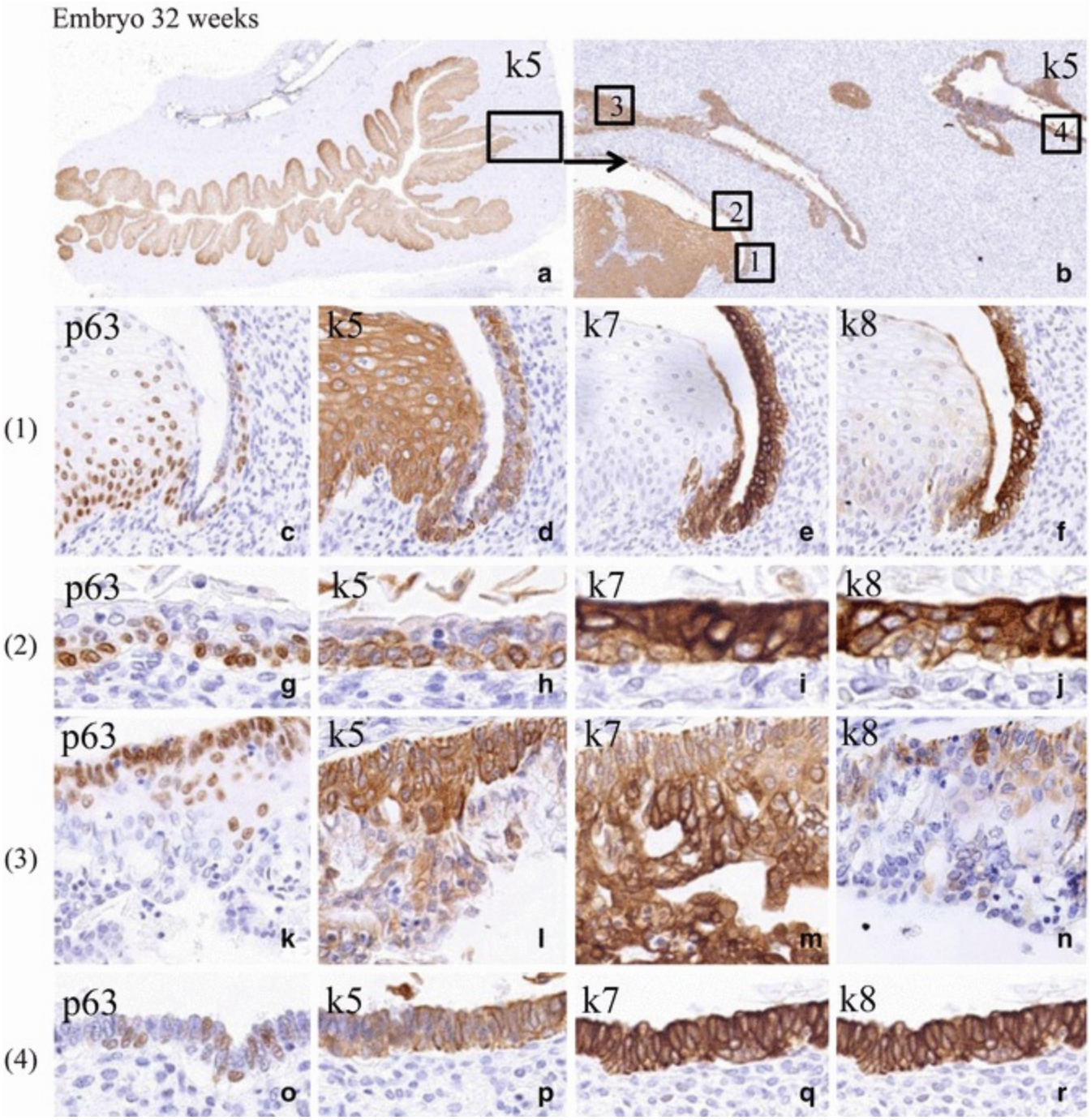

Fig. 1.3 Expression of p63 and (cyto)keratins k5, k7, and k8 in a human fetal cervix at week 32 of gestation. Overview of a sagittal section of the lower genital tract stained for k5, including the vaginal epithelium, the lower part of the Müllerian epithelium, and the squamocolumnar junction (SCJ) (**a**). (**b**) Shown are four boxes focusing on the SCJ (*area 1*), the endocervix (*area 2*), metaplasia close to the SCJ (*area 3*), and more distantly the Müllerian duct epithelium (MDE) (*area 4*). The different markers for the SCJ (**c**–**f**), the endocervix close to the SCJ (**g**–**j**), metaplasia (**k**–**n**), and the MDE (**o**–**r**) are indicated

Close to the squamocolumnar junction (see Fig. 1.3b, box 1), reserve cells beneath k7 and k8 positive cells can be recognized. These are typically k5 and p63 positive and share the k7 and k8 positivity of columnar epithelium, although to a lesser extent (see

Fig. 1.3b, box 2). More cranially from the squamocolumnar junction, the Müllerian duct epithelium is seen in which k5- and p63-positive and k5- and p63-negative cells are still intermingled (see Fig. 1.3b, box 4). Here, the reserve cells have not yet reached their basal position in the columnar epithelium. Close to the SCJ of this embryo, metaplasia is present (see Fig. 1.3b, box 3) in which reserve cells beneath columnar epithelium are programmed to differentiate into squamous epithelium. This is a process that is typical of the adult situation in which columnar epithelium in the TZ undergoes metaplasia to form squamous epithelium. During fetal development this metaplasia may be induced by maternal estrogens.

In the adult situation reserve cells beneath normal columnar epithelium typically combine markers for squamous (p63, k5, and k17) and glandular (k5 and k8) differentiation (see Fig. 1.4a–c). Furthermore these cells are protected against apoptosis by a high concentration of BCL2 (see Fig. 1.4d). In nearly all routine biopsies that include the squamocolumnar junction, reserve cells can be recognized by immunostaining either as stretches of cells or as single cells [34].

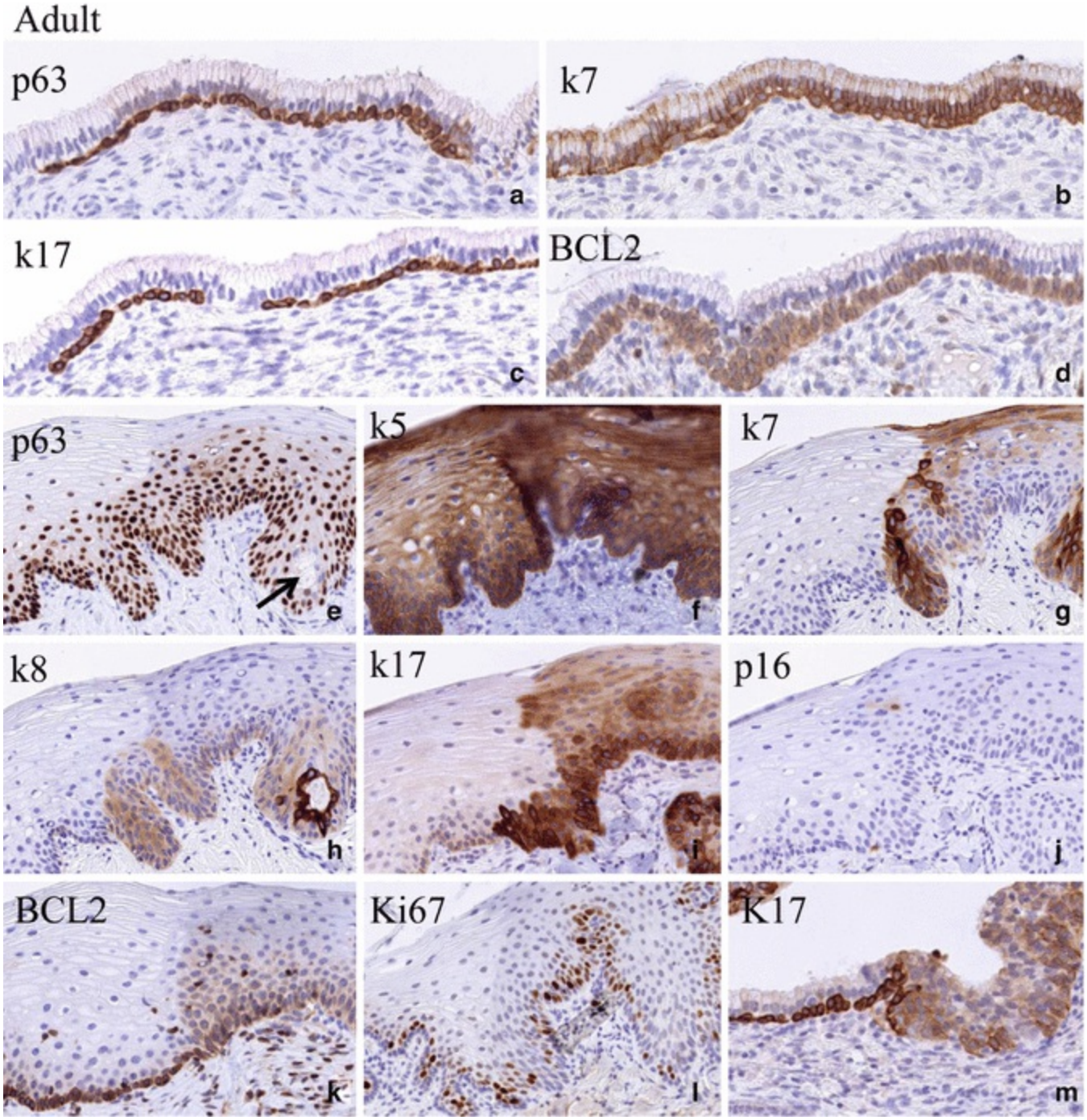

Fig. 1.4 Expression of p63; (cyto)keratins k5, k7, k8, and k17; BCL2; and Ki67 in normal adult endocervix and ectocervix. In the normal endocervix, p63, k7, k17, and BCL2 are strongly expressed in a stretch of reserve cells underlying the columnar epithelium (**a**–**d**). The reserve cells and columnar epithelium show very low proliferative activity (not shown). K7 is only positive in the columnar epithelium. In the normal ectocervix, p63 is present in the basal compartment of the squamous epithelium and k5 in the entire epithelium, while k7 and k8 are negative (**e**–**h**). Basal cells are negative or weakly positive for k17 (**i**), and BCL2 occurs in the nonproliferating basal cells (**k**), while the parabasal cells are Ki67 positive (**l**). The *arrow* in (**e**) refers to a remnant of a gland below the squamous epithelium in the ectocervix with strong staining for k7 and k8. The staining patterns for k17 and k7 (**g**, **i**) of the cells adjacent to this remnant indicate (im)mature metaplasia. These cells are not infected by HPV as indicated by the negative p16 staining (**j**). In M k17 staining is shown in a transition area between reserve cells and the formation of squamous epithelium

Based on the immunophenotyping studies described above, we propose a model for the hierarchical order of cell lineages developing during embryological and fetal growth, in which Müllerian cell types differentiate into endocervical columnar cells and reserve cells (see Fig. 1.5) [1, 22]. To date there is a consensus that the reserve cell is the progenitor for the squamous cell formed during metaplasia in the endocervix. However, whether the reserve cell in the adult situation is a remnant of the embryological population or is formed de novo from a columnar cell is still debated (see below).

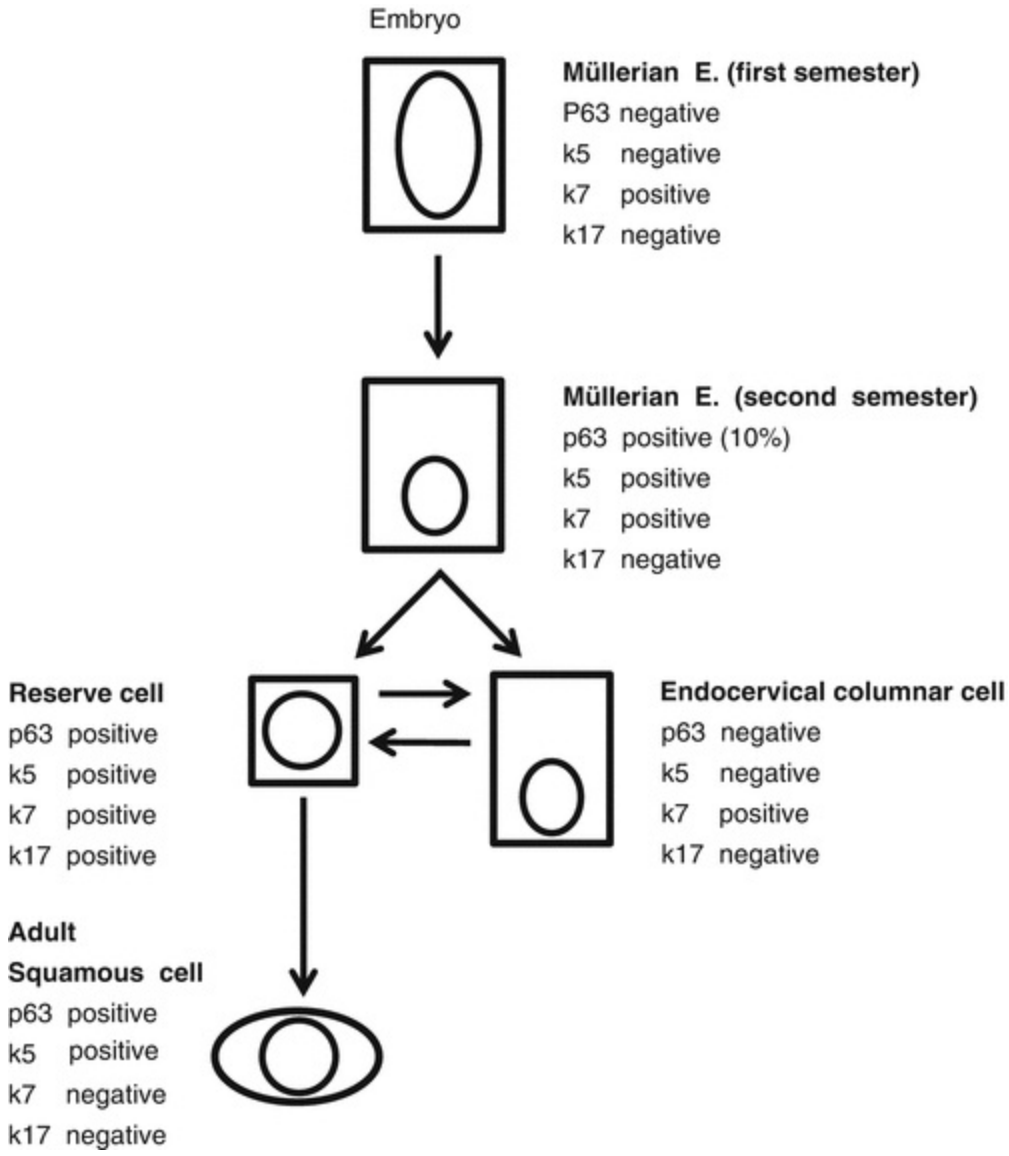

Fig. 1.5 Hierarchical model for cell lineages in the human uterine cervix based on the expression of molecular markers during fetal development. Molecular markers and their expression during fetal development. Early Müllerian duct epithelium (first semester) expresses only the simple (cyto)keratins (e.g., k7). At approximately 20 weeks of gestation, expression of p63 and the basal cell marker k5 is initiated in a small fraction of these cells (Müllerian duct epithelium, second trimester). Simultaneously, the first reserve cells under this second semester duct epithelium are recognized. Initially, the p63 and cytokeratin phenotype of both cells is identical. However, with increasing gestational age, the reserve cells additionally express k17 weakly. At approximately this time, true endocervical columnar cells

appear. These cells arise either directly from the second semester Müllerian cells during which p63 and k5 are lost or from reserve cells. Whether endocervical columnar cells give rise to reserve cells directly or are formed from more primitive Müllerian cells is not yet known [1, 35]. Finally, the reserve cell can undergo squamous differentiation, with loss of k7 and k17 expression

Progenitor Cells in the Adult Squamocolumnar Junction

The two types of epithelia that meet at the squamocolumnar junction each have their own progenitor cells in the adult situation. It can be anticipated that these progenitor cell compartments comprise (1) a real stem cell fraction, from which (2) the basal layer of the squamous epithelium and (3) the reserve cells underlying the glandular epithelium arise. In the ectocervical squamous epithelium, a (4) transient-amplifying (parabasal) cell compartment arises upon proliferation of basal cells.

Extensive and strong Ki67 staining is seen in these parabasal cells, while the basal cells show only very low proliferative activity, as indicated by the fact that only sporadic cells are Ki67 positive in this compartment (see Fig. 1.4l). These basal cells are, however, heavily protected against apoptosis by strong expression of BCL2 (see Fig. 1.4k) [31]. On the contrary, the highly proliferative parabasal cells are negative for BCL2. These mutually exclusive expression patterns of Ki67 and BCL2 indicate that the processes of extensive proliferation on the one hand, and protection from apoptosis on the other, are meticulously separated within the squamous epithelium. These immunophenotyping results are supported by earlier findings that the generation cycle of basal cells is about 30 days, while under physiological conditions the generation time for parabasal cells is about 3 days [36]. In both the endocervical columnar cells, as well as reserve cells beneath the columnar epithelium (see Fig. 1.4a–d), very little proliferative activity is observed under normal physiological conditions. This indicates that the turnover of the endocervical epithelium is apparently low compared to the ectocervical epithelium. The reserve cells, which are recognized throughout the entire endocervical canal up to the endocervix-isthmus junction, show strong positivity for BCL2, while the columnar epithelial cells are BCL2 negative (see Fig. 1.4d) [34, 37, 38]. These observations suggest that the reserve cells are part of a pool of progenitor cells protected against apoptotic cell death and required to ensure survival of the endocervical epithelium. Both columnar and reserve cells, however, can proliferate independently as has been shown in regenerating endocervical epithelium, although the reserve cells are not required a priori for normal regeneration of secretory columnar epithelium [39]. Whether the reserve cells should be regarded as the default progenitor cell for the columnar epithelium, or vice versa, the columnar epithelium as progenitor for the reserve cells can be questioned. In the case of microglandular hyperplasia, it has been proposed that reserve cells are created in adulthood during specialized columnar proliferations [35].

The combined phenotype of the reserve cells expressing both markers of glandular

(k7, k8) and squamous differentiation (p63, k5, and k17) is a strong indicator for the consensus that reserve cells are also the progenitors for (mature and immature) squamous metaplasia [14, 28]. As can be seen in Fig. 1.4f–m, remnants of squamous metaplasia express k7, k8, and k17, which are not found in the normal ectocervical squamous epithelium.

Recently, it was reported that an immature cuboidal progenitor endocervical cell type can be recognized by high levels of k7 expression, situated close to the squamocolumnar junction. After infection by an oncogenic HPV type, this cell type may develop into a high-grade squamous intraepithelial lesion [4, 40] (see also section "Squamous Metaplasia").

Currently no specific markers are available for the real stem cells in the columnar and squamous epithelium. The fact, however, that both the reserve cell and the basal cell of the squamous epithelium express BCL2 and p63 could be an indicator that the real stem cell of the uterine cervix could also express these two markers. The cells originating in the fetus around week 20, which have begun to express p63 and BCL2, could act as the first stem cells of the cervix.

Definition of the Cervical Transformation Zone (TZ)

The area between the original and the new squamocolumnar junction is defined as the TZ. This TZ can be visualized by colposcopic inspection and is also the area in which approximately 90% of (pre)neoplastic lesions develop [11–13, 41]. The TZ is a dynamic entity formed during puberty and is the area where the glandular epithelium is replaced by metaplastic squamous epithelium [42]. Furthermore the presence of endocervical glands underlying the squamous epithelium is an indicator of the position of the TZ [43]. The last gland can serve as a landmark for the position of the original squamocolumnar junction [13]. The junction between the metaplastic squamous epithelium and the glandular cells defines the new squamocolumnar junction and the cranial limit of the transformation zone. At birth and during the premenarchal years, the squamocolumnar junction resides at or very close to the external os [5]. During puberty the endocervical mucosa everts onto the ectocervix as a result of hormonal stimulation and swelling of the stroma of the cervix. Reproductive hormones also influence the production of ectopy during late fetal life and pregnancy and as a result of the use of oral contraceptives. Ectopy is modified over time by squamous metaplasia and epithelialization, low pH, trauma, and possibly cervical infection [12, 42, 44]. As a result of this eversion, the squamocolumnar junction becomes located on the ectocervix, and the exposed endocervical mucosa (ectropion) shows the gradual replacement of the columnar epithelium by squamous epithelium. This represents a protective response to the exposure of the glandular epithelium to the vaginal environment [12, 42, 45, 46]. Reserve cells beneath the columnar epithelium are the progenitor cells for the newly

formed squamous epithelium. A consequence of this development is the formation of a new squamocolumnar junction (see Fig. 1.1e). During late reproductive life and after the menopause, decreasing hormone levels lead to shrinkage of the cervix, and the new squamocolumnar junction comes to lie in the endocervical canal [12].

Squamous Metaplasia

Three different types of squamous metaplastic lesions can be recognized in the cervix, i.e., (1) immature squamous metaplasia, (2) mature squamous metaplasia, and (3) atypical immature metaplasia [13, 42, 45, 47–50]. There are two histogenetic mechanisms by which the endocervical mucosa is replaced by squamous epithelium [5]. The first is the direct ingrowth of squamous epithelium in the direction of the endocervix, which is referred to as squamous epithelialization. The other route is through proliferation of subcolumnar reserve cells (reserve cell hyperplasia) and their subsequent maturation into squamous epithelium. Both mechanisms result in a squamous epithelium overlying endocervical mucus-producing glands [44]. In the first phase, reserve cells proliferate and stratify. Subsequently, these cells undergo a squamous differentiation process that is at first incomplete, with persistence of the columnar epithelium, which is often seen as a residual layer on the surface [45]. Later, metaplastic cells can mature to keratinocytes that are indistinguishable from the suprabasal cells of the pluristratified epithelium, resulting in mature squamous metaplasia.

Typical immunohistochemical staining patterns for p63, k5, k7, k17, and BCL2 in immature squamous metaplasia are shown in Fig. 1.6, while Fig. 1.7 shows a schematic overview of the changes that this marker profile undergoes during the formation of the metaplastic lesions. The biomarker expression pattern of immature metaplasia strongly resembles that of the reserve cell, with the exception of BCL2, which is significantly reduced in intensity as compared to that of the reserve cell. Although the precise mechanisms underlying the induction of squamous metaplasia are still obscure, it seems that cytokines and growth factors present in the metaplastic microenvironment might alter the transcription factor profile of reserve cells. It has been proposed that metaplastic transformation results from the release of cytokines and other soluble factors by both epithelial and inflammatory cells [45].

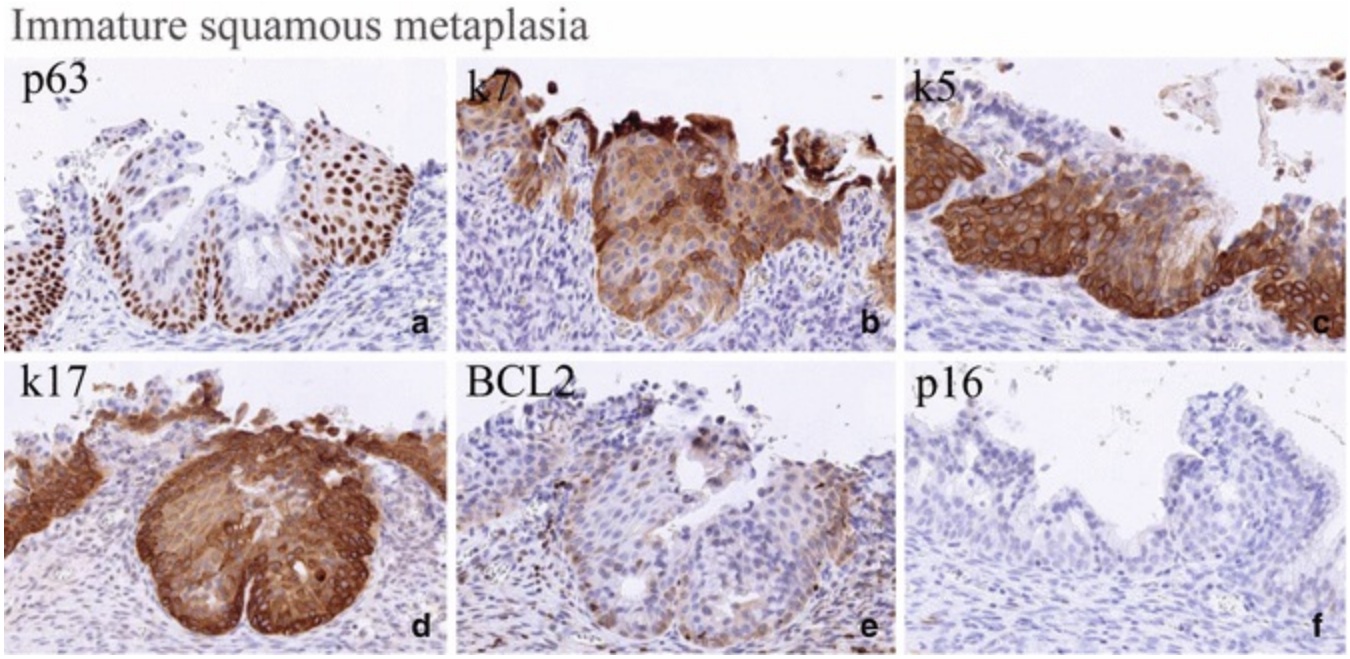

Fig. 1.6 Expression of p63; (cyto)keratins k5, k7, and k17; and BCL2 in immature squamous metaplasia. In immature squamous metaplasia, p63, k5, and k17 show a typical expression pattern in the basal compartment (**a**, **c**, **d**), while k7 staining in this compartment is reduced, but positive in the superficial layer (**b**). BCL2 expression is reduced compared to the expression in reserve cells (**e**), probably as a result of the proliferative activity of these cells associated with squamous differentiation. (**f**) p16 as a surrogate marker for the presence of a high-risk HPV infection is absent

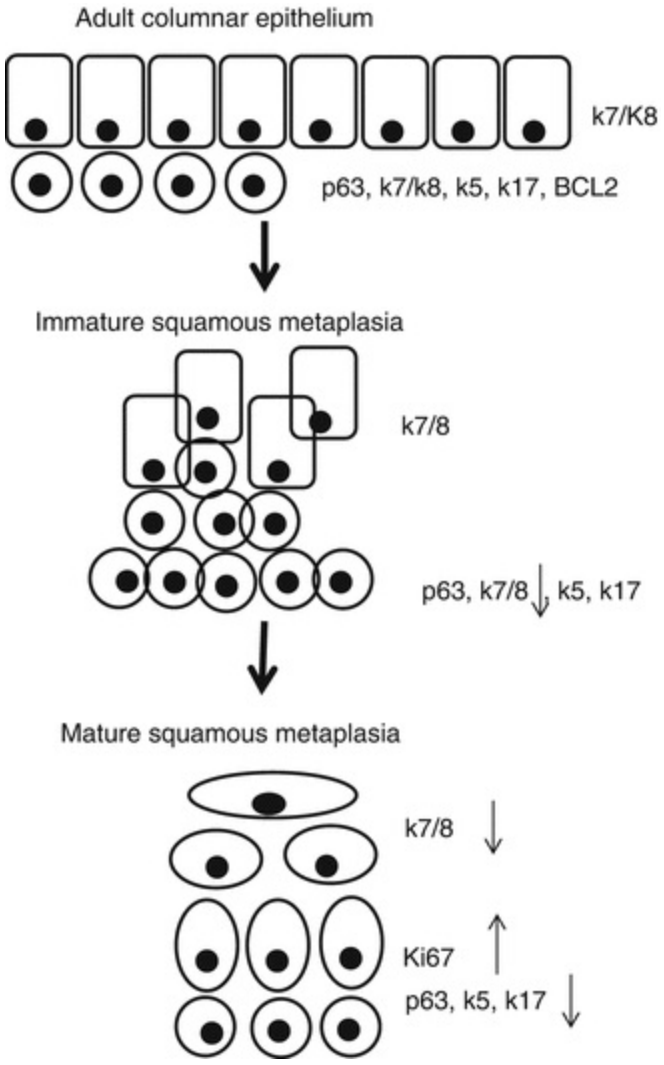

Fig. 1.7 Schematic overview of the changing phenotype during metaplastic transformation of the adult columnar epithelium. During the metaplastic process, k7 and k8 expression is lost in the basal reserve cell compartment, while in the superficial layer this loss is delayed. Loss of k17 expression follows k7

Atypical immature metaplasia is a poorly reproducible diagnosis, which spans a morphological spectrum and has features both of metaplasia and atypia. It is difficult to distinguish from low-grade cervical neoplasia. These lesions may arise as a result of reactive or inflammatory processes or through high-risk HPV infections of true precursors, resulting in cervical carcinoma [46, 49, 51–54].

Potential Target Cells for HPV Infection

In general, the intact cervical epithelium is resistant to viral infections. However, chemical or mechanical disruption of the integrity of this epithelium enables HPV entry. As has been analyzed in experimental animal systems, a simple brush with a Cytobrush cell collector or the application of a spermicide (nonoxynol-9) resulted in abrasions that enabled binding of the virus to the basement membrane prior to its transfer to the

basal cells [55]. Adsorption to the basal surface of the epithelial cells and reestablishment of contact with the basement membrane during repair of the damaged epithelium might further promote preferential infection of basal cells. It has been found that heparan sulfate in the extracellular matrix and on the surface of these cells acts as an initial attachment receptor for HPV. It was furthermore postulated that the adult reserve cells with CD49f (α 6-integrin) expression could be preferentially targeted by high-risk HPV types during cervical carcinogenesis (see also Chap. 2 for the biology of HPV infection) [9, 55, 56].

The fact that high-risk HPV can target both nondividing basal cells of the squamous epithelium and reserve cells of the endocervical epithelium, together with the fact that the entry of HPV DNA into the cell nucleus has been shown to be dependent on the cell cycle, has fundamental implications [56].

Infection by HPV is dependent on proliferative activity since the final delivery of the HPV genome into the nucleus depends on nuclear envelope breakdown among other factors [56]. The fact that the two potential target cells for HPV infection are largely nonproliferative in the normal epithelium, combined with the assumption that dividing cells are pivotal for the initiation of HPV replication, poses a theoretical conflict. However, it has been suggested that in the epidermis, the basal cells can undergo a round of HPV replication independent of the cell cycle. This infected cell is thought then to leave the basal compartment and enter the transient-amplifying compartment of the epithelium where replication is maintained [9].

In the case of abrasions of the squamous epithelium (see Fig. 1.8b, c, arrow 1), and in immature squamous metaplasia, HPV infection might take place in dividing basal cells and/or reserve cells (see Fig. 1.8b, arrow 2). In the latter situation the reserve cell will not be recognized as such since it has undergone metaplasia, resulting in atypical immature metaplasia (see below). The observation that high-risk (HR) HPV is often found to be integrated in early endocervical adenocarcinoma in situ (AIS) suggests that the target cell for HPV in such a scenario could be either a columnar cell or a reserve cell that is committed to columnar differentiation (see Fig. 1.8b, arrow 3) [57]. It has been observed that the columnar epithelium adjacent to the transformation zone shows a marked increase in sensitivity to infection when disrupted by, e.g., a spermicide which disturbs the normal architecture of animal and human cervical epithelium [55].

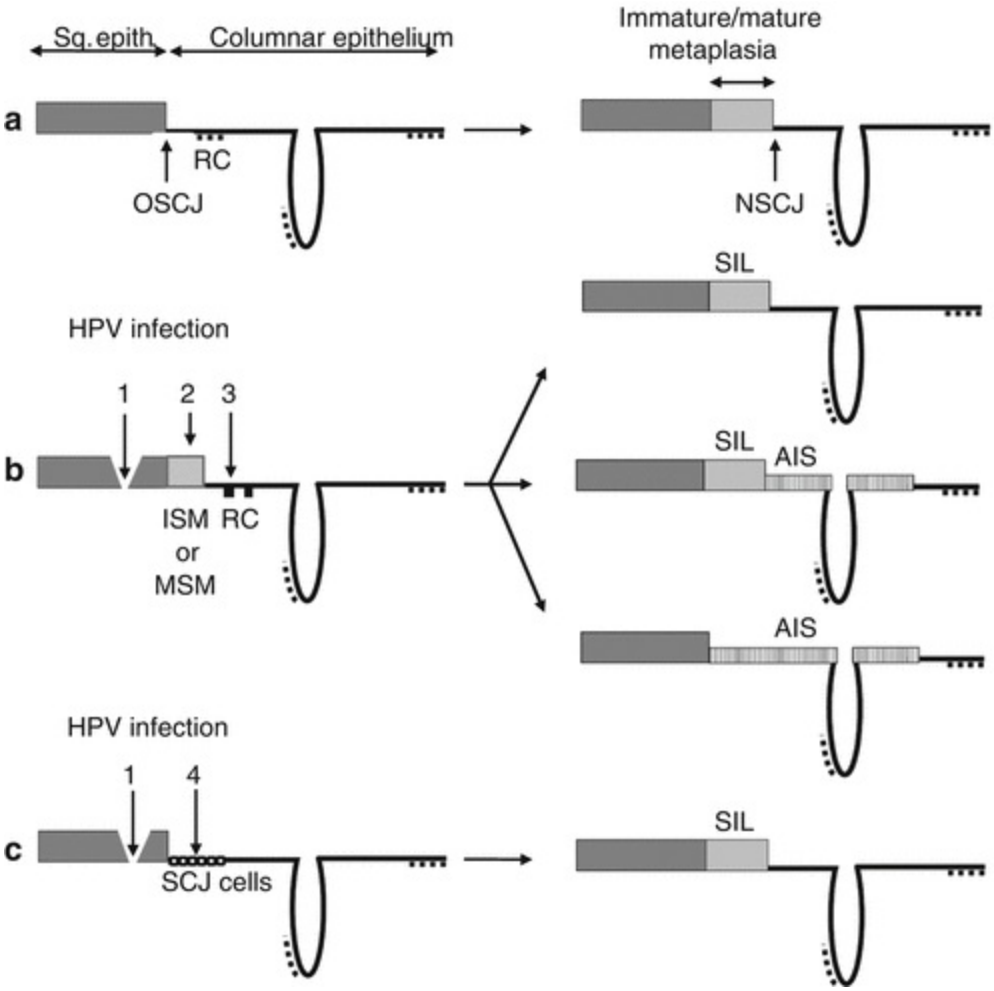

Fig. 1.8 Schematic overview of the potential target cells for HPV infection, resulting in the different types of cervical preneoplasia. (**a**) Topographic localization of the original squamocolumnar junction (*OSCJ*) at the interface between squamous epithelium and columnar epithelium. During the process of metaplasia, the reserve cells (*RC*) generate a new squamous epithelium, via immature and mature metaplasia, resulting in a new squamocolumnar junction (*NSCJ*). (**b**, **c**) HPV infection via microtrauma in the normal squamous epithelium, in which basal cells are infected (**b** *arrow 1* and **c** *arrow 1*), results in a low-grade squamous intraepithelial lesion (*SIL*). Infection of (im)mature metaplasia (*ISM* and *MSM*) (**b** *arrow 2*) and/or columnar epithelium with reserve cells (**b** *arrow 3*) results in high-grade SIL, combined high-grade SIL/adenocarcinoma in situ (*AIS*) lesions, or solitary AIS, depending on the cell type targeted. (**c**) Infection of specific squamocolumnar junction cells (*SCJ* cells) in the columnar epithelium (**c** *arrow 4*) may result in high-grade SIL

Another potential target cell for HPV infection has been recently proposed by the group of Crum and collaborators [4]. These authors suggest that a limited stretch of k7-positive columnar cells in close proximity to the squamocolumnar junction (squamocolumnar junction cells) is the target for high-risk HPV infection and the progenitor for high-grade squamous epithelial lesions (HSILs) (see Fig. 1.8c, arrow 4). Removal of these cells has been described to prevent the formation of precancerous lesions in the uterine cervix [4, 58, 59]. Also, these k7-positive cells have been described to give rise to reserve cells in a so-called top-down differentiation process

[40].

Premalignant Lesions

The majority of precursor lesions in the uterine cervix will present as low-grade or high-grade squamous intraepithelial lesions (LSIL, HSIL) (see Chap. 6) and less frequently as SIL combined with adenocarcinoma in situ (AIS) or as solitary AIS (see Chap. 8). The morphological distinction of these subtypes is not always straightforward and is complicated by mimics such as atypical immature metaplasia, immature squamous metaplasia, reactive atypia, atrophy, and basal cell hyperplasia [53, 60, 61]. This abundance of morphological appearances of premalignant lesions and their mimics in the cervix makes it difficult to decide which cell(s) was (were) initially infected by HPV. Besides the morphological problems, the complex association between different abnormalities within a single biopsy, such as synchronous LSIL and HSIL, combined SIL-AIS, and clonal differences between different types of preneoplasia within single biopsies, complicates the answer to this question [7, 10, 52, 61, 62]. Furthermore, the progression of LSIL to HSIL, clearance of a viral infection, or persistent infection, single or multiple infections in single lesions, and episomal HPV versus viral integration into the host genome add to the complexity of this process [63, 64]. The right panels in Fig. 1.8 indicate the different types of premalignant lesion that can occur upon HPV infection of the different types of potential target cells.

In model B (see Fig. 1.8b), the reserve cell is central to the formation of both the squamous lesions via immature metaplasia and some adenocarcinoma in situ lesions [61]. On the contrary, in model C (see Fig. 1.8c), the k7-positive squamocolumnar junction cells play a central role in the formation of HSIL [4, 7, 61, 65].

In the following paragraphs the relationship between these potential progenitor cells and the different types of premalignant lesion will be discussed in more detail.

Low-Grade Squamous Intraepithelial Lesion (LSIL)

HPV infections that occur via micro-abrasions, and by which the basal cells in the squamous epithelium are targeted, can result in low-grade squamous intraepithelial lesions (LSIL). As a result these cells can contain a few viral copies which are replicated to high level during migration toward the mucosal surface, in concert with differentiation of the epithelium [6, 7, 9]. Viral replication and shedding occur in the upper layers of the epithelium from which these cells exfoliate. By HPV in situ hybridization, these lesions show nuclei filled with hundreds of episomal HPV copies in the superficial epithelium [66–68] .

In normal ectocervical squamous epithelium, the basal cells are k7 and k17 negative, while in mature squamous metaplastic epithelium, k7 and K17 staining can be

found in the superficial and basal cell layers, respectively (see Figs. 1.4 and 1.7). As can be expected, the immunophenotypic profile of LSIL shows a high expression of markers of squamous differentiation, i.e., p63 and k5. In addition, k7 can be found in the superficial layers, while k17 is detected more basally (see Fig. 1.9a–c). Recently it has been shown by others that 33–59% of LSILs show predominantly superficial/apical k7 staining [65, 69]. For k17 several studies showed lack of expression in the majority of lesions or reported expression in the basal compartment in a minor fraction of LSILs [27, 49, 60, 70]. On the basis of their comparable immunophenotypes, it is suggested that the majority of LSILs develop in the mature squamous epithelium at the transformation zone or in the normal ectocervix (for the k7-negative LSILs). In addition, the k7-positive squamocolumnar junction cells have also been suggested as progenitors for some LSILs (k7 positive).

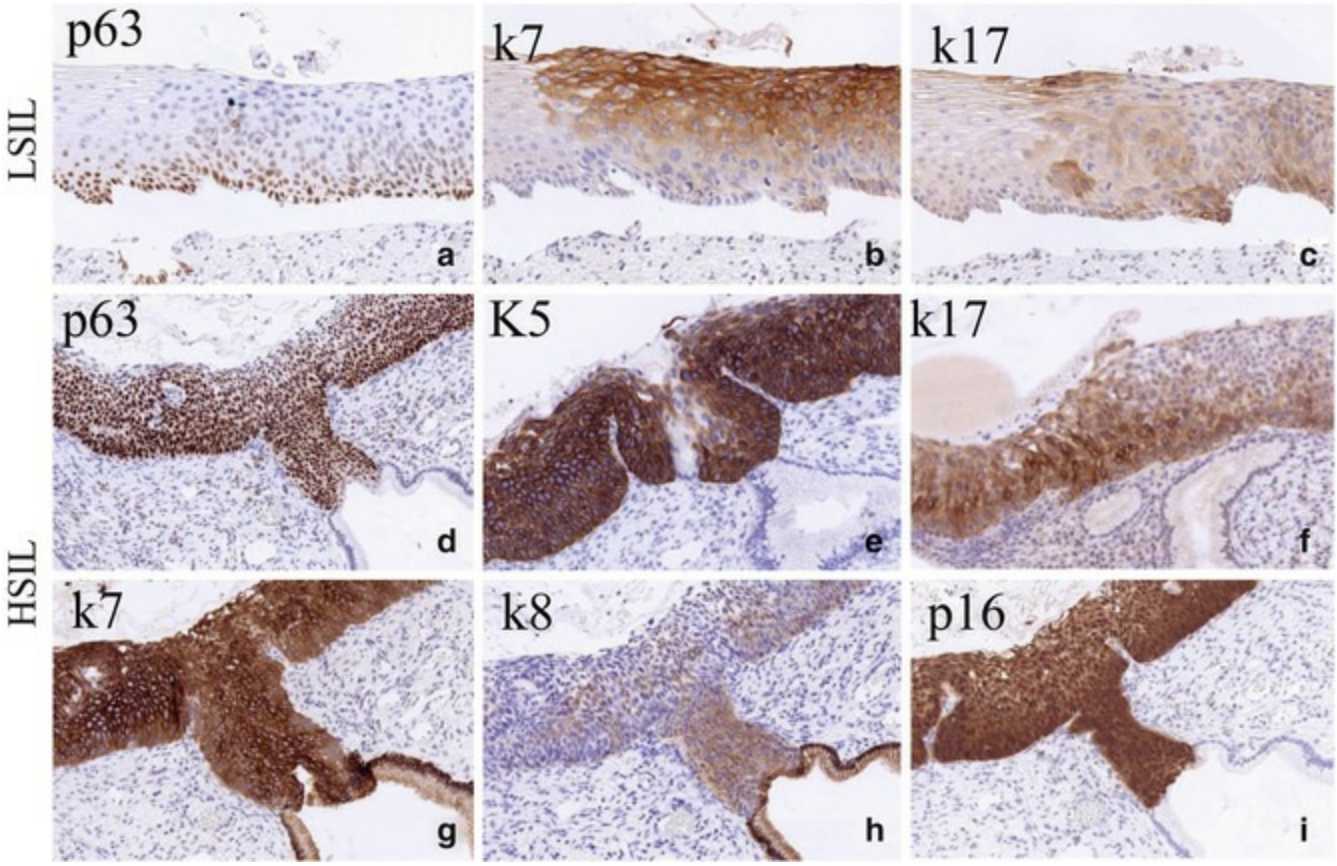

Fig. 1.9 Expression of p63, p16, and (cyto)keratins k5, k7, k8, and k17 in low-grade and high-grade squamous intraepithelial lesions (*LSIL*, *HSIL*). In LSIL the lower compartment is positive for p63 and weakly positive for k17 (**a**, **c**), while the superficial layers are k7 positive (**b**). In addition to strong expression of p63 and k5 (**d**, **e**), HSIL is strongly k17 (**f**) and k7 (**g**) positive throughout the entire thickness of the epithelium. k8 is negative (**h**). These patterns support the view that mature metaplasia/squamous epithelium is targeted by HPV in cases of LSIL, while in HSIL more immature metaplastic squamous epithelium or reserve cells are targeted. Block-positive p16 immunostaining (**i**) indicates infection with high-risk HPV (see Chap. 6)

High-Grade Squamous Intraepithelial Lesion (HSIL)

High-grade squamous intraepithelial lesions (HSILs) are predominantly formed at the transformation zone and characterized by loss of maturation, cytological atypia, and the presence of mitoses in at least the lower two-thirds of the epithelium. In combination with p16 positivity, which serves as a surrogate marker for the presence of HPV, and detection of cell proliferation with Ki67, these high-grade lesions can be easily recognized. These immunomarkers can also help to correctly classify mimics, such as atypical immature metaplasia, that often have histological features overlapping with those of HSIL. The (cyto)keratin profile of HSIL has been extensively studied in the past to determine its cellular origin and type of differentiation [27–29]. In particular, k7 and k17 antibodies were applied to study the potential target cell for HPV [4, 14]. In contrast to LSIL, nearly all HSILs are strongly positive for both k7 and k17 [4, 53, 60, 70, 71] (see Fig. 1.9). This makes the reserve cell, which has a similar immunophenotypic profile (see Figs. 1.4 and 1.7), the candidate progenitor cell for HSIL. Although positivity for p63 and k5 is not typical for HSIL, their expression in LSIL is restricted in most cases to the basal compartment of the epithelium, while in HSIL nearly all cell layers are strongly positive for these markers. As indicated in Fig. 1.8 (model B, arrows 2 and 3), both the reserve cells directly or immature metaplastic epithelium (originating from reserve cells) can be targeted by HPV which gives rise to HSIL. The observation that dynamic metaplasia, rather than the absolute extent of ectopy, increases the risk of viral infection, supports the view that proliferating reserve cells, programmed for the formation of squamous epithelium, are the target cells for HPV [47]. As described above (section "Definition of the Cervical Transformation Zone (TZ)"), atypical squamous metaplasia has been proposed as a pre-stage of HSIL. These metaplastic lesions have a phenotypic profile typical for reserve cells, with k5 and k17 being strongly expressed [49, 51–53].

Recently it was hypothesized that residual embryonic squamocolumnar junction cells with a cuboidal appearance and positive for k7 are targeted by HPV, resulting in HSIL lesions (see Fig. 1.8c arrow 4) [4, 40]. In these studies the cuboidal squamocolumnar junction cells show high expression of k7, while normal columnar epithelium and the reserve cells in these studies were reported to be k7 negative. The fact that HSILs are mostly k7 positive and a large fraction of the LSILs are k7 negative is an important argument pointing to these cuboidal squamocolumnar junction cells as the target cells for HPV and subsequent formation of HSIL.

The two hypotheses for the progenitor cell of HSIL can be integrated by the observation that reserve cells can originate from cuboidal cells during adulthood or columnar cells during fetal development. For this a "top-down differentiation" process is proposed that is also observed during human and mouse fetal squamous differentiation and in microglandular hyperplasia [35, 40]. Focal induction of p63 and

k5 expression in columnar epithelium close to the squamocolumnar junction results in the appearance of reserve cells and squamous metaplasia in this region. According to this model the cuboidal cells are targeted by HPV, but the reserve cells originating from these cuboidal cells are finally responsible for the formation of HSIL.

In our opinion the earlier observation that reserve cells can be found in the human fetus close to the primitive squamocolumnar junction, as well as more cranially (see Fig. 1.3), makes it likely that these cells are present already during puberty or adulthood [1]. The distribution pattern of these cells throughout the endocervix can, however, change during puberty and adulthood. Reserve cells are found in the normal cervix, starting close to the new squamocolumnar junction up to the junction with the isthmic endometrium. In between, an area lined with endocervical columnar cells without underlying reserve cells or at least a lower reserve cell density has been identified [34, 38]. An absence of reserve cells close the squamocolumnar junction could be a result of the metaplastic process in which these cells have differentiated into squamous epithelial cells.

Adenocarcinoma In Situ (AIS)

Adenocarcinoma in situ is characterized by the presence of atypical glandular epithelium without stromal invasion and is much less common than the SILs. It is generally accepted that AIS is the premalignant stage of an adenocarcinoma [72]. Most AIS lesions arise in the transformation zone, and a proportion of these glandular lesions are associated with HSIL [57, 61, 73]. The concurrence of AIS and HSIL in one and the same biopsy supports the hypothesis that both lesions may arise from a single progenitor cell. Reserve cells, capable of undergoing columnar as well as squamous differentiation, may be candidate progenitor cells for the formation of these combined AIS/HSIL lesions [28, 35]. It has been shown that the marker expression profile of solitary AIS lesions is heterogeneous. Two phenotypically distinct types can be identified, i.e., (1) AIS with an endocervical glandular phenotype (p63 negative to weakly positive, k5 negative and k17 sporadically positive, but k7 and k8 positive; see Fig. 1.10a–f) and (2) AIS with a reserve cell phenotype (p63, k5, and k17 positive; see Fig. 1.10h–j). In AIS with a coexisting HSIL, the glandular component often exhibits the reserve cell phenotype (see Fig. 1.11a–f). This observation supports the view that reserve cells are capable of bidirectional premalignant transformation, i.e., into HSIL and reserve cell type AIS, as well as AIS with a coexisting SIL (schematically presented in the right panel of Fig. 1.8b). The endocervical glandular type of AIS is probably a result of HPV infection of glandular epithelium or the unidirectional transformation of a HPV-infected progenitor cell within the glandular cell compartment [61].

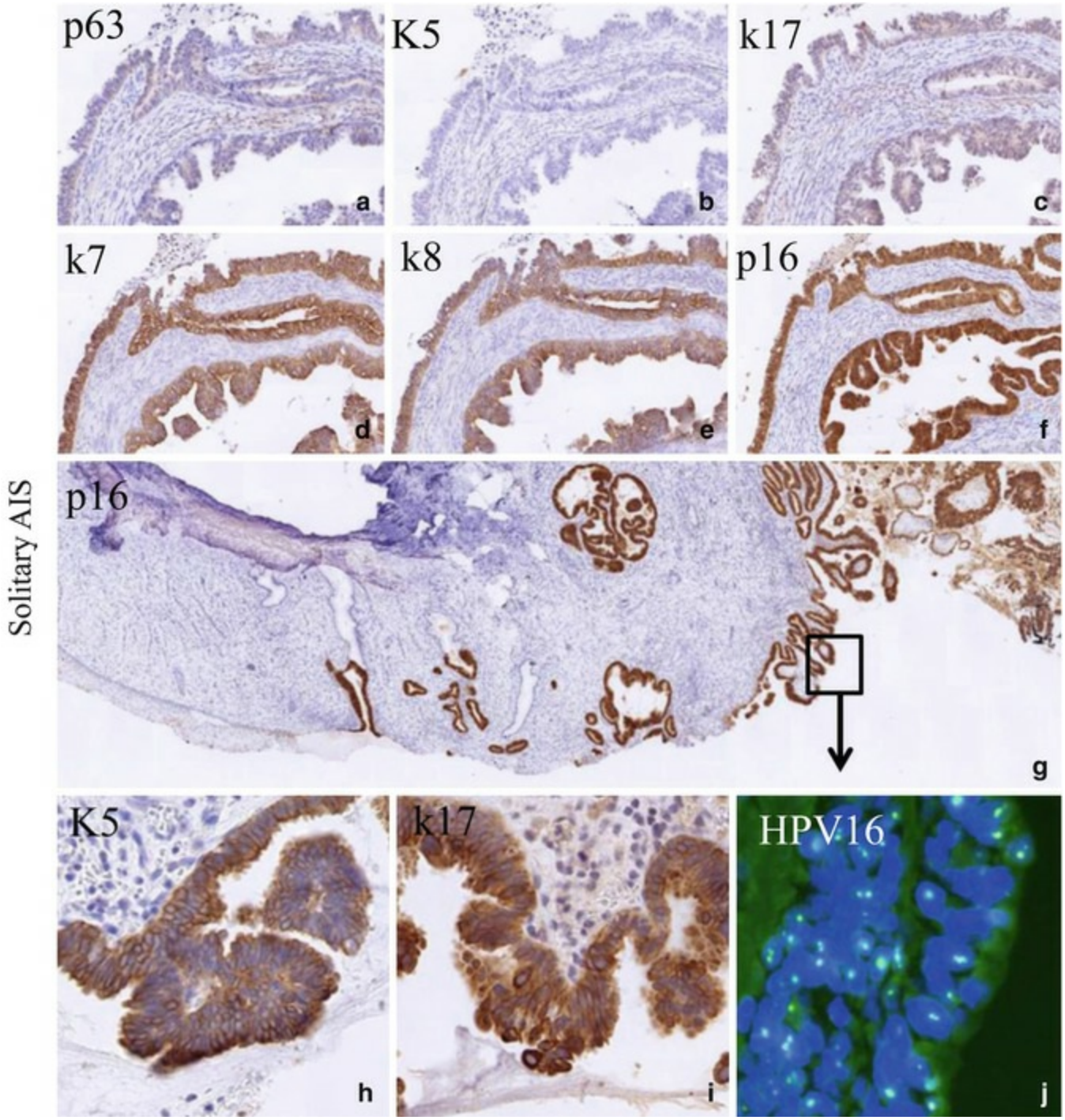

Fig. 1.10 Expression of p63, p16, and (cyto)keratins k5, k7, k8, and k17 in two different phenotypes of solitary adenocarcinoma in situ (*AIS*).(**a**–**f**) Endocervical glandular phenotype in AIS with strong positivity for k7 and k8. Diffuse strong p16 positivity indicates infection with high-risk HPV. (**g**–**j**) Reserve cell phenotype in AIS, with strong immunostaining of k5 and k17 in a HPV-positive lesion, as indicated by p16 immunoreactivity and integrated HPV 16 as detected by means of fluorescence in situ hybridization. These patterns support the view that reserve cells have a bidirectional differentiation potential

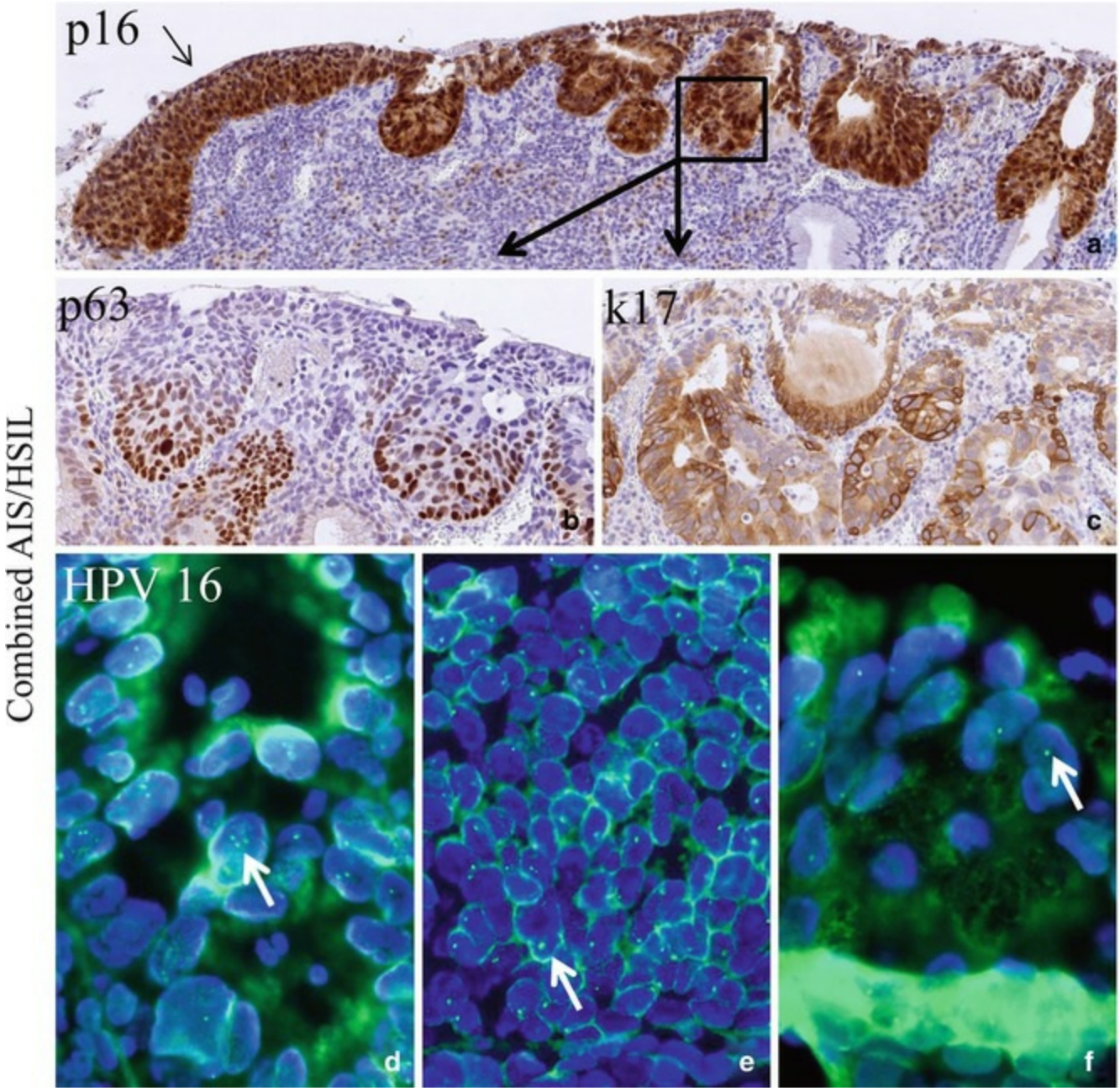

Fig. 1.11 Expression of p63, p16, (cyto)keratin k17, and HPV 16 in a combined adenocarcinoma in situ (AIS) with a high-grade squamous intraepithelial lesion (HSIL). The AIS and HSIL regions are both clearly p16 positive (**a**), indicating infection with high-risk HPV. Positivity of squamous markers p63 and k17 is seen in the glandular portion of this combined lesion (**b**, **c**). HPV 16 fluorescence in situ hybridization shows viral integration in both the glandular components (**d**, **f**) and in the squamous lesion (**e**). *Arrows* point to the nuclear integration site. Both the immunophenotyping and the hybridization results support the hypothesis that reserve cells can undergo a bidirectional transformation

On the basis of the HPV types found in AIS with and without SIL, it is suggested that HSIL coexisting with AIS is etiologically different from solitary HSIL [73]. HPV 18 and 45 showed a preference for AIS, while solitary HSILs contained a wide range of HPV types. Furthermore, for HPV 18 as compared to HPV 16, a difference in the physical

status of the virus is found in the premalignant lesion and in the carcinoma. Integrated HPV 16 is found in a relatively small fraction of HSILs and is almost absent in LSIL, while HPV 18 is frequently integrated in AIS [57, 74]. The examples shown in Figs. 1.10 and 1.11 demonstrate that after integration of the virus, the cancer cell is capable of a bidirectional premalignant transformation. This observation again supports the view of bidirectional differentiation of reserve cells in these lesions.

Acknowledgments

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References

1. Martens JE, et al. Reserve cells in human uterine cervical epithelium are derived from mullerian epithelium at midgestational age. Int J Gynecol Pathol. 2007;26:463–8.
[Crossref][PubMed]

2. Ince TA, et al. p63 Coordinates anogenital modeling and epithelial cell differentiation in the developing female urogenital tract. Am J Pathol. 2002;161:1111–7.
[Crossref][PubMed][PubMedCentral]

3. Kurita T. Developmental origin of vaginal epithelium. Differentiation. 2010;80:99–105.
[Crossref][PubMed][PubMedCentral]

4. Herfs M, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci U S A. 2012;109:10516–21.
[Crossref][PubMed][PubMedCentral]

5. Reich O, Fritsch H. The developmental origin of cervical and vaginal epithelium and their clinical consequences: a systematic review. J Low Genit Tract Dis. 2014;18:358–60.
[Crossref][PubMed]

6. Doorbar J, et al. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25:2–23.
[Crossref][PubMed][PubMedCentral]

7. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7:11–22.
[Crossref][PubMed]

8. Schiffman M, et al. Human papillomavirus and cervical cancer. Lancet. 2007;370:890–907.
[Crossref][PubMed]

9. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev. 2012;25:215–22.

[Crossref][PubMed][PubMedCentral]

10. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2:342–50.

11. Wright TC, Kurman RJ, Ferenczy A. Precancerous lesions of the cervix. In: Blaustein A, Kurman RJ, editors. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer; 2002.

12. Jacobson DL, et al. Cervical ectopy and the transformation zone measured by computerized planimetry in adolescents. Int J Gynaecol Obstet. 1999;66:7–17.
[Crossref][PubMed]

13. Burghardt E, Ostor AG. Site and origin of squamous cervical cancer: a histomorphologic study. Obstet Gynecol. 1983;62:117–27.
[PubMed]

14. Martens JE, et al. Cytokeratin 17 and p63 are markers of the HPV target cell, the cervical stem cell. Anticancer Res. 2004;24:771–5.
[PubMed]

15. Mirkovic J, et al. Carcinogenic HPV infection in the cervical squamo-columnar junction. J Pathol. 2015;236:265–71.
[Crossref][PubMed][PubMedCentral]

16. Klattig J, Englert C. The Mullerian duct: recent insights into its development and regression. Sex Dev. 2007;1:271–8.
[Crossref][PubMed]

17. Sanchez-Ferrer ML, et al. Experimental contributions to the study of the embryology of the vagina. Hum Reprod. 2006;21:1623–8.
[Crossref][PubMed]

18. Cai Y. Revisiting old vaginal topics: conversion of the Mullerian vagina and origin of the "sinus" vagina. Int J Dev Biol. 2009;53:925–34.
[Crossref][PubMed]

19. Drews U. Helper function of the Wolffian ducts and role of androgens in the development of the vagina. Sex Dev. 2007;1:100–10.
[Crossref][PubMed]

20. Kobayashi A, Behringer RR. Developmental genetics of the female reproductive tract in mammals. Nat Rev Genet. 2003;4:969–80.
[Crossref][PubMed]

21. Fritsch H, Richter E, Adam N. Molecular characteristics and alterations during early development of the human vagina. J Anat. 2012;220:363–71.
[Crossref][PubMed][PubMedCentral]

22. Fritsch H, et al. Development of epithelial and mesenchymal regionalization of the human fetal utero-vaginal anlagen. J Anat. 2013;222:462–72.
[Crossref][PubMed][PubMedCentral]

23. Kurita T, Mills AA, Cunha GR. Roles of p63 in the diethylstilbestrol-induced cervicovaginal adenosis. Development. 2004;131:1639–49.

[Crossref][PubMed]

24. Kurita T, et al. Differential expression of p63 isoforms in female reproductive organs. Mech Dev. 2005;122:1043–55.
[Crossref][PubMed]

25. Laronda MM, et al. The development of cervical and vaginal adenosis as a result of diethylstilbestrol exposure in utero. Differentiation. 2012;84:252–60.
[Crossref][PubMed][PubMedCentral]

26. Smedts F, et al. Detection of keratin subtypes in routinely processed cervical tissue: implications for tumour classification and the study of cervix cancer aetiology. Virchows Arch. 1994;425:145–55.
[Crossref][PubMed]

27. Smedts F, et al. Keratin expression in cervical-cancer. Am J Pathol. 1992;141:497–511.
[PubMed][PubMedCentral]

28. Smedts F, et al. Basal-cell keratins in cervical reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. Am J Pathol. 1992;140:601–12.
[PubMed][PubMedCentral]

29. Smedts F, Ramaekers FC, Vooijs PG. The dynamics of keratin expression in malignant transformation of cervical epithelium: a review. Obstet Gynecol. 1993;82:465.
[PubMed]

30. van Dorst EB, et al. The limited difference between keratin patterns of squamous cell carcinomas and adenocarcinomas is explicable by both cell lineage and state of differentiation of tumour cells. J Clin Pathol. 1998;51:679–84.
[Crossref][PubMed][PubMedCentral]

31. van der Heijden M, et al. Bcl-2 is a critical mediator of intestinal transformation. Nat Commun. 2016;7:10916.
[Crossref][PubMed][PubMedCentral]

32. Spencer TE, Dunlap KA, Filant J. Comparative developmental biology of the uterus: insights into mechanisms and developmental disruption. Mol Cell Endocrinol. 2012;354:34–53.
[Crossref][PubMed]

33. Elson DA, et al. Sensitivity of the cervical transformation zone to estrogen-induced squamous carcinogenesis. Cancer Res. 2000;60:1267–75.
[PubMed]

34. Martens JE, et al. Distribution pattern and marker profile show two subpopulations of reserve cells in the endocervical canal. Int J Gynecol Pathol. 2009;28:381–8.
[Crossref][PubMed]

35. Witkiewicz AK, et al. Microglandular hyperplasia: a model for the de novo emergence and evolution of endocervical reserve cells. Hum Pathol. 2005;36:154–61.
[Crossref][PubMed]

36. Stegner HE, Pape C. Ultramicoscopy studies on the dysplastic epithelium of the cervix and on carcinoma in situ. Fortschr Med. 1973;91:603–6.
[PubMed]

37. Ter Harmsel B, et al. BCL-2 immunoreactivity increases with severity of CIN: a study of normal cervical epithelia, CIN, and cervical carcinoma. J Pathol. 1996;179:26–30.
[Crossref][PubMed]

38. Hoogduin KJ, et al. BCL2 and keratin 5 define the uterine-cervix-isthmus junction, a transition between endocervical and tubal-like epithelium. Int J Gynecol Pathol. 2013;32:122–30.
[Crossref][PubMed]

39. Hiersche HD, Nagl W. Regeneration of secretory epithelium in the human endocervix. Arch Gynecol. 1980;229:83–90.
[Crossref][PubMed]

40. Herfs M, et al. A novel blueprint for 'top down' differentiation defines the cervical squamocolumnar junction during development, reproductive life, and neoplasia. J Pathol. 2013;229:460–8.
[Crossref][PubMed]

41. Tamussino K, Girardi F, Reich O. Burghardt's colposcopy and cervical pathology. 4th ed. New York: Thieme; 2015.

42. McNairn AJ, Guasch G. Epithelial transition zones: merging microenvironments, niches, and cellular transformation. Eur J Dermatol. 2011;21:21–8.
[PubMed]

43. Mukonoweshuro P, Oriowolo A, Smith M. Audit of the histological definition of cervical transformation zone. J Clin Pathol. 2005;58:671.
[PubMed][PubMedCentral]

44. Ferenczy A, Wright T. Anatomy and histology of the cervix. In: Kurman R, editor. Blaustein's pathology of the female genital tract. New York: Springer; 1994. p. 185–201.
[Crossref]

45. Herfs M, Hubert P, Delvenne P. Epithelial metaplasia: adult stem cell reprogramming and (pre)neoplastic transformation mediated by inflammation? Trends Mol Med. 2009;15:245–53.
[Crossref][PubMed]

46. Herfs M, et al. Mucosal junctions: open doors to HPV and HIV infections? Trends Microbiol. 2011;19:114–20.
[Crossref][PubMed]

47. Hwang LY, et al. Active squamous metaplasia of the cervical epithelium is associated with subsequent acquisition of human papillomavirus 16 infection among healthy young women. J Infect Dis. 2012;206:504–11.
[Crossref][PubMed][PubMedCentral]

48. Ferenczy A, Wright T. Anatomy and histology of the cervix. In: Kurman R, editor. Blaustein's pathology of the female genital tract. New York: Springer; 2002. p. 207–25.

49. Regauer S, Reich O. CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). Histopathology. 2007;50:629–35.
[Crossref][PubMed][PubMedCentral]

50. Tsutsumi K, et al. In vitro and in vivo analysis of cellular origin of cervical squamous metaplasia. Am J Pathol. 1993;143:1150–8.
[PubMed][PubMedCentral]

51.

Miyatake T, et al. Clonality analysis and human papillomavirus infection in squamous metaplasia and atypical immature metaplasia of uterine cervix: is atypical immature metaplasia a precursor to cervical intraepithelial neoplasia 3? Int J Gynecol Pathol. 2007;26:180–7.
[Crossref][PubMed]

52. Ueda Y, et al. Monoclonal expansion with integration of high-risk type human papillomaviruses is an initial step for cervical carcinogenesis: association of clonal status and human papillomavirus infection with clinical outcome in cervical intraepithelial neoplasia. Lab Investig. 2003;83:1517–27.
[Crossref][PubMed]

53. van der Marel J, et al. Oncogenic human papillomavirus-infected immature metaplastic cells and cervical neoplasia. Am J Surg Pathol. 2014;38:470–9.
[Crossref][PubMed]

54. Keating JT, et al. Ki-67, cyclin E, and p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Pathol. 2001;25:884–91.
[Crossref][PubMed]

55. Roberts JN, et al. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. Nat Med. 2007;13:857–61.
[Crossref][PubMed]

56. Lopez J, et al. Human papillomavirus infections and cancer stem cells of tumors from the uterine cervix. Open Virol J. 2012;6:232–40.
[Crossref][PubMed][PubMedCentral]

57. Witkiewicz A, et al. Superficial (early) endocervical adenocarcinoma in situ: a study of 12 cases and comparison to conventional AIS. Am J Surg Pathol. 2005;29:1609–14.
[Crossref][PubMed]

58. Herfs M, Crum CP. Cervical cancer: squamocolumnar junction ablation--tying up loose ends? Nat Rev Clin Oncol. 2015;12:378–80.
[Crossref][PubMed]

59. Herfs M, et al. Unique recurrence patterns of cervical intraepithelial neoplasia after excision of the squamocolumnar junction. Int J Cancer. 2015;136:1043–52.
[Crossref][PubMed]

60. Selvi K, et al. Role of p16, CK17, p63, and human papillomavirus in diagnosis of cervical intraepithelial neoplasia and distinction from its mimics. Int J Surg Pathol. 2014;22:221–30.
[Crossref][PubMed]

61. Smedts F, Ramaekers FC, Hopman AH. The two faces of cervical adenocarcinoma in situ. Int J Gynecol Pathol. 2010;29:378–85.
[Crossref][PubMed]

62. Ponten J, Guo Z. Precancer of the human cervix. Cancer Surv. 1998;32:201–29.
[PubMed]

63. Quint W, et al. One virus, one lesion – individual components of CIN lesions contain a specific HPV type. J Pathol. 2012;227:62–71.
[Crossref][PubMed]

64.

Litjens RJ, et al. The majority of metachronous CIN1 and CIN3 lesions are caused by different human papillomavirus genotypes, indicating that the presence of CIN1 seems not to determine the risk for subsequent detection of CIN3. Hum Pathol. 2014;45:221–6.
[Crossref][PubMed]

65. Herfs M, et al. Cervical squamocolumnar junction-specific markers define distinct, clinically relevant subsets of low-grade squamous intraepithelial lesions. Am J Surg Pathol. 2013;37:1311–8.
[Crossref][PubMed][PubMedCentral]

66. Guo M, et al. Evaluation of a commercialized in situ hybridization assay for detecting human papillomavirus DNA in tissue specimens from patients with cervical intraepithelial neoplasia and cervical carcinoma. J Clin Microbiol. 2008;46:274–80.
[Crossref][PubMed]

67. Hopman AH, et al. HPV in situ hybridization: impact of different protocols on the detection of integrated HPV. Int J Cancer. 2005;115:419–28.
[Crossref][PubMed]

68. Evans MF, et al. Biotinyl-tyramide-based in situ hybridization signal patterns distinguish human papillomavirus type and grade of cervical intraepithelial neoplasia. Mod Pathol. 2002;15:1339–47.
[Crossref][PubMed]

69. Paquette C, Mills AM, Stoler MH. Predictive value of cytokeratin 7 immunohistochemistry in cervical low-grade squamous intraepithelial lesion as a marker for risk of progression to a high-grade lesion. Am J Surg Pathol. 2016;40:236–43.
[PubMed]

70. Escobar-Hoyos LF, et al. Keratin 17 in premalignant and malignant squamous lesions of the cervix: proteomic discovery and immunohistochemical validation as a diagnostic and prognostic biomarker. Mod Pathol. 2014;27:621–30.
[Crossref][PubMed]

71. Smedts F, Ramaekers FC, Hopman AH. CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia. Histopathology. 2008;52:515–6.
[Crossref][PubMed]

72. Zaino RJ. Glandular lesions of the uterine cervix. Mod Pathol. 2000;13:261–74.
[Crossref][PubMed]

73. Bekkers RL, et al. Coexisting high-grade glandular and squamous cervical lesions and human papillomavirus infections. Br J Cancer. 2003;89:886–90.
[Crossref][PubMed][PubMedCentral]

74. Theelen W, et al. Human papillomavirus multiplex ligation-dependent probe amplification assay for the assessment of viral load, integration, and gain of telomerase-related genes in cervical malignancies. Hum Pathol. 2013;44:2410–8.
[Crossref][PubMed]

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2. Human Papillomaviruses (HPVs)

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Abstract
HPVs are epitheliotropic viruses with double-stranded DNA genomes and eight coding genes defined as “early” or “late” depending on when they are expressed. Over 200 HPVs have been identified with 13 types considered oncogenic or high risk (HR). HPV type 16 confers the greatest risk, being responsible for around 60% of cervical cancers. HPV infection is common with a global point prevalence of around 10% and most infections are transient. The life cycle of HPV is inextricably linked with squamous epithelial differentiation and, during a productive infection, involves tightly regulated sequential gene expression at the separate epithelial layers before particle release. Some persistent infections can lead to cancer; in this scenario the productive life cycle is not completed, and deregulated expression of early oncoproteins E6 and E7 stimulates uncontrolled cellular proliferation while abrogating tumor suppressor function.

Prophylactic HPV immunization and the use of molecular HPV testing as a primary cervical screening test have been implemented in several settings. Immunization has led to a significant decrease in HPV infection and associated disease at the population level, and the high sensitivity and reproducibility of HPV testing enables screening intervals to be extended (for those who test negative) and provides options for self-sampling. Future challenges will include how to integrate and implement immunization and contemporary screening practices most optimally.

Keywords HPV – Cervical cancer – Molecular testing – Immunization

Introduction

Papillomaviruses (PVs) are small, non-enveloped double-stranded (ds) deoxyribonucleic acid (DNA) viruses belonging to the *Papillomaviridae* family. Apart from being site specific, PVs are also highly species specific [1]. Infecting most mammals and birds, more than 300 PV types have been identified including over 200 human papillomaviruses (HPV) (see [2] and Papillomavirus Episteme (PaVE); http://pave.niaid.nih.gov/#home).

HPV infection is extremely common and over 80% of individuals will be infected at some point in their lifetime. The course of infection is generally subclinical, and only a minority of those infected are affected by their exposure. As a strictly epitheliotropic virus, its transmission is sometimes referred to as "skin to skin" where packaged virions are transferred from one individual to another inside terminally differentiated cells – thus requiring a level of contact.

Classification

The members of the PV family are formally classified based on sequence homology of the L1 open reading frame (ORF) – the main structural gene of the virus which is highly conserved, including for the risk association of the virus [3]. The family is divided into genera denoted by Greek letters which have less than 60% homology between the sequences. The human PVs belong in the *alpha, beta, gamma, mu,* and *nu* genera. HPV "species" then aggregate groups of related HPV "types" and are denoted by their host genus, and then a number, for example, HPV 16, is contained (alongside HPVs 31, 33, 35, 52, 58) in *alpha* species group 9 (see Fig. 2.1, adapted from [4]). Species groups contain 61–70% sequence homology. After species come types which have 71–89% homology. Within types are subtypes which have 90–98% homology, and finally within subtypes there are variants which have more than 98% homology. Certainly, the common currency for describing HPVs is at the "type" level (rather than as species), with types being allocated their respective number according to when they were formally ratified by the International HPV Reference Center.

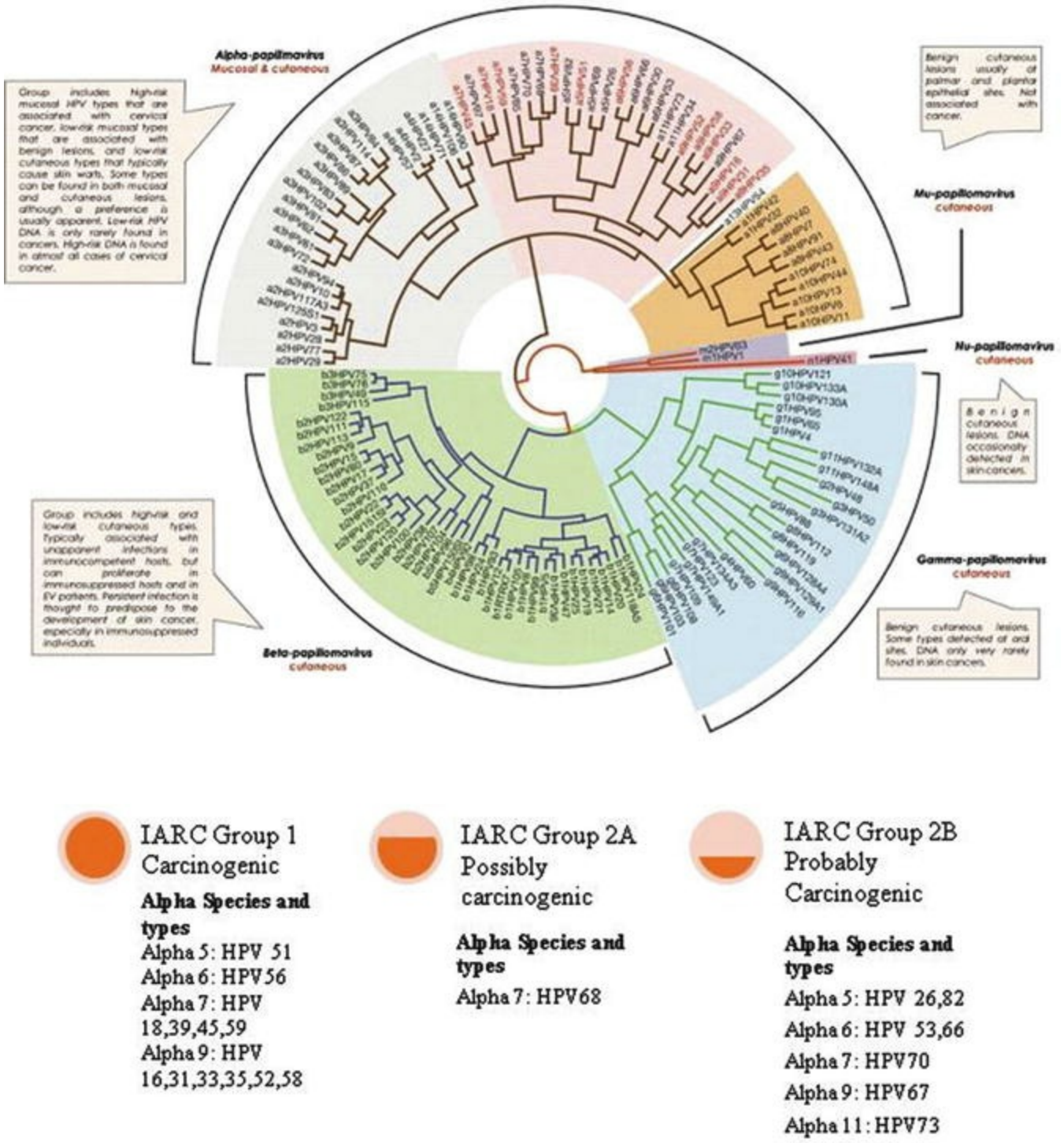


Fig. 2.1 Classification of PVs based on phylogeny and risk status. Maximum likelihood phylogenetic tree for E1, E2, L1 and L2 genes of 132 HPVs at the amino acid level (Reprinted from Doorbar et al. [4], Copyright 2012, with permission from Elsevier)

The *alpha* genus contains the bulk of clinically relevant HPVs including the oncogenic types which can infect the genital mucosa. However, within the *alpha* genus, there are some HPV types which also infect cutaneous epithelium causing benign skin warts such as types −2 and −57. The *beta, gamma, mu, and nu* viruses generally infect

cutaneous epithelia with *beta* HPVs involved in epidermodysplasia verruciformis (EV)-specific lesions. Table 2.1 lists the HPV types within each genus and some of the common manifestations of the different viral types.

Table 2.1 Summary of clinical manifestations of HPV types

Genus	Tropism	Risk classification	Common types
Alphapapillomavirus	Mucosal	High risk	16,18,31,33,35,39,45,51,52,56,58,59,66,68
	Mucosal	Low risk	6,7,11,13,26,28,29,30,32,34,40,42,43,44,53,54,61,62,67,69,70,71,72,73,
	Cutaneous	Low risk	2,3,10,27,57
Betapapillomavirus	Cutaneous		5,8,9,12,14,15,17,19,20,21,22,23,24,24,25,36,37,38,47,49,75,76,80,92,9

Gammapapillomavirus	Cutaneous		4,48,50,60,65,88,95,101,103,108,109,112,116,119,121,123,128A4,129
Mupapillomavirus	Cutaneous		1,63
Nupapillomavirus	Cutaneous		41

In addition to a systematic approach to classifying HPVs according to primary sequence, HPV types are aggregated according to the strength of their association with malignancy. The International Agency on Research on Cancer (IARC) set up a working group to consider the evidence on the risk status of the various HPV types through the assessment of global epidemiological data with an emphasis on case control studies. Currently, 12 HPV types are considered Group 1 carcinogens, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (Fig. 2.1), with a further one (HPV 68) and seven types

(26, 53, 66,67,70,73, 82) considered “probably” or “possibly” carcinogenic respectively according to IARC definition [5].

The concept of low-risk types is also well established, with perhaps the most clinically relevant being HPVs 6 and 11 which are responsible for around 90% of genital warts and also a proportion of CIN1. See Fig. 2.2 for representative images of genital warts [6]. There are reports of “possibly carcinogenic” types and even low-risk types being the only type (or types) detectable in cancers. With this said, given that 96% of all HPV-positive cervical cancers are attributable to types in Groups 1 and 2A alone, it is reasonable to accept that any types beyond this are rarely associated with significant disease. Finally, it is important to emphasize that the IARC categories are somewhat mutable and subject to further change as evidence accumulates – for example, HPV 66 while originally considered a Group 1 carcinogen in 2005 was relocated to the “possibly carcinogenic” category based on updated analysis of the evidence [5].

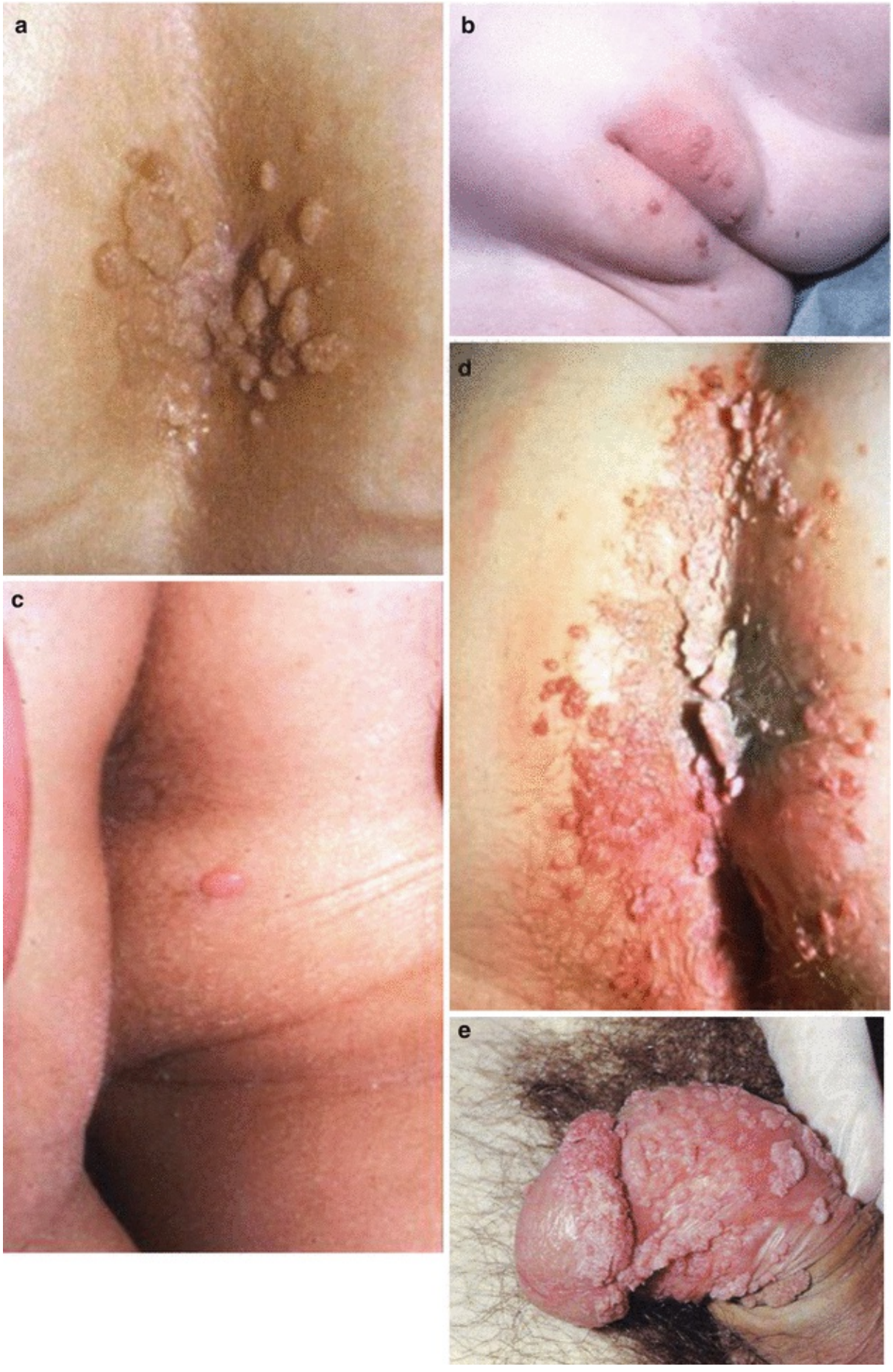
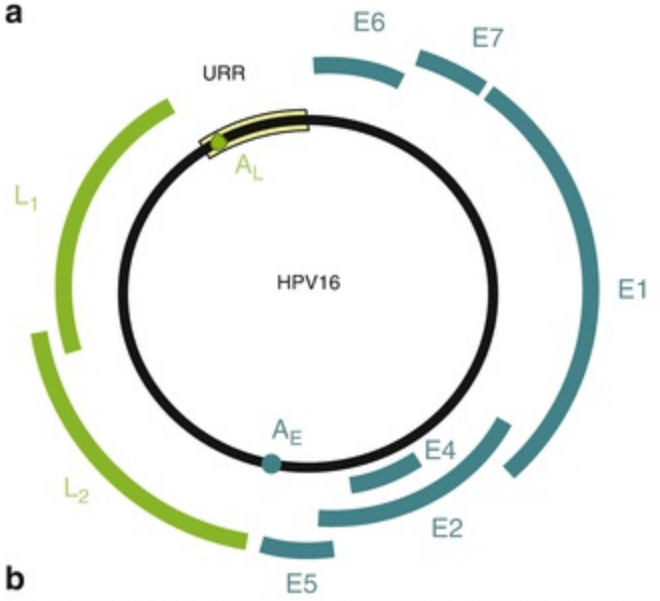

a
b
c
d
e

Fig. 2.2 Anogenital warts. (**a**) Anogenital warts in adult. (**b**) Anogenital warts on the labia of a child. (**c**) Single genital wart on the buttock of a child. (**d**) Extensive perianal warts in renal allograft recipient. (**e**) Condylomata acuminata on the glans penis and foreskin (Reprinted from Cubie [6], Copyright 2013, with permission from Elsevier)

HPV Genome Organization and Life Cycle

The HPV genome is made of approximately eight kilobases (Kb) of circular episomal dsDNA. It is divided into three regions: (1) a long control region (LCR), containing the origin of replication (Ori) and promoter sites for transcription of genes which is variable between different species of HPV indicating the diverse evolution of the virus; (2) an early region coding region for the genes – E1, E2, E4, E5, E6, and E7; and (3) a late region coding for genes L1 and L2. While the late genes, particularly L1, are highly conserved between different PVs, there is much heterogeneity associated with the early genes. For example, the E5 gene does not exist in beta PVs, and the E6 gene is absent in some gamma PVs such as HPVs 101, 103, and 108 [7]. Figure 2.3 lists the major HPV ORFs and their functions [8].

a



b

Protein	Functions
E1	Involved in HPV genome replication.
E2	HPV genome replication and transcription factor for E6 and E7. Also has a role in genome segregation.
E4	Expressed as E1^E4 fusion protein. Disrupts cytokeratins and aids in virion release.
E5	Small transmembrane protein which activates EGFR signalling pathway and has a role in evading immune response.
E6	Oncoprotein which interacts with and degrades p53 and activates telomerase activity. Involved in cell proliferation, avoiding apoptosis and immune evasion.
E7	Oncoprotein which drives cell cycle through binding and degradation of pRB. Causes chromosomal instability and transformation.
L1	Major capsid protein involved in encapsidation of the virus and viral entry and nuclear trafficking.
L2	Minor capsid protein involved in encapsidation of the virus and viral entry and nuclear trafficking.

Fig. 2.3 (**a**) Schematic representation of the genomic organization of HPV-16. The genome contains early (in *blue*) and late (in *green*) regions, which relate to the timing of expression during the viral life cycle. The genome also contains an upstream regulatory region (URR) and two promoters for early (AE) and late (AL) gene expression. (**b**) The main functions of each of the viral proteins are listed in the table (Reprinted from Stanley [8], Copyright 2010, with permission from Elsevier)

Insights into the life cycle of HPV are mostly gained through an understanding of *alpha* PVs. However, the broad principles can be extended to other PVs. The life cycle of HPV is very closely related to differentiating epithelium (Fig. 2.4) [9]. Infection of the virus is thought to occur through microwounds in the epithelial layer providing access to basal stem cells. It is well established that the transformation zone, and particularly the metaplastic region, is susceptible to infection due to increased accessibility and proliferation of the basal cell layers (see also Chap. 1). However,

more recently, the discovery of a discrete population of squamocolumnar junction (SCJ) cells which may be prone to infection has also been suggested [10]. Virus particles bind to glycosaminoglycan (GAG) chains of heparin sulfate proteoglycan (HSPGs) which leads to a conformational change in the virion. Additional receptors are thought to be required for viral entry but these have yet to be properly characterized [11]. Upon infection, E1 and E2 are the first viral proteins expressed leading to establishment of episomal copy number of between 50 and 200. Maintenance of a low level of viral episomes in the basal layer which can then reactivate has been demonstrated in animal models. Reactivation may be as a consequence of changes in immune surveillance, alterations in hormone levels and/or growth factors, UV irradiation, or abrasion/wounding. There is also evidence to suggest that a latent, rather than productive, infection may be more likely to occur in anatomical sites where the complete life cycle can be supported only poorly. Like many other viruses, the notion of latent infection with HPV without clinical or microscopic signs of disease is credible.

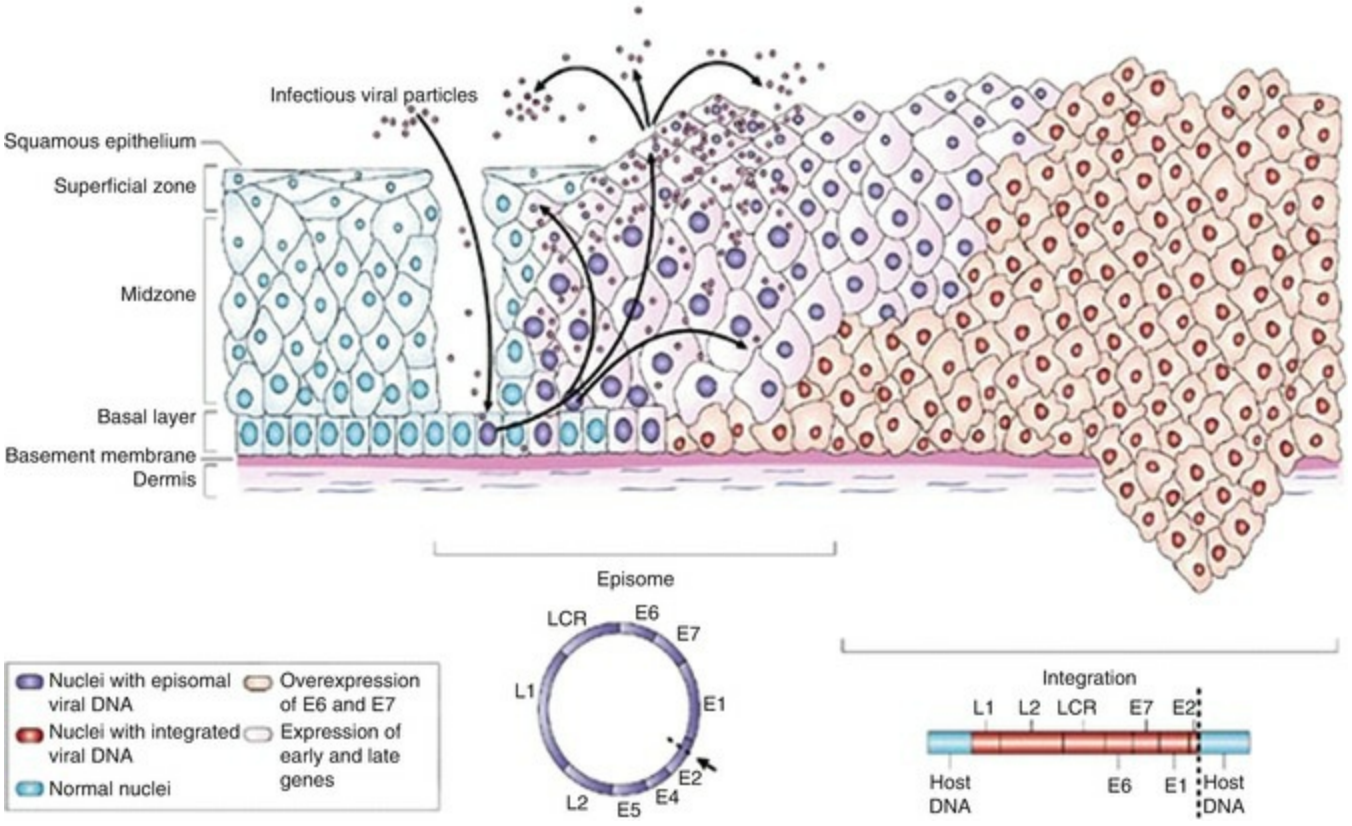


Fig. 2.4 HPV life cycle in differentiating epithelium. The figure shows normal differentiation of stratified squamous epithelium in the leftmost corner (*light blue*). In the middle panel, HPV-infected basal keratinocytes (shown in *dark blue*) with episomal viral genome divide and move on to suprabasal layers which remain in the cell cycle and continue to proliferate. Virions are produced and shed from the uppermost layer of the epithelium. Cells with integrated genomes (*red*) are shown on the right panel. Integration can cause immortalization of cells and continuous proliferation leading to malignancy (Reprinted by permission from Macmillan Publishers Ltd.: Woodman et al. [9], copyright 2007)

In the "productive" life cycle of HPV (i.e., the ability of the virus to make daughter

viruses), E2 protein along with cellular tethering proteins such as bromodomain-containing protein 4 (Brd4) attaches the viral episome to the cellular genome, and viral replication occurs along with cell replication. E1 and E2 proteins are also responsible for the regulation and transcription of other early proteins E5, E6, and E7. The initial proliferation of basal cells by cellular factors is essential for driving expression of the viral proteins during a productive life cycle, and E5, E6, and E7 proteins modify the cellular environment to allow viral genome amplification. As the infected cells move to the upper layers of epithelium, the expression of late proteins E4, L1, and L2 occurs. The virions are initially assembled by recruitment of L2 protein to the replicating episome with the major and minor capsid proteins (L1 and L2) then incorporated at a 5:1 ratio to create the viral particle. The E4 protein disrupts the keratin structure and aids virion release in the topmost layers of the epithelium. This life cycle of the virus is seen in most low-risk HPV infections and a component of high-risk infections. Of note, the E5, E6, and E7 proteins associated with high-risk types differ from those of the low-risk types, particularly in their capacity to drive cell proliferation in the basal cell compartment, interact with tumor suppressor proteins and in their capacity for immune evasion.

The reasons why some infections do not follow a productive course are not fully understood. Integration of the viral DNA into the host genome is considered a risk factor for a nonproductive and potentially transforming infection, although integrated viral HPV DNA can been found in normal cervical tissue. Most cancers have “mixed” forms of the virus (i.e., episomal and integrated), although a component contains episomal forms exclusively. There is evidence to suggest that the presence of an intact E2 gene in episomally driven cancers can hinder the effect exerted by E6 and E7, with E5 exerting a more significant role in this group [12].

Integration is randomly distributed, and although the characteristics of potentially “fragile” areas of the genome have been described, no specific preferential integration site on the genome has been defined [13]. However, it has been observed that disruption of the E2 gene of HPV is a consequence of integration [14]. E2 controls the transcription of viral oncogenes E6 and E7, and its levels within the different layers of the epithelium are tightly regulated to control the viral life cycle. Release of E6 and E7 from the transcriptional control mediated by E2 leads to high-level expression of E6 and E7, which induces a series of cellular processes that can lead to the immortalization and malignant transformation of cells.

E7 is a small protein and one of its main functions is its binding and inactivation of cell cycle regulator retinoblastoma (RB). The E7 protein of high-risk *alpha* HPVs exerts a stronger binding affinity to pRB due to structural subtleties in the N terminus of the protein (compared to low-risk types). In G0 and early G1 phases of cell cycle, RB binds to and inactivates the E2F family of transcription factors which mediate the transcription of genes responsible for S-phase progression. During late G1, RB is

phosphorylated by cyclin-dependent kinases (CDKs) and hyperphosphorylated RB releases E2F. The continuous expression of E2F-responsive genes leads to uncontrolled cell division. Protein phosphatase PP1c acts by competing with CDKs to dephosphorylate RB in order to maintain control over cell cycle. E7 associated with HR-HPV binds to RB and releases E2F for constitutive expression of S-phase genes for continued cell proliferation. It also binds weakly to other proteins involved in cellular proliferation such as p107 and E2F1. The immortalization and transformation abilities of "high-risk" E7s have been proven, whereas low-risk types lack this ability.

As with E7, E6 proteins associated with HR-HPVs also have a stronger association with a tumor suppressor protein – in this case, p53. Upon activation, p53 transcriptionally activates genes required for apoptosis and cell cycle arrest. However, in cells infected with HR-HPVs, the activity of p53 is modulated by E6. E6 causes ubiquitin-mediated proteolysis of p53 through its interaction with the ubiquitin ligase E6-associated protein (E6AP). The E5 protein, on the other hand, stabilizes and enhances epidermal growth factor (EGF) receptor and signaling and mitogen-activated protein (MAP) kinase activity. The aforementioned processes lead to proliferation of damaged cells lacking capabilities for repair and susceptible to secondary mutation – all of which can predispose to a malignant phenotype [15].

Epidemiology

Given the remit of this book, focus will be on HR-HPV infection in females and implications for cervical disease. However, there are a number of sources where information on epidemiology in other neoplasms and males can be sought [16]. Notably 5–8% of all human cancers are thought to be associated with HPV.

Prevalence in the General Population

While the incidence of HPV-associated morbidity and mortality varies greatly worldwide, global data indicate that the pattern of infection is relatively consistent. From the female perspective, HPV is acquired soon after initiation of sexual activity, with a peak in women up to age 25 and then a decline, until an age range of 35–44 years. While this pattern of "peak and decline" has been demonstrated in several countries, there is evidence for country-specific differences in the amount of circulating HPV infection (overall prevalence) and also type-specific prevalence, even in developed countries which provide similar health-care services [17]. For example, data from the Netherlands indicate that the prevalence of HR-HPV in (unvaccinated) women aged 30–60 attending for routine cervical screening is around 6% [18]. The comparative figure in the UK is around 12%. After adjusting for study design, age, and detection methodology, the worldwide HPV DNA point prevalence has been estimated

at around 10% with the highest estimates in Africa and Latin America (20–30%) and the lowest in Southern Europe and Southeast Asia (6–7%). Reasons for these differences are manifold and likely to do with differing relative contributions of external risk factors, sociodemographic differences, different sexual practices and mores, and, potentially, inherent genetic susceptibility.

A second "peak" of HPV infection has been described in women aged over 45. Although this is not replicated in all country-specific settings, there are sufficient data to indicate that this is a real phenomenon in some. Whether this is brought about by cohort effect, waning immunity to past infection, or changed sexual practices, is not well established [5, 17].

Although the bulk of HPV infection is acquired after the onset of sexual activity, infection with HR-HPV can be acquired in childhood in the absence of abuse. HPV epidemiological and natural history data in children is relatively sparse compared to adults; however, the evidence would suggest that HPV can be acquired vertically, during vaginal delivery and also postpartum through close contact with carer and child. While most of the infections clear at around 6 months of life, longitudinal "family" studies have indicated that a proportion will persist beyond this and infections with *beta* types such as HPV 2 and *alpha* types including HPV 16 have been observed beyond 6 months [19].

While HPV 16 is generally always the most commonly detected high-risk type, the subsequent prevalence/rank of the other 11 Group 1 high-risk types after this also varies between countries. However, when type-specific prevalence is assessed within significant disease (CIN2+) rather than in the general population, the rankings largely converge as will be discussed later. Figure 2.5 shows HR-HPV prevalence in a cross section of women attending for routine cervical screening in Scotland [20]. While data gleaned from the screening programs are undoubtedly helpful given that they incorporate a wide age range and include the peak age for cervical cancer incidence, they do underestimate the extent of infection given that they do not capture women who do not attend screening services, who are at greater risk of infection and disease.

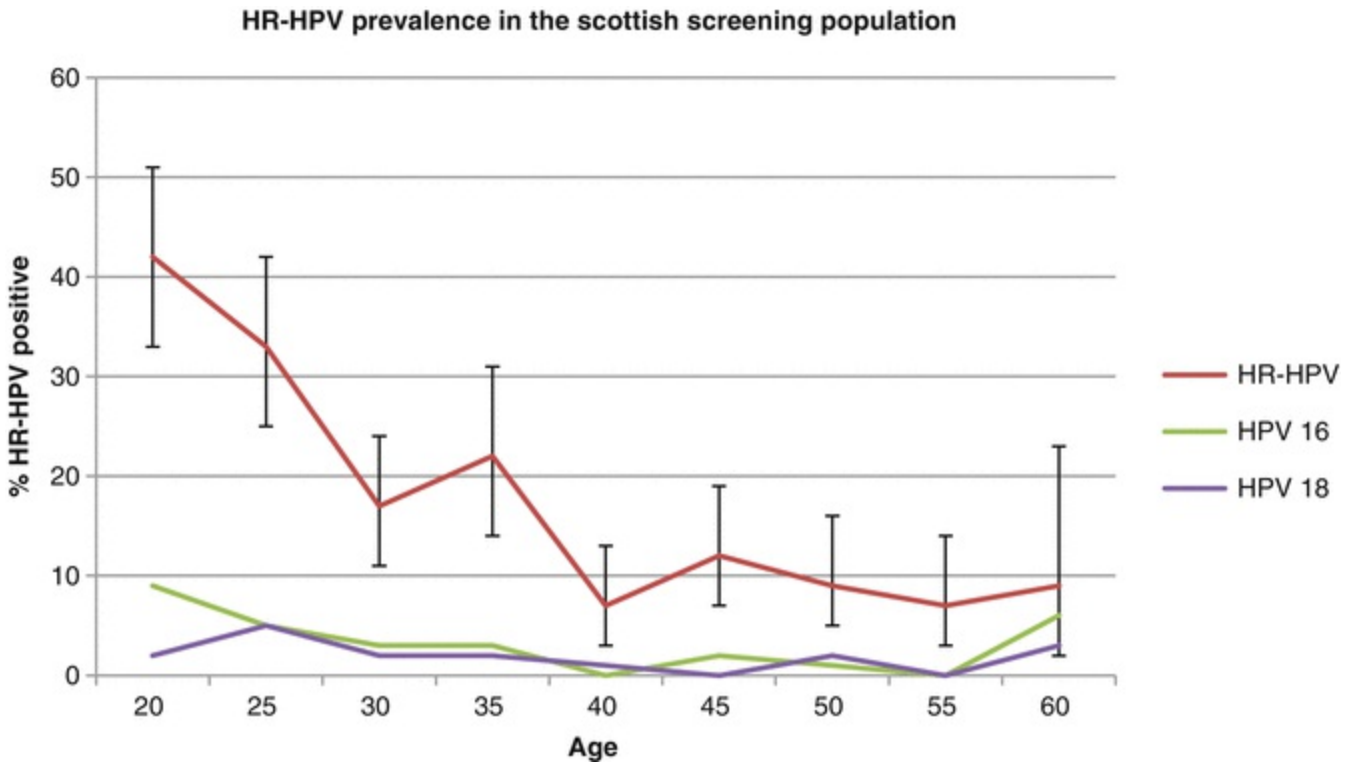


Fig. 2.5 HR-HPV prevalence in women aged 20–60 attending for routine cervical screening in Scotland [20]

Clearance and Risk

Most infections of the genital tract including high-risk infections clear, or become undetectable, within 2 years after infection. However, the likelihood and rate of this clearance is affected by the specific high-risk HPV type and comorbidities such as immune suppression or whether the infection is associated with underlying cervical disease or not. Consistent use of condoms has been shown to reduce transmission, although as HPV is "pan genital" and detectable on the buttocks, scrotum, and perianal region, condoms are, at best, partially protective. Male circumcision has also been demonstrated to reduce the risk of HPV transmission [21].

While HPV acquisition has been demonstrated to reduce with age, there are some reports which indicate that persistent infection is more likely to manifest at older ages (>30 years). However, these observations are somewhat confounded by a lack of consistency as to what defines a persistent infection. Persistent infection is often described in terms of an individual having the same HR-HPV type(s) over two sequential measurements, yet the time between those visits can vary from 6 months or less to several years. Furthermore, the duration of infection prior to the first positive measurement is generally unknown in longitudinal studies. Notwithstanding these issues, women who have "persistent" infection, defined as testing HPV positive over two or more visits, are at increased risk of developing CIN2+ compared to those who have a transient infection.

Differential type-specific risks of the various HR-HPV types for the development of high-grade lesions are also well documented – particularly, the unique "riskiness" of HPV 16. Hazard ratios relating to the development of CIN2+ within 2 years after 6-month persistent infections (6MPI) with HPV 16, HPV 33, HPV 31, HPV 45, and HPV 18 have been reported as 10.44, 9.65, 5.68, 5.38, and 3.8, respectively, compared to women infected with a low-risk HPV type (Fig. 2.6). When CIN3+ is used as an endpoint, women with a 6MPI with HPV 16 and HPV 33 have a 25-fold higher risk of progression to CIN3+ (compared to women infected with a low-risk type). Comparatively, women with a 6MPI infection with HPV 31 have been shown to have a tenfold higher risk of CIN3+, while women infected with HPV 18 or 45 or any of the remaining high-risk types harbor a sixfold and fourfold higher risk, respectively [22, 23].

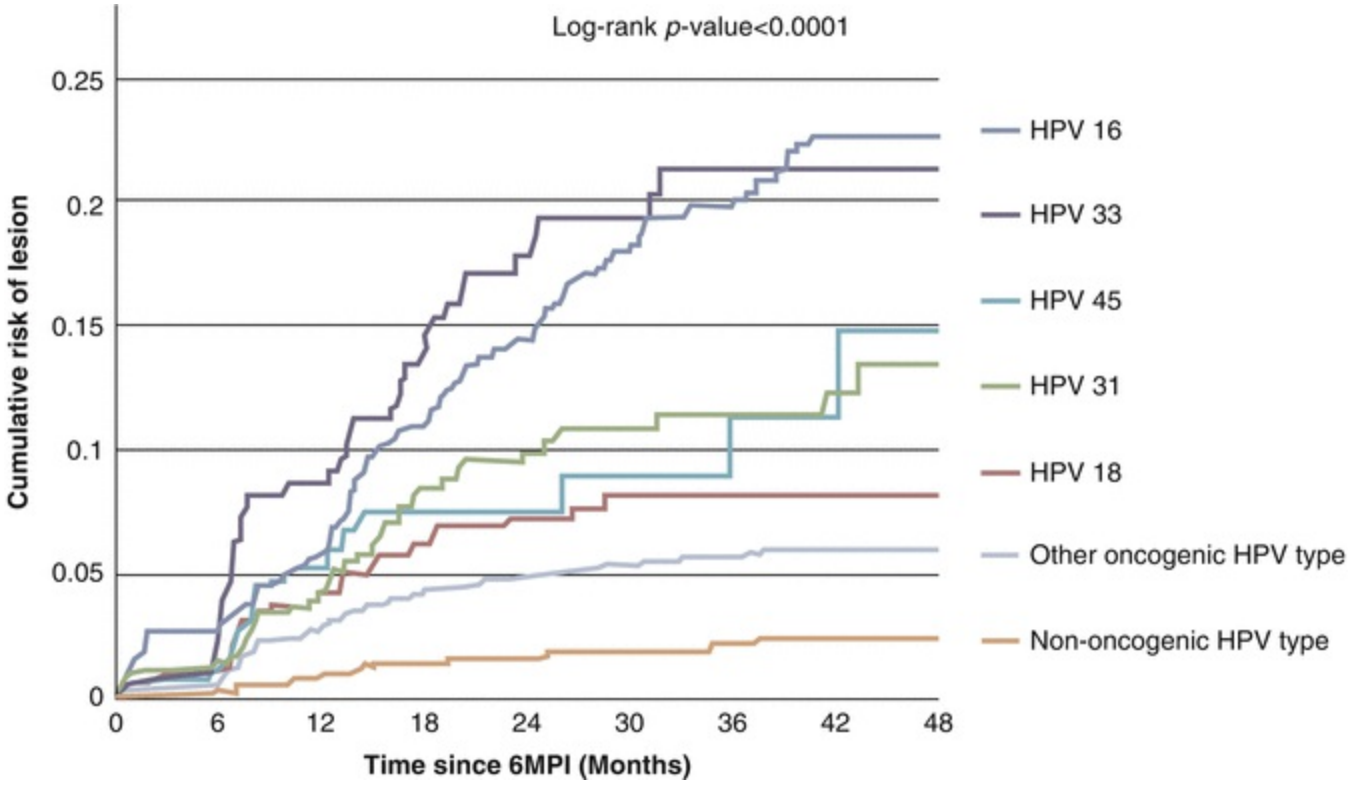


Fig. 2.6: Risk of progression of a 6-month persistent infection (6MPI) for CIN2+ associated with the same HPV type. Kaplan-Meier estimates of cumulative risk (%) of developing CIN2+ lesions associated with the same HPV type were calculated for HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, and other oncogenic HPV types and non-oncogenic HPV types (Figure reproduced with permission from Jaisamrarn et al. [22])

Predictors of incident infections are, unsurprisingly, correlates of sexual behavior of the woman and her partner – these include age at onset of sexual intercourse, number of partners, relationship status (single or not), and smoking. In women who have reported being in a monogamous relationship, the predictors of incident infection have included not living with a partner and the age of that partner. Persistent oral contraceptive use has

been associated with persistence of HR-HPV as has coinfection with *Chlamydia trachomatis*, although there is some disparity in the literature as to the magnitude of this effect after adjustment for behaviors and lifestyle [5]. The implications of multiple infection on acquisition, persistence, and risk of infection are also somewhat unclear; cross-sectional studies indicate that after adjustment for age and underlying pathology, the presence of >1 HR type does not confer an additional risk for subsequent infection and disease, whereas others have indicated that there is a greater chance of acquiring a new HPV type if already infected and that this does indeed confer an additional risk of CIN development. Certainly multiple infection is common, particularly in young women under 30 with around 50% of those who are HR-HPV positive being infected with more than one type [17]. Generally, although an individual may be infected with multiple types, visible lesions are generally clonal (i.e., one type-one lesion), and this has been demonstrated with laser capture dissection methodologies and highly sensitive genotyping technologies.

Prevalence in Disease (Histological)

Most, but not all, CIN2+ lesions contain HR-HPV detectable at the molecular level. Certainly, the majority of cervical cancer is driven by HPV 16, generally followed by HPV 18 with up to 70% of cancers being associated with these types (Fig. 2.7) [24]. When the denominator is restricted to cancers where any HR-HPV has been detected, the percentage positive for HPV 16 and/or 18 can increase to 80%. With respect to morphology, a higher proportion of adenocarcinomas are positive for HPV 18 compared to squamous cell carcinoma. The overwhelming dominance of HPV 16 and 18 informed vaccine design and the first two licensed vaccines used in national immunization programs contained both HPV 16 and 18 as will be discussed in a later section. The prevalence of cervical cancer associated with HPV 16 and 18 has been demonstrated to decline significantly with age; a 2015 meta-analysis of 11,525 cancers indicated that the proportion reduced from 74.8% in women aged 30–39 years to 56.8% in women ≥70 years [25].

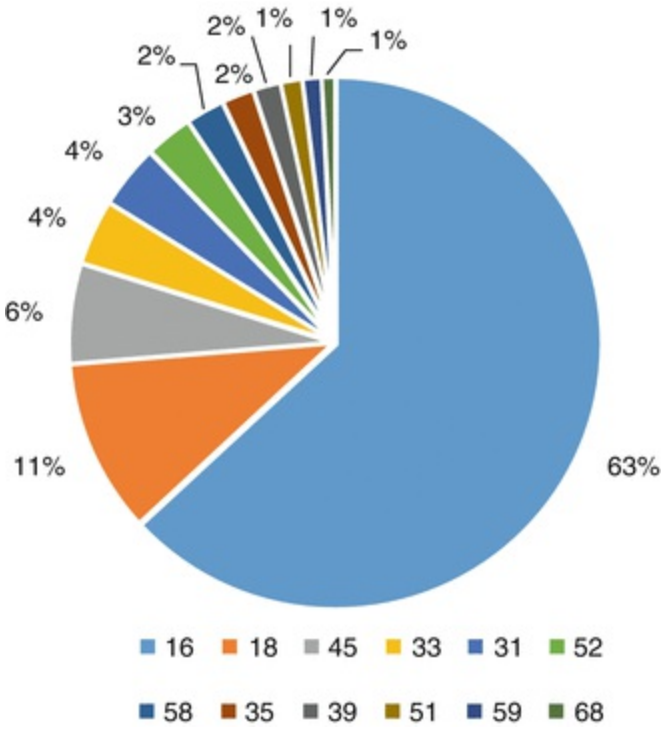


Fig. 2.7 Type-specific prevalence in cervical cancer (Data from Arbyn et al. [24])

As CIN2 (and to a lesser extent CIN3) lesions are a more heterogeneous category pathologically, compared to invasive cancer, the relative proportions of high-risk types associated with them are somewhat different from those observed in cancer. In a recent study of over 370,000 women where type-specific HPV status was related to cervical disease status, HPV 31 was detected in 10% and 11% of CIN2 and CIN3 lesions compared to 3.5% of cancers [24]. Similarly, the attributable fraction of HPV 18 is generally higher in cervical cancer than in CIN2 and CIN3.

The notion of the "HPV-negative" cervical cancer is controversial. Some argue that given that persistent infection with HPV is prerequisite for malignancy, HPV-negative cancers are cases where the virus is simply undetectable due to fragmentation of the viral genome brought about through integration which may be compounded by incapacity of the detection technique. It is feasible that while HPV may initiate and drive transformation, it may become lost later in the process in some cancers. Recent evidence also suggests that HPV is infrequently detected in rare cervical adenocarcinomas (ADC) being associated with 28% of clear cell, 30% of serous, 13% of endometrioid, and no gastric-type carcinomas [26] and see Chap. 9.

Prevalence in Disease (Cytologically Defined)

The prevalence of HR-HPV increases with increasing severity of cytologically defined abnormality. Although this general trend is observed globally, the overall prevalence of HR-HPV according to cytology category varies significantly according to country. For

example, in a global analysis of over 115,000 HPV-positive women, HR-HPV prevalence in women with normal cytology ranged from 8% to 9% in Western/Central Asia and Europe to over 20% in Africa, North America, South/Central America, and Oceania, with an overall prevalence of 12% [17, 27]. With respect to abnormal cytology categories, the global analysis reported overall HR-HPV prevalence(s) in atypical squamous cells of uncertain significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) of 52%, 76%, and 85%, although, again, these prevalences varied according to geography. Consistency and accuracy of cytology are known to vary according to setting and will account for at least part of this variation. Furthermore, it is notable that variation in HR-HPV prevalence, even in histologically defined disease, only diminishes at the level of biopsy-confirmed cancer where the 90% HR-HPV-positive figure is relatively consistent. The prevalence of HR-HPV in the different cytology categories has informed the appropriate application of HR-HPV testing for the management of abnormal cytology – there is little to justify HR-HPV testing of HSIL as nearly all will be HR-HPV positive. However, as around 45–50% of ASCUS are HR-HPV negative, the use of HR-HPV testing for the risk stratification or "triage" of this entity to colposcopy (or more conservative management) is widespread as will be discussed in a later section. As prevalence of HR-HPV LSIL is higher, the effectiveness of HR-HPV triage of LSIL – compared to repeat cytology – is more debatable; however, triage of low-grade disease which includes both categories is also common.

Host Defenses

The host immune response plays a vital role against pathogens, and HPV is no exception with the first line of defense being the epithelia that it specifically infects. HPV keeps a relatively low profile to the immune system. The key mechanism of immune evasion for HPV is through its tightly regulated life cycle, which is inextricably linked to the differentiation process with daughter viruses released through "normal" desquamation. The non-lytic nature of the virus thus generates little inflammation and there is no viremic phase. Furthermore, the epithelial milieu which HPV infects exclusively is relatively sparse in terms of immunological effectors, and, consequently, HPV infections can persist with little exposure to the immune system. This enables the high prevalence of the virus in the population described earlier.

The epithelial keratinocytes, dendritic cells (DCs), Langerhans cells (LCs), and natural killer (NK) cells are among the more important cells involved in the immunological management of HPV infection. The key cells and their functions are described below along with mechanisms of immune evasion employed by HPV.

The basal keratinocytes that are initially infected with HPV act as nonprofessional antigen-presenting cells as they express pathogen recognition receptors (PRRs). These

PRRs, such as the Toll-like receptor (TLR) family, recognize pathogen-associated molecular patterns (PAMPs) and activate the adaptive immune response signaling pathways. TLR activation, particularly TLR9, is necessary for clearance of viral infection; increased levels of TLRs in cervical specimens of HPV-infected women have been shown to be associated with viral clearance [28, 29], and polymorphisms in TLR9 have been associated with increased risk of cervical cancer [30]. Activation of TLRs, in turn, leads to expression of pro-inflammatory cytokines and chemokines such as TNF-α (alpha), CCL2, CCL20, CXCL8, and type I interferons, principally IFN-α (alpha) and IFN-β (beta). In women with HPV infection, a strong pro-inflammatory type 1 cytokine profile is seen which is associated with viral clearance [31, 32]. Cytokines and chemokines recruit innate and adaptive immune cells to the site of infection and create a pro-inflammatory microenvironment containing monocytes, DCs, and NK cells (reviewed in [33]).

This said, HPV is able to counteract the activation of receptors and the effect of secreted cytokines to create an anti-inflammatory microenvironment which favors persistent infection. Expression of TLR9 is reduced in HPV 16-positive cervical cancer samples, and HPV 16 E7-mediated suppression of TLR9 has been suggested, but the mechanism is not fully understood [34]. E6 and E7 also downregulate the expression of monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-3a (MIP-3a), thus inhibiting the translocation of macrophages to the site of infection [35]. In addition, E6 and E7 downregulate the transcription of interferons – E7 through binding to IRF9 and IRF1 and E6 through binding with IRF3 [36].

Dendritic cells or professional antigen-presenting cells are also highly relevant in the initial immune response to infection. In the epithelium, DCs incorporate Langerhans cells (LCs) in the epidermis and three subsets of DCs in the dermis. Viral infection should in theory activate LCs, but evidence would suggest that given that gene expression is confined within keratinocytes, HPV capsids do not activate LCs [37]. Therefore, migration and adhesion of LCs to keratinocytes are necessary for activation – although, again, HPV has developed mechanisms to block this. Downregulation of E-cadherin by E6 and E7 disrupts the adhesion of keratinocytes to LCs [38], and a decrease in the number of LCs is associated with the severity of CIN [39].

Activation of LCs and dendritic cells in the stroma is necessary for recruitment and maturation of CD4+ and CD8+ T cells. In the presence of DCs expressing MHC class I antigen, CD8+ T cells are converted to cytotoxic T lymphocytes (CTLs). Activated by Th1 cytokines such as IL2, IL12, and IFN gamma, CTLs are able to digest HPV-infected cells. On the other hand, CD4+ T cells activated by Th1 or Th2 responses are responsive to DC presenting MHC class 2 antigen. HPV 16 E5 has been shown to suppress the expression of MHC class I and antigen processing via the TAP pathway leading to suppression of CTLs [40]. Th1 cells induce cell-mediated immunity, and this response is considerably reduced in patients with CIN 2+ and severely impaired in

cervical cancer patients. Inversely, a shift to Th2-type chemokines is seen in patients with cervical cancer [41]. Th2 responses are anti-inflammatory, and Th2-type cytokines such as IL10 and TGF-beta (β) 1 can drive such a microenvironment that favors viral persistence and progression.

Another important element of the innate immune response against HPV infection is attributed to natural killer cells (NK cells). In women with HSILs and cervical cancer, the cytolytic ability of NK cells is considerably decreased, although the mechanism remains unknown [42].

Although there are still knowledge gaps in relation to the immune response to HPV infection, its fundamental role is in keeping with the fact that immunocompromised women (including those who are HIV positive) have a higher prevalence of HPV infection and greater risk of associated disease [43]. A better understanding of the mechanisms of immune evasion by the virus is being obtained using virus like particles (VLPs) which have also led to the development of prophylactic vaccines (described in the next section). Undoubtedly, greater detailed insight into the immune response will inform the design of enhanced prophylactic and therapeutic approaches to HPV infection including the creation of improved adjuvants, and other tools, such as short hairpin RNAs, which can target immunosuppressive molecules.

HPV Immunization

One of the most exciting recent developments in HPV-associated disease management is undoubtedly the development and implementation of prophylactic HPV vaccines. Table 2.2 provides an overview of the key characteristics of the current licensed vaccines. All are currently based on VLPs composed of the main structural protein L1, and while they elicit strong neutralizing antibody responses, they are effectively empty shells and do not contain the genetic apparatus for replication.

Table 2.2 Overview of prophylactic HPV vaccines

Vaccine	Types	Adjuvant	Indications	Trade name and manufacturer
Bivalent	16,18	ASO4	Females aged 9–25: Prevention of cervical cancer, cervical intraepithelial neoplasia (CIN) grade 2 or worse and adenocarcinoma in situ, and CIN grade 1, caused by HPV 16 and 18	Cervarix (GSK)
Quadrivalent	16,18,6,11	Allum	Females aged 9–26: Prevention of cervical, vulvar, and vaginal cancers. Males and females 9–26 for the prevention of anal cancer, precancerous or dysplastic lesions, and genital warts caused by HPV 6, 11, 16, and 18	Gardasil or Silagard (SPMSD)

Nonavalent	16,18,31,33,45,52,58, 6,11	Allum	Females aged 9–26: Prevention of cervical, vulvar, vaginal, and anal cancers caused by HPV 16, 18, 31, 33, 45, 52, and 58. Males and females 9–26 for the prevention of anal cancer, precancerous or dysplastic lesions, and genital warts caused by HPV 6, 11, 16, and 18, 31, 33, 45, and 52	Garadsil-9 (SPMSD)

Delivery and Immunogenicity

Current vaccines take the form of suspensions within prefilled syringes and are delivered via intramuscular (IM) injection. IM injection of adjuvant VLPs invokes a significantly more potent immune response compared to that associated with natural infection where, as discussed earlier, HPV is highly adept at evading immune surveillance within the epithelia, an environment relatively limited in its capacity for providing relevant antigen-presenting cells for HPV. Alternatively, the IM route offers swift exposure of the VLPs to the lymphatics. In addition, evidence would suggest that the VLP is "intrinsically very immunogenic" as a consequence of the repeat pattern of L1 capsomers facilitating the adaptive immune response. Consequently, only ~50% of individuals seroconvert as a consequence of natural HPV infection compared to nearly all who receive vaccine via IM immunization [44].

HPV vaccine dosing follows the classic "prime-boost" regimen. The vaccines were initially licensed for three doses implemented at 0 then 1 (or 2) months after baseline with a final dose at 6 months after baseline.

Although the precise mechanism, components, and levels of the immune response required to confer protection through immunization are not fully understood, it is generally accepted that generation of a neutralizing antibody response is important. Immunization is associated with a peak of antibody titers around 1 month after the final dose which then falls over a period of 12–18 months to an eventual plateau. Although the actual level of antibody required to be efficacious clinically is still unknown, encouragingly, the clinical vaccine trials indicate that antibody levels generated after three doses are protective for a minimum of 10–12 years postimmunization. This is consistent with the finding that vaccine-induced serum neutralizing antibody levels persisted around 1 log higher than those associated with natural infection for the duration of the trials [45].

More recently both the quadrivalent and bivalent vaccines have been shown to elicit non-inferior antibody responses to three dose schedules when delivered as a two-dose regimen provided the second dose is given a minimum of 6 months after the first [46, 47]. These data have been translated into policy in some settings; for example, the current guidance from the Joint Committee on Vaccination and Immunisation (JCVI), which sets vaccine policy in the UK, has recommended a two-dose schedule for the UK program since 2014. There are also emerging data to indicate that one dose may offer a

level of protection; this is important given the costs of setting up and delivering immunization programs. Of the countries that offer HPV vaccination programs, the majority are middle or high income.

As the vaccine is most efficacious in those who have not been exposed to HPV, countries which have initiated vaccination programs have generally "targeted" children of late primary or early secondary school age (i.e., prior to the likely onset of sexual activity). However, several countries also offered an initial "catch-up" of older girls and women for the first few years of the program. The maximum age and duration of catch-up ranged according to program, with some extending this to females up to age 26.

While the majority of immunization programs have focused on females, gender-neutral vaccination is now a reality, with Australia being one of the first countries to adopt this approach at the program level [47]. A somewhat contentious area, there is an argument that the value, particularly the cost-effectiveness, of vaccinating males is diminished when vaccine uptake in females is high (over 80%) given the indirect herd benefits which males will incur as a result of lower transmission rates from females. Certainly a reduction of genital warts in the heterosexual male population was observed in Australia, which was attributed to what was then the female-only vaccination policy of the time [48]. However, this argument is countered by the fact that establishing and maintaining high uptake is challenging. Although some countries have been successful in this endeavor with sustained uptake rates of over 80%, substantially lower rates have also been observed, even in high-income settings [49]. While school-based programs offer logistical advantages for delivery and are generally associated with higher uptake than community-based settings – such a setup is not always feasible. Negative reports of vaccine in the media and/or by anti-vaccine groups have also had negative consequences for uptake. Furthermore, men who have sex with men (MSM) are not protected by female-only vaccination while remaining at significantly higher risk of genital warts and anal cancer. As a consequence, targeted programs for MSM have been recommended in certain countries.

Therapeutic Vaccines

Given the nature of prophylactic vaccines, which confer optimal efficacy in those who have not been exposed, there is still a requirement for robust therapeutic vaccines given the millions of individuals who have already been exposed to the virus and/or have associated disease. However, compared to prophylactic vaccines, which are already making significant impact on HPV infection and disease (see below), the traction and application of therapeutic vaccines is somewhat behind. This has largely been attributable to the fact that while the immune response is clearly critical in HPV clearance and lesion regression, the key correlates of what drives this are not fully defined. This said, given the intracellular nature of HPV, it is reasonable to assume that

therapeutic vaccines need to engender the production of cytotoxic T cells which recognize MHC molecules bound to viral peptides. Relevant viral proteins/antigens to achieve this have included, unsurprisingly, E6 and E7 but also L1, L2, and E2 and various combinations thereof [50]. Candidates for therapeutic vaccines have included DNA vaccines (delivered by electroporation) and peptide vaccines, and both have been shown to induce T cell responses. A recent trial where a synthetic long peptide vaccine (full-length E6 and E7) was delivered in patients with CIN showed 50% had either total or partial regression at 3 months from vaccination with a correlation between the levels of vaccine-driven T cell responses and clinical outcome. Interestingly, the same vaccine did not show an equivalent, beneficial clinical response in cancer patients; this reconciles with the contention that alteration of systemic and local immunity as a consequence of cancer has a negative impact on T cell response, production, and function [51]. Different approaches for the management of preinvasive and invasive disease by therapeutic agents may thus be warranted.

Impact (Prophylactic Vaccines)

The prophylactic vaccines appear highly efficacious; trial data indicate over 95% efficacy for lesions associated with vaccine types in individuals who were negative for HPV vaccine types prior to immunization. In addition a welcome cross protective effect, particularly against HPV types 31, 33, and 45, has been observed, although the extent of cross protection may differ with the vaccine applied [52].

Efficacy measurements garnered from trial settings are not directly transferable to that which can be expected in national programs given that the latter will be inherently larger, more diverse, and complex. However, it is encouraging to see that the impact of national HPV vaccine programs that have incorporated the bivalent and/or the quadrivalent vaccine is now being realized at the population level. Specifically, a significant decrease in vaccine-type HPV infection and cross protective types has been observed, in addition to a dramatic reduction in genital wart cases (in countries where the quadrivalent vaccine was offered) with Australia demonstrating a 90% reduction of warts in the vaccine-targeted age group. An impact on cytologically defined high-grade abnormalities and CIN of all grades has also been seen, with a recent UK study reporting a 30% reduction of CIN1 and a 50–55% reduction of CIN2+ in women vaccinated with the bivalent vaccine aged 14–18 as part of a catch-up strategy [53]. This is consistent with data from Denmark which indicated a 44% reduction in CIN2+ lesions in women vaccinated with the quadrivalent vaccine, again as part of a catch-up strategy [54]. It is reasonable to expect that the impact on CIN2+ may be even greater in women vaccinated at target age (and therefore less likely to have been exposed to HPV). As the nonavalent vaccine has been licensed only recently (at the time of this publication), program data on its impact are not available. However, extrapolations

from existing datasets on type-specific prevalence in CIN and cancers indicate that it could protect against up to 85% of CIN3 and 90% of cervical cancers [55].

In summary, prophylactic vaccines appear highly efficacious, and the main hurdles remain the cost of implementation and the maintenance of high uptake to ensure optimal population-level effects. While therapeutic vaccines are behind in terms of application, their evolution and development will be enhanced given that systems for boosting and refining relevant T cell populations are also developing.

HPV Testing

Justification for HPV Testing

Molecular HPV testing is being used increasingly for the detection and management of cervical disease [56]. This is underpinned by the following reasons:

1. Persistent HPV infection with HR-HPV is requisite for the development of the vast majority of cervical cancers.

2. The likelihood of having significant disease (CIN2+) and being HPV negative is very low – lower than that in women who have negative cytology, particularly over the longer term.

3. In line with 2, the sensitivity of HPV testing for CIN2+ is very high (~95%).

4. HPV testing can be performed in diverse biospecimens including self-taken samples.

5. HPV testing is objective and its consistency, particularly by taking a global view, is higher than that of cytology.

6. Certain high-risk HPV types are riskier than others – particularly HPV 16.

HPV tests that are used for screening and management are generally high-throughput molecular assays that can detect 13–14 high-risk HPV types including all group 1 and 2A types and provide a consensus readout (i.e., high-risk HPV detected or not detected) or a consensus readout in addition to a limited genotyping result. The limited genotyping capability is often confined to HPV 16/18, although capacity is increasing beyond these two types on some platforms [57]. Although genotyping within the HR-HPV category can provide insight into risk particularly if performed longitudinally, for pragmatic

reasons women are managed according to HR-HPV-positive or HR-HPV-negative status particularly in guideline-driven organized screening programs, where the onus is to minimize complexity where possible. Testing for low-risk HPV types is not valuable for cervical disease management, and, although group 2B types can be associated with cancer, they are not generally incorporated into clinically validated assays given that the loss in specificity is not offset by what is a small increment in sensitivity. The last 10 years has seen an almost exponential increase in the number of commercially available HPV tests; however, the amount and quality of evidence on their clinical performance varies widely [58]. In reaction to this, the scientific community devised performance standards, which HPV assays should fulfill to be considered validated for use in cervical screening. These standards incorporated minimum sensitivity and specificity for the detection of CIN2+ and also reproducibility [59]. An increasing number of HPV assays that demonstrate similar clinical performance are now clinically validated. Choice of which assay may be integrated into a health-care system may thus be influenced by pragmatic yet non-trivial attributes such as cost, ease of use, throughput, and flexibility of the platform for other applications.

With respect to application of HPV testing for patient management, ensuring that the context in which testing is delivered is appropriate is fundamental to avoid misuse of what are very sensitive tests, even when using robust clinically validated assays. The three accepted main indications for HPV testing are (1) risk stratification or "triage" of equivocal or low-grade cytological abnormalities to inform the route to colposcopy; (2) monitoring the success of treatment after removal of cervical lesions, often referred to as "test of cure"; and (3) primary cervical screening. Indications 1 and 2 have been established within both organized and opportunistic screening programs for several years. Indication 3, primary screening by HPV testing, represents the most fundamental change and is a more recent phenomenon. However, this is now accepted as the optimal modality for screening, with several countries committing to and implementing this approach.

The advantages and disadvantages of HR-HPV testing for the three main indications are summarized in Table 2.3. Simply put, the benefit of HPV testing is more immediately conferred on those who test negative as they are at a relatively low risk of disease. Managing the positives is more challenging given that, as discussed earlier, the majority of HR-HPV infections are subclinical, particularly in women under 30. Consequently, the disadvantages of HR-HPV testing largely converge around its low specificity and positive predictive value (PPV). HPV testing for longitudinal epidemiology and surveillance monitoring programs of vaccine impact is also important but is not a screening/clinical indication, and the type and characteristics of tests used for this context are generally different from those applied for cervical screening.

Table 2.3 Summary of indications for clinical HPV testing for cervical screening and disease management –

advantages and limitations

Indication	Advantages	Disadvantages
Primary screening	Sensitive for the detection of CIN2+ – longer screening intervals possible after an HPV-negative result Opportunities for self-sampling in hard-to-reach populations	Although sensitive, will not detect all CIN2+
	Less affected by the impact of vaccination compared to cytology	Prevalence of "screen" HR-HPV positives higher than screen cytology positives
	Objective – more practical for countries which do not have the infrastructure for cytology-based screening	Low PPV of HR-HPV test for significant disease requires additional triage
Triage of low-grade abnormalities	Sensitive for the detection of CIN2+ Reduced intensity of follow-up in those who test HPV negative and minimization of unnecessary colposcopy referrals	Low PPV, particularly in LSIL where prevalence of HR-HPV can be 70–80%
Post treatment monitoring (test of cure)	Sensitive for the detection of residual CIN2+ Reduced intensity of follow-up for the majority who test HPV negative	Low PPV of those who test HR-HPV positive after treatment (~15–20% at 6 months)

Targets and Types of HR-HPV Test

As described earlier, the bulk of clinically applied HPV tests are molecular and rely on nucleic acid amplification, often of DNA of the L1, E6, or E7 genes. E6/E7 mRNA tests also exist, and data suggest these may offer advantages in terms of specificity given that the expression of oncogenes is targeted, giving an indication of viral activity rather than simply presence [60, 61]. E6/E7 mRNA is however detected in infections that follow a benign course so this is not a solution to the specificity issue in itself. While helpful for natural history studies, serological assays are not used diagnostically as they are too insensitive given the low levels of antibodies produced as a consequence of natural infection. In situ hybridization assays are also available – while these can be helpful to determine localization of infection, they are relatively labor intensive compared to molecular assays and thus not generally used for high-throughput testing within screening.

The use of quantification/viral load to inform management is somewhat nebulous, and validated molecular assays are generally interpreted at the qualitative (i.e., presence/absence) level. Normalization of viral load to cellular content provides technical challenges given the wide range of cell content present in cervical biospecimens; furthermore, one-off readings of viral load have been shown to have utility for certain, but not all HR-HPV types. Finally, coinfection with multiple types where only one type is clinically significant confounds the usefulness of total viral load measurement. Recent evidence would suggest that while determination of a clinically

relevant viral load threshold may be elusive at a single time point, sequential viral load measurement and assessment of the longitudinal dynamics therein (through examination of slope) can be informative – with a type-specific exponential increase indicating a clonal, potentially "transforming" pattern [62]. However, one of the challenges of this approach is that clearly more than one reading/sample is required.

Tools and Biomarkers for the Risk Stratification of HR-HPV Infection

There is a significant appetite for development of an HPV test that can separate benign from clinically significant infection. This requirement is particularly prescient given the move to HPV primary screening where the number of HPV screen positives will be at least double that which would have been detected by cytology. Primary screening algorithms which start with HPV first behoove additional risk stratification or "triage" tests to inform route to colposcopy as referral on the back of a single HR-HPV-positive result would not be justified. Current suggested triage approaches which are evidenced include cytology and limited genotyping of HPV 16/18 – however, as two thirds of HR-HPV screen positives will be cytologically negative, the challenge of how to manage HPV-positive/cytologically negative women remains. While limited genotyping indicates referral in those who are HPV 16/18 positive and therefore at greatest risk, those who are HR-HPV positive for one of the 11–12 other types cannot be treated in the same manner as those who are HR-HPV negative. In addition the limited typing approach may have diminishing returns in those countries which offer immunization programs – given that the prevalence of 16/18 infection will decrease. Biomarker-type strategies that can delineate risks which are agnostic to underlying type are thus of value. To this end the most evidenced is p16INK4a (p16) – a cyclin-dependent kinase inhibitor, cell cycling modulator, and surrogate of deregulated E7 protein expression which has been shown to accumulate in tandem with severity of underlying CIN [63]. p16 can be detected via immunohistochemistry (IHC) in biopsies but also in cytology preparations either alone or in combination with another marker of cellular proliferation – Ki67. While a level of human interpretation is still required, the use of adjunctive staining has been shown to simplify and enhance the interpretation and clinical performance of cytology, respectively, and has been applied in primary screening and also low-grade triage contexts.

Detection of methylated sequences has also been shown to have potential as a biomarker approach. Addition of methyl (CH_3) groups via dimethyl transferases to areas of the genome which are rich in C and G nucleotides is a fundamental process which influences gene regulation and transcription. Furthermore, abnormal methylation is a key feature of oncogenesis and can lead to chromosomal instability in addition to faulty gene regulation and expression. Methylation is also considered a characteristic

host response to “silence” foreign agents including viral nucleic acid. Characteristic methylation patterns of both the virus (particularly the late regions) and host which have prognostic capabilities have been described. With respect to the former, hypermethylation of L1 has been shown to be a surrogate of viral persistence and oncogenic risk, with the percentage of methylated HPV L1 CG dinucleotides in normal tissue being around 5–10% compared to 40–80% in cancer. Clinically relevant host methylation targets (including cellular adhesion molecular 1 (CADM-1)) have included T-lymphocyte maturation-associated protein (MAL) and erythrocyte membrane protein band 4.1-like 3 (EPB41L3) [64]. Moreover, the methodology for detecting methylation is becoming increasingly straightforward given the increasing power, rapidity, and simplicity of sequencing technologies. Although there is less evidence on the clinical performance of methylation targets for screening/clinical use compared to p16 and limited typing, this will undoubtedly accumulate in time.

HPV Testing in Various Biospecimens

One of the advantages of HPV testing is its amenability to diverse biospecimens; molecular HPV testing has been extensively performed on the residual volume of liquid-based cervical cytology samples but is also validated on clinician-taken swabs in viral transport media using devices validated by the manufacturer of the particular assay. Furthermore, unlike cytology, HPV testing is also amenable to self-taken samples. Much interest has been devoted to the application of vaginal self-sampling to improve uptake in screening for those who are “hard to reach” because they cannot attend or do not wish to attend for traditional screening. However, self-sampling is also being increasingly seen as a potential alternative option for women beyond the hard-to-reach groups. The evidence indicates that if a molecular amplification type test is used, the clinical performance of HPV testing using self-taken samples is similar to that observed in clinician-taken samples, particularly in terms of sensitivity [65]. The devices and approaches for self-sampling are manifold and include basic tipped swabs (which are then packaged dry or in preservation media), retractable sheathed brushes of various dimensions, miniature douche-type devices, filter-paper mounted on cassette, and tampons. Urine has also been assessed as a biospecimen for HPV testing, and while the current evidence suggests it is less optimal compared to a vaginal sample, particularly if random rather than first void, the development of collection and concentration devices may improve the performance of this approach [66]. Self-taken samples clearly do not permit reflex cytology on residual sample, so in algorithms where this is stipulated, women who test positive would need to have an additional sample taken for cytology. This said, in scenario of HPV primary screening where the triage approach is not cytology but another molecular-type assay, this challenge/issue may not be relevant.

HPV Testing in Immunized Populations

Undoubtedly, the prevalence and pattern of HPV infection and associated disease is already changing as a consequence of prophylactic immunization in those countries which can afford it. The significantly lower prevalence of disease will make the remaining fewer cases of significant disease more challenging to detect – particularly using subjective approaches, such as cytology given the altered signal-to-noise ratio and reader fatigue [67]. Indeed, a negative impact on the performance of cytology in some settings has already been observed as a consequence of immunization particularly that of the positive predictive value of low-grade dyskaryosis for CIN2+. Additionally, the number of women who need to be referred to colposcopy (as a consequence of preceding cytological abnormalities) to detect one case of CIN2+ is significantly higher in immunized compared to unimmunized women [68]. Consequently, how to detect and manage residual cervical disease optimally in a vaccinated era will be a key challenge – and the demand for objective approaches calibrated to post immunization disease levels becomes pressing.

Conclusion A greater understanding on the natural history, epidemiology, life cycle, and immune response to HPV (as described above) has been gained, in no small, part by (1) the increasing sophistication of in vitro technologies which have facilitated analysis of the virus and its interactions and (2) the advent of high-throughput, reliable molecular assays for the detection of HPV in clinical samples. We are now increasingly seeing translation of this knowledge in the form of primary and secondary prevention strategies – namely, prophylactic vaccination and HPV testing for cervical screening. Global application of these strategies will likely incur a significant change in the prevalence and pattern of residual infection and associated disease.

References

1. Lowy DR. History of papillomavirus research. In: Garcea RL, DiMaio D, editors. The papillomaviruses. New York: Springer; 2007. p. 13–28.
[Crossref]

2. Bernard H-U, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70–79.

3. de Villiers E-M, Fauquet C, Broker TR. Classification of papillomaviruses. Virology. 2004;324(1):17–27.
[Crossref][PubMed]

4. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human

papillomaviruses. Vaccine. 2012;30(Suppl 5):F55–70.
[Crossref][PubMed]

5. IARC. Monographs on the evaluation of carcinogenic risks to humans, no. 100B. IARC Working Group on the evaluation of carcinogenic risk to humans. International Agency for Research on Cancer: Lyon; 2012.

6. Cubie HA. Diseases associated with human papillomavirus infection. Virology. 2013;445(1–2):21–34.
[Crossref][PubMed]

7. Zheng Z-M, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci J Virtual Libr. 2006;11:2286–302.
[Crossref]

8. Stanley M. Pathology and epidemiology of HPV infection in females. Gynecol Oncol. 2010;117(2 Suppl):5–10.
[Crossref]

9. Woodman CBJ, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7(1):11–22.
[Crossref][PubMed]

10. Herfs M, Yamamoto Y, Laury A, Wang X, Nucci MR, McLaughlin-Drubin ME, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci. 2012;109(26):10516–21.
[Crossref][PubMed][PubMedCentral]

11. Day PM, Schelhaas M. Concepts of papillomavirus entry into host cells. Curr Opin Virol. 2014;4:24–31.
[Crossref][PubMed]

12. Venuti A, Paolini F, Nasir L, Corteggio A, Roperto S, Campo MS, et al. Papillomavirus E5: the smallest oncoprotein with many functions. Mol Cancer. 2011;10:140.
[Crossref][PubMed][PubMedCentral]

13. Wentzensen N, Vinokurova S, von Knebel Doeberitz M. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. Cancer Res. 2004;64(11):3878–84.
[Crossref][PubMed]

14. Vietri M, Bianchi M, Ludlow JW, Mittnacht S, Villa-Moruzzi E. Direct interaction between the catalytic subunit of Protein Phosphatase 1 and pRb. Cancer Cell Int. 2006;6:3.
[Crossref][PubMed][PubMedCentral]

15. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiol Mol Biol Rev. 2004;68(2):362–72.
[Crossref][PubMed][PubMedCentral]

16. Hebnes JB, Olesen TB, Duun-Henriksen AK, Munk C, Norrild B, Kjaer SK. Prevalence of genital human papillomavirus among men in Europe: systematic review and meta-analysis. J Sex Med. 2014;11(11):2630–44.
[Crossref][PubMed]

17. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M, Franceschi S. Global burden of human papillomavirus and related diseases. Vaccine. 2012;30(Suppl 5):F12–23.
[Crossref][PubMed]

18. Veldhuijzen NJ, Berkhof J, Gillio-Tos A, De Marco L, Carozzi F, Del Mistro A, Snijders PJ, Meijer CJ, Ronco G. The age distribution of type-specific high-risk human papillomavirus incidence in two population-based screening trials. Cancer Epidemiol Biomark Prev. 2015;24(1):111–8.
[Crossref]

19. Koskimaa HM, Paaso AE, Welters MJ, Grénman SE, Syrjänen KJ, van der Burg SH, Syrjänen SM. Human papillomavirus 16 E2-, E6- and E7-specific T-cell responses in children and their mothers who developed incident cervical intraepithelial neoplasia during a 14-year follow-up of the Finnish Family HPV cohort. J Transl Med. 2014;12:44.
[Crossref][PubMed][PubMedCentral]

20. Cuschieri K, Geraets DT, Moore C, Quint W, Duvall E, Arbyn M. Clinical and analytical performance of the onclarity HPV assay using the VALGENT framework. J Clin Microbiol. 2015;53(10):3272–9.
[Crossref][PubMed][PubMedCentral]

21. Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect Dis. 2010;10(12):862–74. Erratum in: Lancet Infect Dis. 2015;15(10):1130.

22. Jaisamrarn U, Castellsagué X, Garland SM, Naud P, Palmroth J, Del Rosario-Raymundo MR, Wheeler CM, Salmerón J, Chow SN, Apter D, Teixeira JC, Skinner SR, Hedrick J, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, de Carvalho NS, Germar MJ, Peters K, Paavonen J, Bozonnat MC, Descamps D, Struyf F, Dubin GO, Rosillon D, Baril L; HPV PATRICIA Study Group. Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomised PATRICIA study. PLoS One. 2013;8(11):e79260.

23. Skinner SR, Wheeler CM, Romanowski B, Castellsagué X, Lazcano-Ponce E, Del Rosario-Raymundo MR, Vallejos C, Minkina G, Da Silva DP, McNeil S, Prilepskaya V, Gogotadze I, Money D, Garland SM, Romanenko V, Harper DM, Levin MJ, Chatterjee A, Geeraerts B, Struyf F, Dubin G, Bozonnat MC, Rosillon D, Baril L; VIVIANE study group. Progression of HPV infection to detectable cervical lesions or clearance in adult women: analysis of the control arm of the VIVIANE study. Int J Cancer. 2016;138:2428–38.

24. Arbyn M, Tommasino M, Depuydt C, Dillner J. Are 20 human papillomavirus types causing cervical cancer? J Pathol. 2014;234(4):431–5.
[Crossref][PubMed]

25. Hammer A, Rositch A, Qeadan F, Gravitt PE, Blaakaer J. Age-specific prevalence of HPV16/18 genotypes in cervical cancer: a systematic review and meta-analysis. Int J Cancer. 2015

26. Molijn A, Jenkins D, Chen W, Zhang X, Pirog E, Enqi W, Liu B, Schmidt J, Cui J, Qiao Y, Quint W, Chinese HPV Typing Group. The complex relationship between human papillomavirus and cervical adenocarcinoma. Int J Cancer. 2016;138(2):409–16.
[Crossref][PubMed]

27. Clifford GM, Gallus S, Herrero R, Muñoz N, Snijders PJ, Vaccarella S, Anh PT, Ferreccio C, Hieu NT, Matos E, Molano M, Rajkumar R, Ronco G, de Sanjosé S, Shin HR, Sukvirach S, Thomas JO, Tunsakul S, Meijer CJ, Franceschi S, IARC HPV Prevalence Surveys Study Group. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005;366(9490):991–8.
[Crossref][PubMed]

28. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki A-B. Association between toll-like receptor expression and human papillomavirus type 16 persistence. Int J Cancer. 2011;128(4):879–86.
[Crossref][PubMed][PubMedCentral]

29. Scott ME, Ma Y, Farhat S, Moscicki A-B. Expression of nucleic acid-sensing Toll-like receptors predicts HPV16 clearance associated with an E6-directed cell-mediated response. Int J Cancer. 2015;136(10):2402–8. [Crossref][PubMed]

30. Roszak A, Lianeri M, Sowińska A, Jagodziński PP. Involvement of toll-like receptor 9 polymorphism in cervical cancer development. Mol Biol Rep. 2012;39(8):8425–30. [Crossref][PubMed][PubMedCentral]

31. Scott M, Stites DP, Moscicki A-B. Th1 cytokine patterns in cervical human papillomavirus infection. Clin Diagn Lab Immunol. 1999;6(5):751–5. [PubMed][PubMedCentral]

32. Paradkar PH, Joshi JV, Mertia PN, Agashe SV, Vaidya RA. Role of cytokines in genesis, progression and prognosis of cervical cancer. Asian Pac J Cancer Prev APJCP. 2014;15(9):3851–64. [Crossref][PubMed]

33. Moerman-Herzog A, Nakagawa M. Early defensive mechanisms against human papillomavirus infection. Clin Vaccine Immunol. 2015;22(8):850–7. [Crossref][PubMed][PubMedCentral]

34. Hasan UA, Bates E, Takeshita F, Biliato A, Accardi R, Bouvard V, et al. TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. J Immunol Baltim Md 1950. 2007;178(5):3186–97.

35. Kleine-Lowinski K, Rheinwald JG, Fichorova RN, Anderson DJ, Basile J, Münger K, et al. Selective suppression of monocyte chemoattractant protein-1 expression by human papillomavirus E6 and E7 oncoproteins in human cervical epithelial and epidermal cells. Int J Cancer. 2003;107(3):407–15. [Crossref][PubMed]

36. Stanley MA. Epithelial cell responses to infection with human papillomavirus. Clin Microbiol Rev. 2012;25(2):215–22. [Crossref][PubMed][PubMedCentral]

37. Fausch SC, Da Silva DM, Rudolf MP, Kast WM. Human papillomavirus virus-like particles do not activate Langerhans cells: a possible immune escape mechanism used by human papillomaviruses. J Immunol Baltim Md 1950. 2002;169(6):3242–9.

38. Hubert P, Caberg J-H, Gilles C, Bousarghin L, Franzen-Detrooz E, Boniver J, et al. E-cadherin-dependent adhesion of dendritic and Langerhans cells to keratinocytes is defective in cervical human papillomavirus-associated (pre)neoplastic lesions. J Pathol. 2005;206(3):346–55. [Crossref][PubMed]

39. Mota FF, Rayment NB, Kanan JH, Singer A, Chain BM. Differential regulation of HLA-DQ expression by keratinocytes and Langerhans cells in normal and premalignant cervical epithelium. Tissue Antigens. 1998;52(3):286–93. [Crossref][PubMed]

40. Campo MS, Graham SV, Cortese MS, Ashrafi GH, Araibi EH, Dornan ES, et al. HPV-16 E5 down-regulates expression of surface HLA class I and reduces recognition by CD8 T cells. Virology. 2010;407(1):137–42. [Crossref][PubMed]

41. Alcocer-González JM, Berumen J, Taméz-Guerra R, Bermúdez-Morales V, Peralta-Zaragoza O, Hernández-

Pando R, et al. In vivo expression of immunosuppressive cytokines in human papillomavirus-transformed cervical cancer cells. Viral Immunol. 2006;19(3):481–91.
[Crossref][PubMed]

42. Garcia-Iglesias T, Del Toro-Arreola A, Albarran-Somoza B, Del Toro-Arreola S, Sanchez-Hernandez PE, Ramirez-Dueñas MG, et al. Low NKp30, NKp46 and NKG2D expression and reduced cytotoxic activity on NK cells in cervical cancer and precursor lesions. BMC Cancer. 2009;9:186.
[Crossref][PubMed][PubMedCentral]

43. Duerr A, Kieke B, Warren D, Shah K, Burk R, Peipert JF, et al. Human papillomavirus-associated cervical cytologic abnormalities among women with or at risk of infection with human immunodeficiency virus. Am J Obstet Gynecol. 2001;184(4):584–90.
[Crossref][PubMed]

44. Stanley M. HPV – immune response to infection and vaccination. Infect Agent Cancer. 2010;5:19.

45. Skinner SR, Apter D, De Carvalho N, Harper DM, Konno R, Paavonen J, Romanowski B, Roteli-Martins C, Burlet N, Mihalyi A, Struyf F. Human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for the prevention of cervical cancer and HPV-related diseases. Expert Rev Vaccines. 2016;15(3):367–87.
[PubMed]

46. Basu P, Bhatla N, Ngoma T, Sankaranarayanan R. Less than 3 doses of the HPV vaccine – review of efficacy against virological and disease end points. Hum Vaccin Immunother. 2016;12:1394–402.
[Crossref][PubMed][PubMedCentral]

47. Brotherton JM, Ogilvie GS. Current status of human papillomavirus vaccination. Curr Opin Oncol. 2015;27(5):399–404.
[Crossref][PubMed]

48. Fairley CK, Hocking JS, Gurrin LC, Chen MY, Donovan B, Bradshaw CS. Rapid decline in presentations of genital warts after the implementation of a national quadrivalent human papillomavirus vaccination programme for young women. Sex Transm Infect. 2009;85(7):499–502.
[Crossref][PubMed]

49. Beavis AL, Levinson KL. Preventing cervical cancer in the United States: barriers and resolutions for HPV vaccination. Front Oncol. 2016;6:19.
[Crossref][PubMed][PubMedCentral]

50. Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. J Clin Invest. 2015;125(9):3401–12.
[Crossref][PubMed][PubMedCentral]

51. McKee SJ, Bergot AS, Leggatt GR. Recent progress in vaccination against human papillomavirus-mediated cervical cancer. Rev Med Virol. 2015;25(Suppl 1):54–71.
[Crossref][PubMed]

52. Drolet M, Bénard É, Boily MC, Ali H, Baandrup L, Bauer H, Beddows S, Brisson J, Brotherton JM, Cummings T, Donovan B, Fairley CK, Flagg EW, Johnson AM, Kahn JA, Kavanagh K, Kjaer SK, Kliewer EV, Lemieux-Mellouki P, Markowitz L, Mboup A, Mesher D, Niccolai L, Oliphant J, Pollock KG, Soldan K, Sonnenberg P, Tabrizi SN, Tanton C, Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 2015;15(5):565–80.

53. Pollock KG, Kavanagh K, Potts A, Love J, Cuschieri K, Cubie H, Robertson C, Cruickshank M, Palmer TJ, Nicoll

S, Donaghy M. Reduction of low- and high-grade cervical abnormalities associated with high uptake of the HPV bivalent vaccine in Scotland. Br J Cancer. 2014;111(9):1824–30.
[Crossref][PubMed][PubMedCentral]

54. Baldur-Felskov B, Dehlendorff C, Munk C, Kjaer SK. Early impact of human papillomavirus vaccination on cervical neoplasia – nationwide follow-up of young Danish women. J Natl Cancer Inst. 2014;106(3):djt460.
[Crossref][PubMed]

55. Mesher D, Cuschieri K, Hibbitts S, Jamison J, Sargent A, Pollock KG, Powell N, Wilson R, McCall F, Fiander A, Soldan K. Type-specific HPV prevalence in invasive cervical cancer in the UK prior to national HPV immunisation programme: baseline for monitoring the effects of immunisation. J Clin Pathol. 2015;68(2):135–40.
[Crossref][PubMed]

56. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, Koliopoulos G, Naucler P, Sankaranarayanan R, Peto J. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine. 2012;30 Suppl 5:F88–99. Review. Erratum in: Vaccine. 2013;31(52):6266.

57. Cubie HA, Cuschieri K. Understanding HPV tests and their appropriate applications. Cytopathology. 2013;24(5):289–308.
[PubMed]

58. Poljak M, Kocjan BJ, Oštrbenk A, Seme K. Commercially available molecular tests for human papillomaviruses (HPV): 2015 update. J Clin Virol. 2016;76(Suppl 1):S3–S13.

59. Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M, Bosch FX, Cuzick J, Dillner J, Heideman DA, Snijders PJ. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 2009;124(3):516–20.
[Crossref][PubMed][PubMedCentral]

60. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJ; International HPV screening working group. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32. Epub 2013 Nov 3. Erratum in: Lancet. 2015;386(10002):1446.

61. Reid JL, Wright Jr TC, Stoler MH, Cuzick J, Castle PE, Dockter J, Getman D, Giachetti C. Human papillomavirus oncogenic mRNA testing for cervical cancer screening: baseline and longitudinal results from the CLEAR study. Am J Clin Pathol. 2015;144(3):473–83.
[Crossref][PubMed]

62. Depuydt CE, Jonckheere J, Berth M, Salembier GM, Vereecken AJ, Bogers JJ. Serial type-specific human papillomavirus (HPV) load measurement allows differentiation between regressing cervical lesions and serial virion productive transient infections. Cancer Med. 2015;4(8):1294–302.
[Crossref][PubMed][PubMedCentral]

63. Bergeron C, Ronco G, Reuschenbach M, Wentzensen N, Arbyn M, Stoler M, von Knebel Doeberitz M. The clinical impact of using p16(INK4a) immunochemistry in cervical histopathology and cytology: an update of recent developments. Int J Cancer. 2015;136(12):2741–51.
[Crossref][PubMed]

64. Lorincz AT. Cancer diagnostic classifiers based on quantitative DNA methylation. Expert Rev Mol Diagn. 2014;14(3):293–305.
[Crossref][PubMed][PubMedCentral]

65. Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao FH, Hillemanns P, Anttila A. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol. 2014;15(2):172–83. [Crossref][PubMed]

66. Vorsters A, Van den Bergh J, Micalessi I, Biesmans S, Bogers J, Hens A, De Coster I, Ieven M, Van Damme P. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014;33(11):2005–14.

67. Franco EL, Mahmud SM, Tota J, Ferenczy A, Coutlée F. The expected impact of HPV vaccination on the accuracy of cervical cancer screening: the need for a paradigm change. Arch Med Res. 2009;40(6):478–85. [Crossref][PubMed]

68. Palmer TJ, McFadden M, Pollock KG, Kavanagh K, Cuschieri K, Cruickshank Cotton S, Nicoll S, Robertson C. HPV immunisation and cervical screening-confirmation of changed performance of cytology as a screening test in immunised women: a retrospective population-based cohort study. Br J Cancer. 2016;114(5):582–9. [Crossref][PubMed][PubMedCentral]

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3. Cervical Screening: History, Current Algorithms, and Future Directions

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Abstract
This chapter describes the principles and evaluation of cancer screening programs, the evolution and history of cytology-based cervical cancer screening programs in the UK, past and contemporary terminology and algorithms for the management of abnormal cytology results, and the future application of HPV and other molecular technology in cervical cancer screening.

Keywords Gynecological cytology – Pap tests – Terminology – Bethesda system – Squamous intraepithelial lesion – CIN – CGIN – Sensitivity – Specificity – HPV – Vaccination – Cancer screening

Principles of Screening

The criteria for appraising the validity of a screening program were first described by Wilson and Jungner for the World Health Organization (WHO) in 1968 and relate to the disease in question, the test applied, the treatment available, and the cost of intervention as shown below [1]:

1. The condition being screened for should be an important health problem.

2. The natural history of the condition should be well understood.

3. There should be a detectable early stage.

4. Treatment at an early stage should be of more benefit than at a later stage.

5. A suitable test should be devised for the early stage.

6. The test should be acceptable.

7. Intervals for repeating the test should be determined.

8. Adequate health service provision should be made for the extra clinical workload resulting from screening.

9. The risks, both physical and psychological, should be less than the benefits.

10. The costs should be balanced against the benefits.

Subsequently these criteria were expanded and embellished by the UK National Screening Committee to encompass not only the validity but also the effectiveness and appropriateness of any screening program as follows [2]:

The Condition

1. The condition should be an important health problem.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood, and there should be a detectable risk factor, disease marker, latent period, or early symptomatic stage.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable.

4. If the carriers of a mutation are identified as a result of screening, the natural history of people with this status should be understood, including the psychological

implications.

The Test

1. There should be a simple, safe, precise, and validated screening test.

2. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.

3. The test should be acceptable to the population.

4. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.

5. If the test is for mutations, the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested for, should be clearly set out.

The Treatment

1. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.

2. There should be agreed evidence-based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

3. Clinical management of the condition and patient outcomes should be optimized in all healthcare providers prior to participation in a screening program.

The Screening Program

1. There should be evidence from high-quality randomized controlled trials that the screening program is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (e.g., Down's syndrome and cystic fibrosis carrier screening), there must be evidence from high-quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.

2. There should be evidence that the complete screening program (test, diagnostic procedures, treatment/intervention) is clinically, socially, and ethically acceptable to health professionals and the public.

3. The benefit from the screening program should outweigh the physical and psychological harm (caused by the test, diagnostic procedures, and treatment).

4. The opportunity cost of the screening program (including testing, diagnosis and treatment, administration, training, and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e., value for money).

5. There should be a plan for managing and monitoring the screening program and an agreed set of quality assurance standards.

6. Adequate staffing and facilities for testing, diagnosis, treatment, and program management should be available prior to the commencement of the screening program.

7. All other options for managing the condition should have been considered (e.g., improving treatment and providing other services), to ensure that no more cost-effective intervention could be introduced or current interventions increased within the resources available.

8. Evidence-based information, explaining the consequences of testing, investigation, and treatment, should be made available to potential participants to assist them in making an informed choice.

9. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.

10. If screening is for a mutation, the program should be acceptable to people identified as carriers and to other family members.

11. As described in Chap. 2, the etiology and pathogenesis of cervical neoplasia is well established and the natural history understood. While cytology-based screening for cervical precancer meets many of the Wilson and Jungner criteria, it remains open to the criticism that it has never been subjected to high-quality randomized clinical trials, in contrast, for example, to breast cancer screening [3, 4].

Epidemiology of Cervical Cancer

Globally, cervical cancer remains a major public health problem. Worldwide, cervical cancer is the fourth most common cancer in women, and the seventh most common overall, with an estimated 528,000 new cases in 2012. More than 85% of the global burden occurs in developing countries where it accounts for almost 12% of all female cancers. High-risk regions, with estimated age-standardized rates over 30 per 100,000, include Eastern Africa (42.7), Melanesia (33.3), Southern Africa (31.5), and Middle Africa (30.6) while rates are lowest in Western Europe (7.3), Northern America (6.6), Australia and New Zealand (5.5), and Western Asia (4.4) reflecting in part the success of cytology-based population screening programs in the latter. Cervical cancer remains the most common cancer in women in Eastern and Middle Africa.

There were an estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths, and 87% of cervical cancer deaths occur in less developed regions. The average risk of dying from cervical cancer before age 75 is three times higher in less than in more developed regions. Mortality varies 18-fold between the different regions of the world, ranging from less than 2 per 100,000 in Western Asia, Western Europe, and Australia and New Zealand to above 20 per 100,000 in Melanesia (20.6), Middle Africa (22.2), and Eastern Africa (27.6) [5].

Papanicolaou and the Development of Cytology-Based

Population Screening

Donne (1844) and Pouchet (1847) first described the cytology of vaginal secretion in the mid-nineteenth century but neither description related to the diagnosis of cervical cancer [6, 7]. In 1869 Dickenson examined discharges from women with cervical cancer, but failed to find diagnostic cells [8]. It was not until 1871 that Richardson in the USA recommended cytological examination in cases of suspected cervical carcinoma and wrote: "In suspected cancer of the womb ... a small portion of the secretion from the os uteri, or from the ulcerated surface of the growth itself, should such exist, must therefore be removed by means of a probe or pair of forceps introduced through a speculum, and on examination with a power of 200 diameters will probably disclose at least a few cells on each slide, which will indicate with more or less certainty the character of the morbid formation." In 1886 Friedlaender also used this method but warned against diagnosing carcinoma from the cytology alone [6].

Papanicolaou first systematically used the vaginal smear, and ever since the technique has been associated with his name as the "Pap test" or "Pap smear." George N. Papanicolaou qualified in medicine in Athens in 1904 and as a junior postgraduate specialized in the experimental study of reproduction. In 1913 he emigrated to New York, where he studied the estrous cycle in animals and the human menstrual cycle by examination of vaginal smears [9]. During his studies on patients in the Women's Hospital, New York, Papanicolaou identified malignant cells in vaginal smears, and in 1928 he gave his first paper on this subject at a conference, entitling it "New cancer diagnosis" [10].

Simultaneously, and independently, cancer cells were recognized in cervical smears by the Romanian pathologist Aurel Babes in Bucharest. Babes and Daniel first presented their new method for the diagnosis of carcinoma of the cervix, using a platinum loop to transfer material from the affected area to glass slides which were then air-dried and stained by the Giemsa technique, to the Bucharest Gynaecological Society in 1927 and the results were published the following year [11–13]. Simultaneously, the Italian gynecologist Odorico Viana, influenced by Babes, reported on the successful diagnosis of cervical cancer by the smear technique [14, 15].

Although the lesion we now recognize as cervical intraepithelial neoplasia (CIN)/squamous intraepithelial lesion (SIL) (see Chap. 6), the precursor of invasive squamous cell carcinoma of the cervix, had been recognized by the third decade of the twentieth century, Papanicolaou's report and the others referred to above received little attention, and Papanicolaou returned to a study of reproductive endocrinology in the 1930s. In 1939 Joseph Hinsey was appointed to the department of anatomy at Cornell and encouraged Papanicolaou to return to his work on cancer detection using the vaginal smear. Hinsey also arranged collaboration with Herbert Traut, a gynecologist trained in pathology, and Andrew Marchetti, chairman of the department of obstetrics and

gynecology at Cornell, such that every woman admitted to the gynecology service at Cornell was required to have a vaginal smear, and these samples were made available for Papanicolaou to examine. In 1940 Papanicolaou obtained funding from the Commonwealth Fund, which enabled him to develop a new staining technique which included wet fixation in an ether-alcohol solution [16]. He subsequently demonstrated that the vaginal smear permitted an earlier diagnosis of cervical cancer and that this was made possible because vaginal smears had been taken repeatedly. Papanicolaou described the technique as exfoliative cytology from the Greek *ex*, away, and Latin *folium*, leaf, the analogy of vaginal smears being to that of leaves falling from a tree [17]. In 1941, Papanicolaou and Traut published their seminal paper entitled "The diagnostic value of vaginal smears in carcinoma of the uterus," and this was followed 2 years later by the monograph *Diagnosis of Uterine Cancer by the Vaginal Smear*, funded by the Commonwealth Fund and beautifully illustrated with camera lucida watercolor drawings by Murayama [18]. It should be noted that the Papanicolaou classification provided a measure of the likelihood of the presence of invasive cervical cancer, whereas contemporary cervical cytology classifications provides an evaluation of the likelihood of the presence of preinvasive disease (see below).

These publications altered the opinion of the medical profession, and many gynecologists became enthusiastic about the possibility of identifying cancer of the cervix at an early and curable stage. Cervical cancer detection by cytology was strongly supported by the American Cancer Society and the National Cancer Institute, and subsequent studies confirmed the value of cytology, to detect not only cancer but also precancerous changes [18–23]. In 1947 Papanicolaou began offering cytology training courses at Cornell, the First National Cytology Conference was held in Boston in 1948, the forerunner of the American Society of Cytopathology was founded in 1951, the first International Cancer Cytology Congress was held in Chicago in 1956, and the International Academy of Cytology was founded in 1957. The emergence of a body of trained exfoliative cytologists made possible the rapid development of population screening, first by the vaginal smear and soon after by cervical scraping using a wooden spatula introduced by Ayre [24]. Ruth M. Graham published the first modern comprehensive cytology text, *The Cytologic Diagnosis of Cancer*, in 1950 and Papanicolaou published his *Atlas of Exfoliative Cytology* in 1954 [25].

By the mid-1950s screening for cervical cancer by exfoliative cytology had been widely introduced in North America and elsewhere, and the evidence of its benefits in terms of reduction in mortality progressively accumulated: the reduction in mortality was clearly directly related to the intensity of screening [26–35].

Cervical Cancer Screening in the UK (1950–1985)

After the Second World War, a small number of British gynecologists and pathologists,

aware of the introduction of exfoliative cytology for cervical cancer screening in North America, began to explore the possibility of introducing a similar screening program in the recently established National Health Service. In 1951 an initial discussion was held by the section of obstetrics and gynecology of the Royal Society of Medicine after which a number of cytologists went to North America to visit Papanicolaou, Ruth Graham, Ayre, and others. Following a further conference at the Royal College of Obstetricians and Gynaecologists in 1955, Sir William Gilliatt and Dame Hilda Lloyd, both past presidents of the College, established a committee to look into the matter and further developed links with Papanicolaou and Ruth Graham and also with Professor Alex Agnew, H. Fidler, and D. A. Boyes in Vancouver.

Prior to the establishment of a comprehensive national population-based cervical cancer screening program, a number of well-known gynecologists were instrumental in the establishment of exfoliative cytology of the female genital tract at various centers: Chassar Moir in Oxford; McLaren in Birmingham; Way in Newcastle; Anderson in Edinburgh; Nixon at University College Hospital, London; Miss G. Hill at the Royal Free Hospital, London; McClure Browne at the Hammersmith Hospital; and Sir Dugald Baird in Aberdeen. In 1960 Sir Dugald Baird initiated the first population screening program to cover all women at risk of developing cervical cancer in North East Scotland, and Dr. J. Elizabeth Macgregor was appointed to manage the program and the laboratory [31, 33–36]. Dr. Erica Wachtel, who worked with Prof. McClure Browne at the Hammersmith Hospital, practiced exclusively in cytopathology and was the first practitioner to be appointed professor of cytopathology in the UK [37]. The first NHS consultant cytopathologist, O. A. N. Husain, was appointed to St. Stephen's Hospital, London, in 1961 [38].

Following the reports of J. M. G. Wilson of the DHSS [39, 40], a comprehensive National Cervical Cytology Screening Service was established in 1967. In 1964, in preparation for this service, five training schools were set up to teach the skills of cytodiagnosis – at the Hammersmith and Royal Free Hospitals, London; Birmingham; Manchester; and Newcastle. A national request/report form (HMR101) was introduced in 1967, which in modified form persists until today, and in the first year of the service half a million smear tests were performed. Expansion was rapid and by 1970 nearly 2.5 million tests per year were being recorded, increasing to 3.9 million in 1986. Most of the increase in the number of smears had been from general practitioners, rising from 27% of all smears in 1973 to 43% in 1980.

Women aged 35–60 years were screened at five yearly intervals with some opportunistic screening of women in antenatal and sexual health clinics. A manual record card-based screening registry for England was established at Southport to recall women for repeat tests.

UK Terminology of Cervical Cytology and Histology

The Papanicolaou classification system for cytological diagnosis introduced in 1954 was intended to apply to all types of cytology specimen to indicate the degree of certainty that cancer was present or absent: there was no correlation with cytology in the context of a program intended to identify precancerous lesions [25] (Table 3.1).

Table 3.1 Papanicolaou classification of cytology reports

Class I	Negative	Absence of atypical or abnormal cells
Class II	Negative	Atypical cells present but without abnormal features
Class III	Suspicious	Cells with abnormal features suggestive but not conclusive for malignancy
Class IV	Positive	Cells and cell clusters fairly conclusive for malignancy
Class V	Positive	Cells and cell clusters conclusive for malignancy

The entity of carcinoma in situ, the immediate precursor lesion of invasive squamous cell carcinoma of the cervix, in which the constituent cells morphologically looked like the cells found in invasive squamous cell carcinoma, had been recognized from the late nineteenth century [41–44]. However by the early 1950s, surface lesions of the cervix with abnormal but less marked histological features had been identified, for which a number of terms were suggested including anaplasia, basal cell hyperplasia, atypical metaplasia, and atypical hyperplasia. In 1953 Regan proposed the term dysplasia, from the Greek *dys*, bad, and *plasia*, molding, which he divided into three grades, mild, moderate, and severe. This proposal was endorsed by the First International Congress of Exfoliative Cytology and the World Health Organization: in the latter the abnormal cells were described in terms of their histological correlation [45, 46]. Dysplasia appeared to have a lower risk of progression to cancer than carcinoma in situ, and consequently, at that time, women found to have carcinoma in situ were recommended to have a hysterectomy, while those with dysplasia were not immediately treated [47, 48].

During the establishment of the cervical screening program in the UK, it became apparent that a variety of terminology was being used to describe the morphological appearances of neoplastic cells derived from in situ and invasive cervical squamous lesions. In particular the practice in many laboratories of calling cells thought to be derived from carcinoma in situ “malignant cells” and using “dyskaryosis” to imply that nothing more than dysplasia was present began to be questioned in the light of the conclusive evidence from Richart that dysplasia and carcinoma in situ of the cervix were a “lesional continuum” [49]. A working party of the British Society for Clinical Cytology (BSCC) recommended that the terminology in the WHO publication *Cytology of the Female Genital Tract* be adopted for normal cellular components of a cervical

smear (e.g., superficial, intermediate, and parabasal squamous cells; endocervical cells; endometrial cells) and the term "dyskaryosis" adopted for neoplastic squamous and glandular cells, irrespective of whether the cytologist thought that they were derived from an in situ or invasive lesion [50] (Table 3.2).

Table 3.2 Definition of dyskaryosis

Disproportionate nuclear enlargement
Irregularity in nuclear form and outline
Hyperchromasia
Multinucleation
Irregular chromatin distribution, which may be stippled, clumped, or stranded with condensation beneath the nuclear membrane
Abnormalities of the number, size, and form of nucleoli

Eight years later, a second BSCC working party endorsed the recommendation of dyskaryosis as the preferred terminology and recommended a three-grade system of mild, moderate, and severe dyskaryosis, based on the nuclear-cytoplasmic area of the dyskaryotic cells, which correlated with cells from the surface of CIN 1, CIN 2, and CIN 3, respectively. They also provided guidance on cytological features which were suggestive of the presence of invasive squamous carcinoma. This recommendation was universally adopted in the UK cervical cancer screening programs [51] (Table 3.3).

Table 3.3 BSCC terminology in gynecological cytopathology (1986)

Grade	Morphological features	Histological correlate
Mild dyskaryosis	The abnormal nucleus occupies less than half the area of the cell, which has plentiful thin translucent cytoplasm with angular borders resembling a superficial or intermediate squamous cell	CIN 1
Moderate dyskaryosis	The abnormal nucleus occupies one half to two-thirds of the area of the cell. There is more disproportionate nuclear enlargement than in mild dyskaryosis, and nuclear morphology tends to be more abnormal than in mild dyskaryosis. The cytoplasm resembles that of intermediate, parabasal, or superficial cells.	CIN 2
Severe dyskaryosis	The abnormal nucleus practically fills the cell or at least two-thirds of its area and is surrounded by a narrow rim of thick dense cytoplasm. Affected cells may be round, oval, elongate, or polygonal	CIN 3

The 1986 working party also recognized that "There are smears in which the evidence is such that it is impossible to decide if the cells are the product of inflammation or if they have neoplastic potential" and suggested that such samples be described as showing borderline abnormalities. In 1994, a joint working party of the National Health Service Cervical Screening Programme (NHSCSP), the BSCC, and

Royal College of Pathologists provided guidance on the diagnosis and management of borderline nuclear changes in squamous and glandular cells and their distinction from reactive or inflammatory change and neoplastic change [52].

In 2002, conscious of the widespread adoption of the two-tiered Bethesda system for reporting cervical cytology, originally developed in 1988 and subsequently modified in 2001, which reflected clinical practice and management in terms of low- and high-grade abnormality, the BSCC held a conference at which it was agreed that a two-tier system should also be introduced in the NHSCSP [53–58]. The revised BSCC terminology for cervical cytology was published in 2008 [59] and implemented in the NHSCSP in 2013. This terminology aligns closely with the Bethesda system, reflects contemporary understanding of the biology of human papillomavirus (HPV) infection, and permits international comparison of data (Table 3.4). The principal change introduced by this terminology is that while dyskaryosis is retained as the descriptor of neoplastic cell nuclear morphology, it is graded by evaluation of nuclear: cytoplasmic diameter rather than area, as previous studies had shown that the former was a more reliable discriminator of mild from moderate or severe dyskaryosis, i.e. low-grade from high-grade dyskaryosis, in both conventional and liquid-based cervical cytology preparations [60].

Table 3.4 BSCC terminology (2008): comparison with other terminologies (from Denton et al. [59])

BSCC 1986 and NHSCSP	BSCC proposed new terminology	The Bethesda system 2001	ECTP terminology	AMBS 2004
Negative	Negative	Negative for intraepithelial lesion or malignancy	Within normal limits	Negative
Inadequate	Inadequate	Unsatisfactory for evaluation	Unsatisfactory due to	Unsatisfactory
Borderline nuclear change	Borderline change, squamous, but not otherwise specified	Atypical squamous cells of undetermined significance (ASC-US)	Koilocytes (without changes suggestive of intraepithelial neoplasia) Squamous cell changes (not definitely neoplastic but merit early repeat)	Possible low-grade squamous intraepithelial lesion
	Borderline change, high-grade dyskaryosis not excluded	ASC-H (cannot exclude HSIL)		Possible high-grade squamous intraepithelial lesion
	Borderline change in endocervical cells	Atypical endocervical, endometrial, or glandular (NOS or specify in comments)	Atypical glandular cells (qualify)	Atypical endocervical cells of undetermined significance Atypical glandular cells of undetermined significance

		Atypical endocervical or glandular cells, favor neoplastic		
Mild dyskaryosis	Low-grade dyskaryosis (includes all cases of koilocytosis provided that no high-grade dyskaryosis is present)	Low-grade squamous intra-epithelial lesion (LSIL)	Mild dysplasia (CIN1)	Low-grade squamous intraepithelial lesion
Moderate dyskaryosis	High-grade dyskaryosis	High-grade squamous intra-epithelial lesion (HSIL)	Moderate dysplasia (CIN2)	High-grade squamous intraepithelial lesion
Severe dyskaryosis		HSIL	1. Severe dysplasia (CIN3) 2. Carcinoma in situ (CIN3)	
Severe dyskaryosis? invasive	High-grade dyskaryosis? invasive	Squamous cell carcinoma	1. Severe dysplasia? invasive 2. Invasive squamous cell carcinoma	Squamous cell carcinoma
? Glandular neoplasia	? Glandular neoplasia, endocervical, non-cervical	1. Endocervical carcinoma in situ 2. Adenocarcinoma – endocervical, endometrial, extrauterine, not otherwise specified	Adenocarcinoma AIS, endocervical, endometrial, extrauterine NOS	Endocervical adenocarcinoma *in situ* Adenocarcinoma

BSCC British Society for Clinical Cytology, *ECTP* European Commission Training Programme, *AMBS* Australian Modified Bethesda System

The NHS Cervical Screening Program (1986–2004)

Despite the establishment of the cervical screening program as described above, it was clear by the mid-1980s that it had had little impact on the incidence or mortality from cervical cancer. In 1985 a leading article in *The Lancet* drew attention to this fact and specifically commented that the most successful cancer screening programs are organized as public health cancer control programs, specifically directed toward a reduction of mortality; call the age group at greatest and most immediate risk (30 years +) based on population registers and keep on trying to call persistent non-attenders; concentrate first upon women who have never had a smear; and put "someone in charge" (a manager) of the process who can be held to account [61]. In 1988 health circular HC (88)1 directed District Health Authorities to give priority to screening for prevention of cervical cancer and in particular implementation of a call and recall

system from lists of women held on Family Practitioner Committee (primary care) computers starting not later than 31 March 1988. All women aged 20–64 were to be invited for screening at least every 5 years (some health authorities elected to invite women every 3 years) and adequate facilities made available for prompt investigation treatment and follow-up of women with abnormal smear results [62]. General practitioners were also offered a financial incentive based on the proportion of their practice female population eligible for cervical screening that were tested. Initially the NHS cervical screening program was managed by a multidisciplinary National Coordinating Network but subsequently a director, Professor Julietta Patnick, and support staff were appointed in 1994 [63, 64]. Over the succeeding two decades, in collaboration with the relevant professional bodies, the NHSCSP produced a comprehensive series of guidance documents related to all aspects of the cervical cancer screening process from invitation to attend screening to treatment of identified abnormality. In particular, the first NHSCSP commissioned guidance entitled *Achievable standards, benchmarks for reporting and criteria for evaluation* and, thereby henceforth known as ABC 1, gave guidance on specimen adequacy, management of smear abnormality, evaluation of the program, internal quality control (IQC), and external quality assurance (EQA) [65] (Table 3.5). In relation to IQC and EQA, ABC 1 introduced achievable standard ranges for cytology reporting by laboratories and individuals, and in subsequent years these ranges were amended based on the mandatory returns (KC61) submitted by laboratories in the preceding year (Table 3.6). In the first of two subsequent editions of ABC, published in 2000, guidance on reporting of cervical smears was reinforced and where necessary revised, new performance indicators were introduced, and pitfalls in cytological diagnosis leading to false-positive and false-negative results described [66, 67]. In the second subsequent edition of ABC, published in 2013, adoption of the revised BSCC terminology for cervical cytology was mandated, management of cytological abnormality updated following the implementation of HPV triage and test of cure, and performance indicators for evaluating cervical cytopathology expanded to encompass not only individual and laboratory cytology performance but also the performance of related colposcopy and histopathology services [68–70].

Table 3.5 ABC 1: recommendations for management

Management	Cytology result
Routine recall	Negative
Repeat smear at shorter interval than recommended routine recall	Inadequate sample and the first occurrence of mild dyskaryosis or borderline change A second repeat sample may be requested for inadequate samples or borderline change, but after three such smears colposcopy must be recommended. The repeat interval may vary between 3 and 12 months but is usually 6 months Annual repeat smears are recommended for 5 years after treatment of CIN 2 and CIN 3 At least two negative smears at least 6 months apart, after mild dyskaryosis, borderline

	change or treatment of CIN 1 before a woman returns to routine screening or screening is ceased at age 65
Referral for gynecological opinion	Moderate, severe, and ungraded dyskaryosis; invasive squamous and glandular neoplasia should all be referred on the first occurrence. Colposcopy should be recommended on the second occurrence of mild dyskaryosis

Herbert et al. [142]

Table 3.6 ABC 1: criteria for evaluating cervical cytology and monitoring the accuracy of screening

Measurement	Achievable range
Sensitivity of primary screening with respect to the final report after rapid review of all negative and inadequate smears	>90% all abnormalities >95% high-grade abnormality
Laboratory report profile: Inadequate Mild dyskaryosis and borderline change Moderate and severe dyskaryosis	7.0 ± 2.0% 1.6 ± 0.4% 5.5 ± 1.5%
Positive predictive value (PPV) of moderate or severe dyskaryosis for the histological diagnosis of CIN 2 or worse	65–85%

Herbert et al. [142]

The success of the reorganized English cervical screening program as NHSCSP was evidenced by the progressive fall in incidence of cervical cancer in the succeeding two decades: this has now largely stabilized (Fig. 3.1). The increased incidence around 2009 was the result of the increased uptake of screening due to the widely publicized diagnosis and death of a television personality [71, 72].

Fig. 3.1 Age-standardized incidence rates of invasive cervical cancer in the UK (1979–2013) (Source: Cancer Research UK. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/cervical-cancer. Accessed August 2016)

NHSCSP 2004 to the Present

Liquid-Based Cytology (LBC)

From the late 1980s, a number of manufacturers began to investigate the potential to produce monolayer or near-monolayer preparations of cervical cytology samples, with the intention that this would provide an optimized platform on which to employ computer-assisted image analysis microscopy. Production of near-monolayer preparations required samples to be collected in a liquid preservative – hence liquid-based cytology – and then most of the debris, blood, and exudate removed either by filtration or density gradient sedimentation, prior to preparation of the monolayer or near-monolayer sample. By the late 1990s, two systems were widely available: ThinPrep® (Hologic) and SurePath® (BD Diagnostics). In 2000 an initial evaluation by the National Institute for Clinical Excellence (NICE) suggested that LBC might be valuable technology to implement in the NHSCSP [73]. In 2003, following a further evaluation by NICE and an evaluation study in three English laboratories, the Department of Health (DoH) announced that LBC was to be used as the primary means of processing samples in the cervical screening program in England and Wales and full implementation of the new technology was to be achieved by 2008 [74, 75]. Implementation was conducted by a cascade process of laboratory conversion and training, and by late 2008 all cervical screening laboratories in England and Wales had

converted to LBC. Scotland had already adopted LBC and Northern Ireland followed some time later [76].

Importantly, at the same time the DoH also announced changes in screening age range and frequency to be implemented by April 2004: women would in future be invited for their first screening test at age 25, not age 20, and screened thereafter every 3 years until age 49 and every 5 years from age 50 to 64 [77]. This policy change, based on an audit of the screening histories of women with invasive cervical cancer [78], was intended to unify and consolidate considerable variation in practice across England: as noted above the national recommendation was to screen every 5 years but some districts had elected to screen every 3 years. While concerns were raised about the effect of not screening women less than 25 years of age, it has been kept under review through the national audit of screening histories of women who develop cervical cancer: most cervical cancers in women under age 30 years are screen detected as superficially invasive carcinomas (FIGO stage IA) [79–83].

As predicted, progressive implementation of LBC, combined with the change in screening age range and frequency, resulted in a reduction in the number of inadequate samples reported and thereby a decrease in the total number of tests examined: over 246,000 fewer tests were reported as inadequate in 2007–2008 compared with 2003–2004, the last year before LBC implementation. This also occurred against a background of an increased number of women aged 25–64 being screened, reflecting a more efficient screening program with fewer unnecessary tests outside the recommended screening age range [84]. Furthermore, the progressive loss of tests in women aged less than 25 years reduced the number of abnormal tests reported, particularly low-grade abnormalities which are most prevalent in this age group: nearly 19,000 fewer tests were reported as low grade and over 4000 as high grade in 2007–2008 compared with 2003–2004. As a result there was reconfiguration of consultant programmed activities in some laboratories to ensure maintenance of quality standards for the minimum number of abnormal tests examined annually.

Therefore, following a change in the screening age range and frequency and full implementation of LBC, a total of 269,000 fewer cervical cytology samples were examined in England in 2007–2008 compared with 2003–2004. Implementation of LBC also resulted in increased laboratory productivity and efficiency, with no adverse effect on quality. A large laboratory in Manchester reported that nearly 1 min per slide was saved during primary microscopy, and microscopy by cytopathologists, using LBC compared with conventional smear preparations. The uninterrupted hourly rate of slide examination rose from 8.6 slides for conventional smear preparations to 11.7 for LBC preparations, comparable to the data from the Scottish LBC feasibility study [76, 85]. A separate study from Scotland reported a 40% reduction in full primary screening time [86]. In the Sheffield laboratory, individual screener productivity increased by 20% in the first year following full LBC implementation [87], and productivity increases of up

to 50% coupled with decreased numbers of unsatisfactory samples and an increased sensitivity for the detection of cytological abnormalities validated by subsequent histological investigation have been reported [88].

Increased productivity was also reflected in national data showing a progressive increase in the proportion of laboratories reporting results within 2 weeks of specimen receipt, an important achievement in view of the Cancer Reform Strategy objective that all women should receive the results of their test within 2 weeks by 2010 [89, 90].

As a result of LBC implementation, there was a growing mismatch between workload and capacity in some laboratories. However, a NHSCSP workforce survey revealed that over one-third of screening staff were over 50 years of age, and LBC implementation buffered laboratories against this marked demographic change [91]. In fact, some laboratories found no need to replace primary screening staff on retirement, resulting in cash-releasing cost savings.

National implementation of LBC not only resulted in improved laboratory efficiency and productivity but was also the platform for consideration of the implementation of molecular testing and automation in the NHS cervical screening programs.

HPV Testing

The recognition of the strong causal relationship between persistent infection of the genital tract with high-risk human papillomavirus (HPV) types and the occurrence of cervical cancer has resulted in the development of a number of HPV DNA and RNA detection systems in an attempt to refine existing cytology-based cervical cancer screening programs [92–95] (see Chap. 2). LBC provides an ideal platform for application of this and other molecular technologies. Detection of high-risk HPV DNA is considered to be potentially useful in four clinical applications [96]:

1. As a triage test to select which women who have low-grade cytological abnormalities in routine screening require immediate referral for colposcopy rather than cytological surveillance.

2. Follow-up of women with abnormal screening results who are negative at colposcopy and biopsy.

3. Follow-up for women treated for high-grade CIN with local ablative or excisional treatment to more rapidly and accurately identify those who have or have not been cured.

4. As a primary screening test, either alone or in combination with cervical cytology

to detect cervical cancer precursors.

Triage of Low-Grade Abnormality

A meta-analysis of studies published between 1992 and 2010 comparing HPV testing with Hybrid Capture 2 (HC2) with repeat cytology in the management of low-grade cytological abnormality (borderline nuclear change/atypical squamous cells (ASCUS); mild dyskaryosis/low-grade squamous intraepithelial lesion (LSIL)) showed that HPV triage with HC2 of women with borderline nuclear change had significantly higher sensitivity than, and similar specificity to, repeat cytology. In triage of women with mild dyskaryosis, an HC2 test yielded a significantly higher sensitivity, but a significantly lower specificity, compared to repeat cytology [97]. A pilot study conducted within the initial English evaluation of liquid-based cytology demonstrated that, while HPV triage of low-grade abnormality resulted in a reduction in the rate of repeat smears but an increase in rates of referral to colposcopy, it was likely to be cost effective [98, 99]. A further evaluation of HPV triage implementation in six laboratories in the English cervical screening program (the sentinel site study) demonstrated that triaging women with low-grade cytological abnormalities by HPV testing would allow approximately a third of these women to be returned immediately to routine recall, and immediate referral for colposcopy would avoid the need for repeat cytology in the remainder. The HPV-positive rates at the six sites ranged from 34.8% to 73.3% for women with borderline cytology and from 73.4% to 91.6% for women with mild dyskaryosis, and these differences remained after the rates were standardized for age. Overall the HPV-positive rate was higher in sites using ThinPrep® than in those using SurePath® LBC [68.7% and 61.7% respectively ($p < 0.001$)], and the difference remained after adjustment for age group and initial cytology result. LBC technology was, however, confounded by site, and it was therefore not possible to determine whether this difference was due to variation in the reporting of cytology between sites. In the only site which used both technologies, there was no significant difference in positive rates between the two technologies [100]. Based on this data HPV triage of low-grade cytological abnormality was implemented in the English cervical screening program in 2011 using the algorithm developed for the sentinel site study (Fig. 3.2).

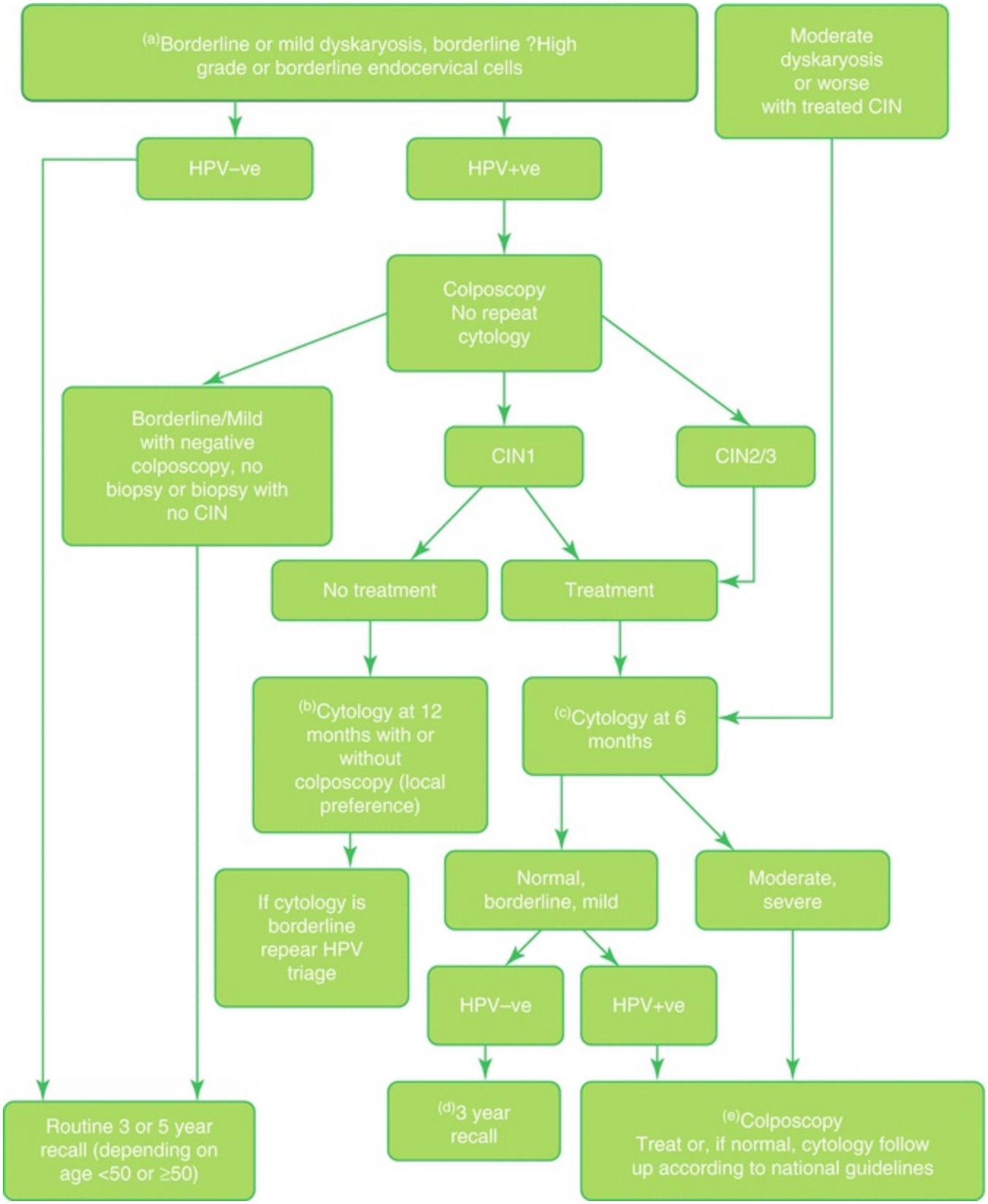

Fig. 3.2 Flow chart: triage and test of cure in the NHSCSP (© Crown Copyright 2016). This information was originally developed by Public Health England Screening (https://www.gov.uk/topic/population-screening-programmes) and is used under the Open Government Licence v3.0

Test of Cure

Prior to 2011 NHSCSP guidance was that women treated for low-grade disease (CIN 1) required follow-up cytology at 6, 12, and 24 months and if all results were negative could return to routine screening. Women treated for high-grade disease (CIN 2 or 3 or cervical glandular intraepithelial neoplasia (CGIN)) required 6- and 12-month follow-up cytology and annual cytology for the subsequent 9 years at least before returning to screening at the routine interval. It has been estimated that in England every year more than 300,000 cytology tests were performed annually for follow-up after treatment, approximately 10% of the annual workload [101]. A number of studies prior to 2007 demonstrated that testing for high-risk HPV infection with Hybrid Capture 2 was more sensitive, though less specific, than repeat cytology in the detection of residual disease following excisional treatment of high-grade CIN, and a large prospective study from the UK showed that a negative result from a high-risk HPV test after treatment was indicative of very low risk of recurrent disease even in the presence of low-grade cytological abnormality: women who were cytology and HPV negative at 6 months could safely be returned to routine three-yearly recall [102, 103]. Evaluation of HPV as test of cure after treatment of CIN in the sentinel study demonstrated that about 85% of treated women were HPV negative at 6 months after treatment [100]. HPV test of cure was implemented with HPV triage of low-grade cytological abnormality in the English cervical screening program in 2011 (Fig. 3.2).

Automation in Cervical Screening

As noted above, LBC provides the platform for computer-assisted evaluation of cervical smears and thereby partial automation of the screening process in the laboratory, a goal which had been sought for over 50 years [104]. Early attempts at automation of the screening process using conventional cervical smears were hampered by difficulties in visualization of the cells if they were obscured by blood, inflammatory cells, or mucus; detection of the boundaries of cells and their nuclei, especially in overlapping cells or three-dimensional cell groups; a recognition that there were more similarities than differences between normal and neoplastic cells; and limited computing capacity which was unable to process the enormous volume of data generated from a single Papanicolaou smear which might contain up to 300,000 cells. LBC presents a monolayer or near monolayer of cells with clearly defined boundaries, largely devoid of obscuring blood, inflammatory cells, or mucus, and this, coupled with developments in computerized image analysis, has made semi-automated slide-scanning devices available for clinical use.

Two systems currently dominate the market and have been approved for primary cervical screening by the US Food and Drug Administration (FDA). Both consist of a highly automated microscope and an image analyzer that presents a restricted number of

fields of view (FOVs) containing abnormal cells for interpretation by laboratory staff.

The BD FocalPoint™ Slide Profiler uses multiple algorithms to assign a score (0.0–1.0) to each slide (either conventional or SurePath) based on the probability of abnormality. Threshold scores are derived by separation of meaningful objects, i.e. irregular-shaped nuclei, from the background and describing each object with a set of measurement values. Slides with scores below the primary threshold, typically about 25% of a population of routine cervical screening samples, can be archived with no need for human microscopic review (no further review – NFR) resulting in a significant reduction in the workload of laboratory screening staff.

This system has been further developed as the BD FocalPoint™ GS Workstation in which the automated primary screening system is combined with an automated microscope which provides the electronic capability of locating diagnostically relevant locations in the samples above the threshold score. After reading a slide barcode, the microscope automatically positions the slide at the first relevant location, and a user-activated footswitch or mouse click moves the microscope to the next position until all locations are screened for suspicious cells or features.

The ThinPrep™ Imaging System rapidly scans and locates 22 areas of interest, known as fields of view (FOVs), in batches of ThinPrep® LBC slides and stores the coordinates which mark the position of the FOVs along with the slide identification information. Once all of the slides in a batch have been imaged, the slides are taken to a review microscope where they are reviewed by a cytology screener. The review scope automatically takes the cytology screener to each FOV in geographic order and, if any abnormalities are identified in the FOVs, the entire slide is reviewed by the cytology screener. If no abnormalities are identified in the FOVs, the slide may be signed out as negative. By directing the cytology screener to the FOVs on a slide, the amount of time required to screen a slide is dramatically reduced.

Both systems were granted FDA approval on the basis of being able to detect an equivalent or higher proportion of high-grade cytological abnormalities compared with manual reading [105, 106]. However an earlier systematic review of the literature published on the clinical and cost effectiveness of automated and semi-automated cervical screening devices including AutoPap, a predecessor of the BD FocalPoint™ Slide Profiler, by the New Zealand Health Technology Assessment program reported that the evidence base was not sufficiently strong for reliable conclusions to be drawn and recommended further trials with robust reference standards [107]. Similarly a systematic review by the UK Health Technology Assessment also concluded that previous studies had not been of sufficiently good quality to allow reliable recommendations [108].

In England, the MAVARIC trial was therefore designed to achieve a rigorous, prospective, unbiased comparison of manual and automation-assisted reading which had been powered to demonstrate non-inferiority in terms of sensitivity to detect CIN 2 or

worse (CIN2+). Other objectives of the study were to compare the specificity of automation-assisted screening relative to manual, to incorporate both automated systems, and to evaluate the reliability of NFR in excluding CIN2+.

The principal finding was that automation-assisted reading was 8% less sensitive than manual reading (relative sensitivity 0.92; 95%CI 0.89–0.95) equivalent to an absolute reduction in sensitivity of approximately 6.3%, assuming the sensitivity of manual reading to be 79%. There was an increase of 0.6% in specificity relative to manual reading (relative specificity 1.006; 95%CI 1.005–1.007).

The inferior sensitivity of automation-assisted reading in the detection of CIN2 or worse combined with an inconsequential increase in specificity suggested that automation-assisted reading could not be recommended for primary cervical screening [109].

Furthermore, a large randomized trial in Finland comparing automation-assisted screening with conventional cytological screening reported no difference in the risk of cervical cancer between the automation-assisted and conventional screening methods [110].

However, in the MAVARIC study, the No Further Review facility on the BD FocalPoint™ Slide Profiler system proved to be reliable in terms of negative predictive value, missing only 1% of CIN2+ lesions associated with routine screening samples. It was considered that it could be a valuable adjunct in primary screening as this module does not require the expensive workstations required for reading the Fields of View and could reduce by up to 25% the number of slides requiring human reading; it has been subsequently utilized in this mode in a few English laboratories [111].

NHSCSP Beyond 2016: Cervical Screening in the Era of HPV Vaccination

HPV Vaccination

HPV vaccination using the bivalent vaccine (Cervarix®) against the two commonest types of HPV implicated in cervical carcinogenesis (HPV types 16 and 18) was introduced into the UK in September 2008 for girls aged 12–13 years, followed in autumn 2009 by a 2-year "catch-up" campaign to vaccinate all girls up to 18 years of age. The vaccine was originally administered as a three-dose schedule over 6 months. In 2012 Cervarix® was replaced by Gardasil®, a quadrivalent vaccine that also protects against HPV types 6 and 11, which cause about 90% of genital warts, and in September 2014 the three-dose schedule was replaced by a two-dose schedule with the doses 1 year apart.

Uptake of this school-based HPV vaccination program has been very good with more than 80% of 12–13-year-olds consistently receiving at least two of the three

scheduled vaccinations, and in the last year for which data is currently available, 2014–2015, the national coverage for the completed priming (first) dose was 89.4% [112]. As a result there will be a progressive increase in the proportion of women in the screening program who have been vaccinated, with an expected decrease in prevalence of cervical neoplasia, but these women will need to continue to participate in screening since the vaccine only offers protection against about 70% of cervical cancer. A nonovalent vaccine, Gardasil 9®, which offers protection against 90% of cervical cancer, has recently been licensed for use in Europe but not yet implemented in the UK [113, 114].

A modeling study from the UK predicted that HPV 16/18 vaccination of a cohort of 12-year-old girls would result over the lifetime of each cohort in a 23% reduction in the number of abnormal cytology tests, a 32% reduction in biopsies, and a 42% reduction in CIN treatments, assuming 100% vaccine coverage. Interestingly these estimates assumed that introduction of vaccination did not also allow a reduction in screening frequency [115]. Studies from Australia and Scotland, where HPV vaccination was introduced before England and Wales, have reported a reduction in prevalence of cytological abnormality [116, 117].

Primary HPV Testing

A progressive reduction in prevalence of cytological abnormality will result in a decrease in positive predictive value (PPV) and an increase in negative predictive value (NPV) of cytology-based programs and is the driver for adoption of high-risk HPV testing as the primary screening test with secondary triage to cytology: HPV testing is a highly standardized assay that maintains its performance characteristics under low prevalence conditions [118]. While there is good evidence that primary screening with HPV is more sensitive for detection of high-grade CIN and cancer, it is less specific, particularly in women less than 30 years of age [119, 120]. Several approaches are under evaluation to deal with the lower specificity of HPV DNA testing as associated with transient infection including HPV typing for HPV-16 and HPV-18/45; surrogate markers of viral integration such as p16; dual staining of cytology preparations with p16 and Ki67, a proliferation marker; mRNA coding for the viral E6 and/or E7 proteins; and DNA methylation with a potential clinical use recommending more aggressive management in those who are positive [121–133]. In countries such as the UK where cytology is of good quality, the most attractive option is to use HPV DNA testing as the sole primary screening modality with cytology triage of HPV-positive women [96]. However, HPV genotyping assays, particularly for HPV 16 and 18, would also permit post-vaccination surveillance to determine overall vaccine effectiveness and prevalence of non-vaccine HPV types in the vaccinated population [95]. Primary HPV screening, possibly combined with secondary molecular marker analysis, might also be

a platform for self-sampling as a means of addressing the falling coverage in young women in the UK and elsewhere [128, 132–137].

Four European randomized trials comparing cytology combined with HPV testing with cytology alone over extended follow-up demonstrated a significant reduction in the incidence of cervical cancer among women screened with HPV, compared with cytology [138]. While the rates were similar until 2.5 years of follow-up, thereafter HPV-based screening provided 60–70% greater protection against cervical cancer compared with cytology alone. In addition, the ARTISTIC trial has provided additional information:

- Cytology and HPV combined would not add significantly to HPV as a stand-alone screen with cytology triage for HPV positives.
- A negative HPV test provides a similar degree of protection against subsequent CIN 2 or worse over the next 6 years as does liquid-based cytology over 3 years, indicating that screening intervals could be extended [139, 140].

A recent analysis of the ARTISTIC study and other UK data showed that HPV primary screening and LBC triage would be cost effective compared with LBC provided there was adherence to the follow-up of HPV-positive cytology-negative women [141].

A pilot study to determine the feasibility of HPV primary screening was established in the sentinel site study laboratories in 2013. In late 2015, having evaluated the available data, the UK National Screening Committee recommended that HPV primary screening should be adopted in the UK cervical screening programs. This recommendation was accepted by health ministers, and in July 2016 a public announcement was made that the UK would adopt primary HPV screening, with full implementation planned to be completed by 2019. The proposed algorithm is shown in Fig. 3.3.

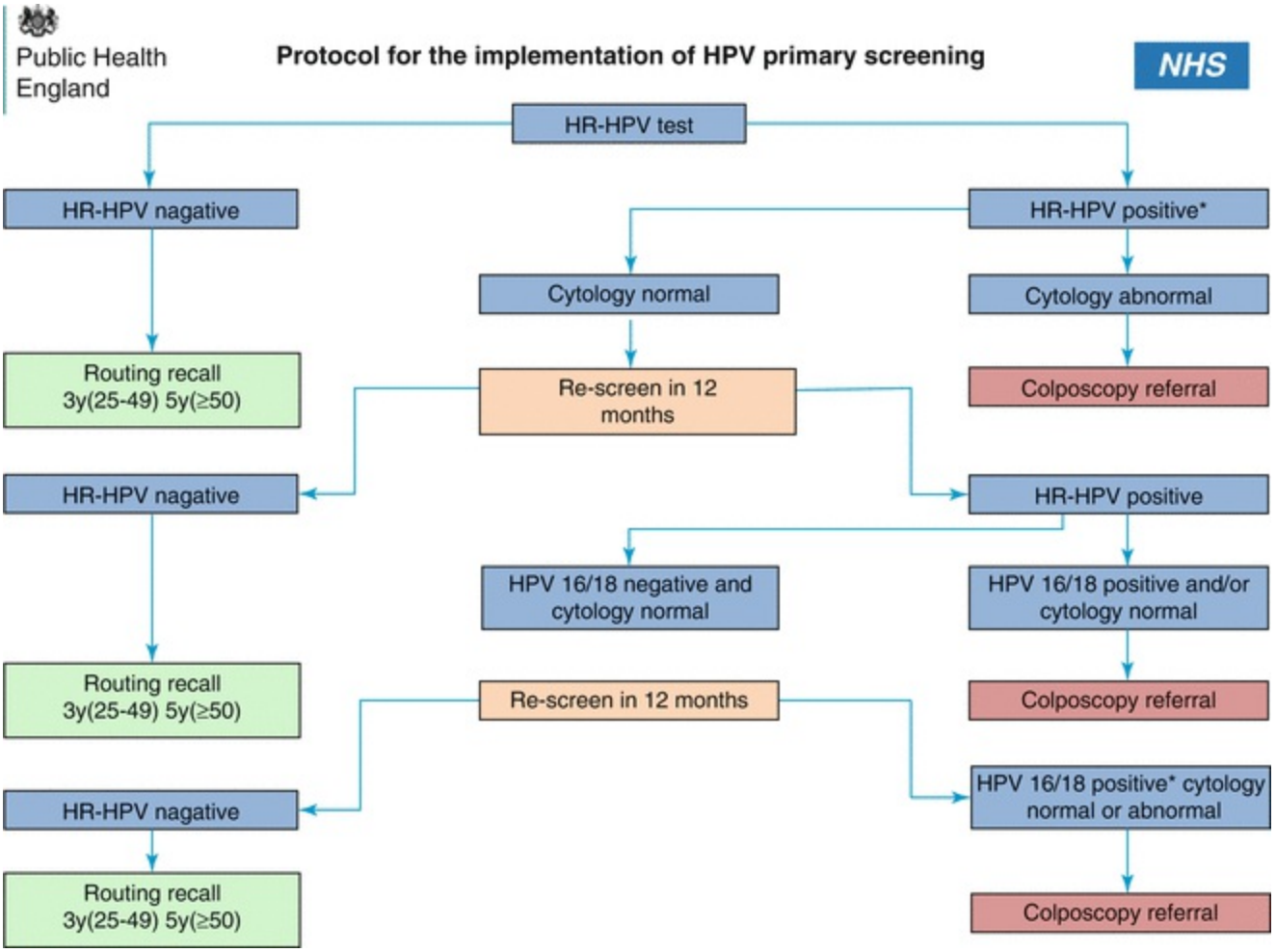

Fig. 3.3 Algorithm for HPV primary screening in the NHSCSP (© Crown Copyright 2016). This information was originally developed by Public Health England Screening (https://www.gov.uk/topic/population-screening-programmes) and is used under the Open Government Licence v3.0. Notes: (1) Applies to all women in the cervical screening program aged 25–64 years on routine call/recall and early recall. (2) Inadequate tests at any screening episode in the pathway will be repeated in 3 months. Three inadequate tests in a row will lead to a colposcopy referral. (3) Women in follow-up for cervical cancer (who still have a cervix) and CGIN/SMILE (without complete excision margins) will be screened annually with HPV testing for 10 years. * HPV16/HPV18 testing not required but may be provided automatically by the HPV test platform

References

1. Wilson JM, Jungner G. Principles and practice of screening for disease. Public Health Papers 34, 1. Geneva: World Health Organisation; 1968.

2. Public Health England. Criteria for appraising the viability, effectiveness and appropriateness of a screening programme. Public Health England. 23-10-2016. 18-7-2016.

3. Frisell J, Glas U, Hellstrom L, Somell A. Randomized mammographic screening for breast cancer in Stockholm. Design, first round results and comparisons. Breast Cancer Res Treat. 1986;8(1):45–54. [PubMed]

4. Anderson TJ, Lamb J, Alexander F, et al. Comparative pathology of prevalent and incident cancers detected by breast screening. Edinburgh Breast Screening Project. Lancet. 1986;1(8480):519–23.
[PubMed]

5. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
[PubMed]

6. Spriggs AI. History of cytodiagnosis. J Clin Pathol. 1977;30(12):1091–102.
[PubMed][PubMedCentral]

7. Koprowska I. Concurrent discoveries of the value of vaginal smears for diagnosis of uterine cancer. Diagn Cytopathol. 1985;1(3):245–8.
[PubMed]

8. Hajdu SI. Cytology from antiquity to Papanicolaou. Acta Cytol. 1977;21(5):668–76.
[PubMed]

9. Stockard CR, Papanicolaou GN. The existence of a typical oestrous cycle in the guinea pig; with a study of its histological and physiological changes. Am J Anat. 1917;22:225–83.

10. Papanicolaou GN. New cancer diagnosis. Proceedings of the 3rd Race Betterment Conference, p. 528–34. 1928. Battle Creek, Michigan, Race Betterment Foundation; 1928.

11. Daniel C, Babes A. Posibilitatea diagnosticului cancerului uterin cu ajutorul frotiului. Proceedings of the Bucharest Gynaecological Society. 23-1-1927.

12. Babes A. Diagnostic du cancer du col uterin par les frottis. Presse Medicale. 1928;36:451–4.

13. Naylor B, Tasca L, Bartziota E, Schneider V. In Romania it's the Methode Babes-Papanicolaou. Acta Cytol. 2002;46(1):1–12.
[PubMed]

14. Viana O. La diagnosi precoce del cancro uterino mediante lo striscio [The early diagnosis of uterine cancer by smears]. La Clinica Ostetrica. 1928;30:781–93.

15. Viana O. The early diagnosis of uterine cancer by smears. Acta Cytol. 1970;14(8):544–9.
[PubMed]

16. Papanicolaou GN. A new procedure for staining vaginal smears. Science. 1942;95(2469):438–9.
[PubMed]

17. Koprowska I, George N. Papanicolaou – as we knew him. Acta Cytol. 1977;21(5):630–8.
[PubMed]

18. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. New York: The Commonwealth Fund; 1943.

19. Ayre JE. A simple office test for uterine cancer diagnosis. Can Med Assoc J. 1944;51(1):17–22.
[PubMed][PubMedCentral]

20. Ayre JE. Cervical cytology in diagnosis of early cancer. JAMA. 1948;136(8):513–7.

21. Foote FW, LI K. Smear diagnosis of in situ carcinoma of the cervix. Am J Obstet Gynecol. 1948;56(2):335–9.

[PubMed]

22. Pund ER, Nieburgs HE. Preinvasive carcinoma of the cervix uteri; seven cases in which it was detected by examination of routine endocervical smears. Arch Pathol (Chic). 1947;44(6):571–7.

23. Pund ER, Nettles JB. Preinvasive and invasive carcinoma of cervix uteri; pathogenesis, detection, differential diagnosis, and the pathologic basis for management. Am J Obstet Gynecol. 1948;55(5):831–7.
[PubMed]

24. Ayre JE. Selective cytology smear for diagnosis of cancer. Am J Obstet Gynecol. 1947;53(4):609–17.
[PubMed]

25. Papanicolaou GN. Atlas of exfoliative cytology. Cambridge: The Commonwealth Fund by Harvard University Press; 1954.

26. Christopherson WM, Lundin Jr FE, Mendez WM, Parker JE. Cervical cancer control: a study of morbidity and mortality trends over a twenty-one-year period. Cancer. 1976;38(3):1357–66.
[PubMed]

27. Christopherson WM, Scott MA. Trends in mortality from uterine cancer in relation to mass screening. Acta Cytol. 1977;21(1):5–9.
[PubMed]

28. Anderson GH, Boyes DA, Benedet JL, et al. Organisation and results of the cervical cytology screening programme in British Columbia, 1955–85. Br Med J (Clin Res Ed). 1988;296(6627):975–8.

29. Hakama M, Louhivuori K. A screening programme for cervical cancer that worked. Cancer Surv. 1988;7(3):403–16.
[PubMed]

30. Macgregor JE, Fraser ME, Mann EM. Improved prognosis for cervical cancers due to comprehensive screening. Acta Cytol. 1972;16(1):14–5.
[PubMed]

31. Macgregor JE. Evaluation of mass screening programmes for cervical cancer in N.E. Scotland. Tumori. 1976;62(3):287–95.
[PubMed]

32. Macgregor JE, Teper S. Mortality from carcinoma of cervix uteri in Britain. Lancet. 1978;2(8093):774–6.
[PubMed]

33. Macgregor JE, Moss SM, Parkin DM, Day NE. A case-control study of cervical cancer screening in north east Scotland. Br Med J (Clin Res Ed). 1985;290(6481):1543–6.

34. Macgregor JE, Moss S, Parkin DM, Day NE. Cervical cancer screening in north-east Scotland. IARC Sci Publ. 1986;76:25–36.

35. Macgregor JE, Campbell MK, Mann EM, Swanson KY. Screening for cervical intraepithelial neoplasia in north east Scotland shows fall in incidence and mortality from invasive cancer with concomitant rise in preinvasive disease. BMJ. 1994;308(6941):1407–11.
[PubMed][PubMedCentral]

36. Macgregor JE, Fraser ME, Mann EM. Improved prognosis of cervical cancer due to comprehensive screening.

Lancet. 1971;1(7689):74–6.
[PubMed]

37. Wachtel E. Screening for cervical cancer. Practitioner. 1973;211(262):137–42.
[PubMed]

38. Kocjan G, Herbert A. Nasseem Husain: homage to a pioneer of cytology automation. Cytopathology. 2015;26(4):211–6.
[PubMed]

39. Wilson JM. Screening for cervical cancer. Mon Bull Minist Health Public Health Lab Serv. 1961;20:214–22.
[PubMed]

40. Wilson JM. Some aspects of the epidemiology of cervical cancer. Mon Bull Minist Health Public Health Lab Serv. 1965;24:72–81.
[PubMed]

41. Williams J. On cancer of the uterus: being the Harveian Lectures for 1886. London: H K Lewis; 1886.

42. Cullen TS. Cancer of the uterus: its pathology, symptomatology, diagnosis, and treatment. New York: Appleton; 1900.

43. Rubin IC. The pathological diagnosis of incipient carcinoma of the cervix. Am J Obstet Gynecol. 1910;62:668–76.

44. Broders AC. Carcinoma in situ contrasted with benign penetrating epithelium. JAMA. 1932;99:1670–4.

45. Wied GL. Editorial. First International Conference of Exfoliative Cytology. Proceedings of the First International Congress of Exfoliative Cytology. Philadelphia: Appleton-Century Crofts.; 1962, p. 297.

46. Ritton G, Christopherson WM. Cytology of the Female Genital Tract. [No 8]. Geneva, World Health Organisation. International Classification of Tumours; 1973.

47. Reagan JW, Hicks DJ. A study of in situ and squamous-cell cancer of the uterine cervix. Cancer. 1953;6(6):1200–14.
[PubMed]

48. Reagan JW, Seidemann IL, Saracusa Y. The cellular morphology of carcinoma in situ and dysplasia or atypical hyperplasia of the uterine cervix. Cancer. 1953;6(2):224–34.
[PubMed]

49. Richart RM. Cervical intraepithelial neoplasia. Pathol Annu. 1973;8:301–28.
[PubMed]

50. Spriggs AI, Butler EB, Evans DMD, et al. Problems of cell nomenclature in cervical cytology smears. J Clin Pathol. 1978;31:1226–7.

51. Evans DM, Hudson EA, Brown CL, et al. Terminology in gynaecological cytopathology: report of the Working Party of the British Society for Clinical Cytology. J Clin Pathol. 1986;39(9):933–44.
[PubMed][PubMedCentral]

52. Borderline nuclear changes in cervical smears: guidelines on their recognition and management. National Coordinating Network (National Cervical Screening Programme), British Society for clinical Cytology, and Royal College of Pathologists' Working Party. J Clin Pathol. 1994;47(6):481–92.

53. The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses. Developed and approved at a National Cancer Institute Workshop, Bethesda, Maryland, U.S.A., December 12–13, 1988. Anal Quant Cytol Histol. 1989;11(5):291–7.

54. Solomon D. The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses: developed and approved at the National Cancer Institute Workshop in Bethesda, Maryland, December 12–13, 1988. Hum Pathol. 1990;21(7):704–8.
[PubMed]

55. The revised Bethesda System for reporting cervical/vaginal cytologic diagnoses: report of the 1991 Bethesda workshop. J Reprod Med. 1992;37(5):383–6.

56. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002;287(16):2114–9.
[PubMed]

57. Smith JH. Bethesda 2001. Cytopathology. 2002;13(1):4–10.
[PubMed]

58. Herbert A, Gray W, Cross P. Terminology of the BSCC, European Community and the Bethesda system: the boundary between low-grade and high-grade cytology. Cytopathology. 2009;20(1):3–4.
[PubMed]

59. Denton KJ, Herbert A, Turnbull LS, et al. The revised BSCC terminology for abnormal cervical cytology. Cytopathology. 2008;19(3):137–57.
[PubMed]

60. Slater DN, Rice S, Stewart R, et al. Proposed Sheffield quantitative criteria in cervical cytology to assist the grading of squamous cell dyskaryosis, as the British Society for Clinical Cytology definitions require amendment. Cytopathology. 2005;16(4):179–92.
[PubMed]

61. Cancer of the cervix: death by incompetence. Lancet 1986;326:363–4.

62. Department of Health and Social Security. HC(88)1. Health Services Management Cervical Cancer Screening. 12-1-1988. London: DHSS.

63. Farmery E, Gray M. Report of the first five years of the NHS cervical screening programme. Oxford: National Co-ordinating Network, Anglia and Oxford Regional Health Authority; 1994.

64. NHS Cancer Screening Programmes. Celebrating 15 years of achievement. NHS Cervical Screening Programme Annual Review 2003. Sheffield: NHS Cancer Screening Programmes; 2003.

65. Herbert A, Johnson J. Achievable standards, benchmarks for reporting and criteria for evaluating cervical cytopathology. 1st ed. Sheffield: NHS Cervical Screening Programme; 1995.

66. Johnson J, Patnick J. Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. Second edition including revised performance indicators. Cytopathology. 2000;11(4):212–41.
[PubMed]

67. Johnson J, Patnick J, editors. Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. Sheffield: NHS Cancer Screening Programmes; 2000.

68. Smith J, Patnick J, editors. Achievable standards, benchmarks for reporting, criteria for evaluating cervical cytopathology. 3rd ed. Sheffield: NHS Cancer Screening Programmes; 2013.

69. Smith JH. ABC3 Part I: a review of the guidelines for terminology, classification and management of cervical cytology in England. Cytopathology. 2012;23(6):353–9.
[PubMed]

70. Blanks RG. ABC3 Part II: a review of the new criteria for evaluating cervical cytology in England. Cytopathology. 2012;23(6):360–70.
[PubMed]

71. Marlow LA, Sangha A, Patnick J, Waller J. The Jade Goody Effect: whose cervical screening decisions were influenced by her story? J Med Screen. 2012;19(4):184–8.
[PubMed]

72. Lancucki L, Sasieni P, Patnick J, Day TJ, Vessey MP. The impact of Jade Goody's diagnosis and death on the NHS Cervical Screening Programme. J Med Screen. 2012;19(2):89–93.
[PubMed][PubMedCentral]

73. National Institute for Clinical Excellence. Guidance on the use of liquid-based cytology for cervical screening. 2000. NICE Technology Appraisal Guidance No5.

74. National Institute for Clinical Excellence. Guidance on the use of liquid based cytology for cervical screening. 2003. Technology Appraisal 69.

75. NHSCSP. Modernising the NHSCSP. NICE appraisal on liquid based cytology published 22 October 2003. Advice to the service. Sheffield: NHSCSP; 2003.

76. National Advisory Group. Steering Group Report on the feasibility of introducing liquid based cytology. Scottish Cervical Screening Programmme; 2002.

77. Luesley D, Leeson S. Colposcopy and programme management. NHSCSP Publication 20. Sheffield: NHSCSP; 2004.

78. Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. Br J Cancer. 2003;89(1):88–93.
[PubMed][PubMedCentral]

79. Rieck GC, Tristram A, Hauke A, Fielder H, Fiander AN. Cervical screening in 20-24-year olds. J Med Screen. 2006;13(2):64–71.
[PubMed]

80. Herbert A, Smith JH. Women under 25 should be offered screening. BMJ. 2007;334(7588):273.
[PubMed][PubMedCentral]

81. Herbert A, Holdsworth G, Kubba AA. Cervical screening: why young women should be encouraged to be screened. J Fam Plann Reprod Health Care. 2008;34(1):21–5.
[PubMed]

82. Fiander AN. Cervical screening in young women aged 20–24 years. J Fam Plann Reprod Health Care. 2008;34(1):19.
[PubMed]

83. Castanon A, Leung VM, Landy R, Lim AW, Sasieni P. Characteristics and screening history of women diagnosed with cervical cancer aged 20–29 years. Br J Cancer. 2013;109(1):35–41.
[PubMed][PubMedCentral]

84. Patnick J, editor. NHSCSP annual review 2006. Sheffield: NHSCSP; 2007.

85. Dowie R, Stoykova B, Crawford D, et al. Liquid-based cytology can improve efficiency of cervical smear readers: evidence from timing surveys in two NHS cytology laboratories. Cytopathology. 2006;17(2):65–72.
[PubMed]

86. Williams AR. Liquid-based cytology and conventional smears compared over two 12-month periods. Cytopathology. 2006;17(2):82–5.
[PubMed]

87. Gregory L, Dudding N, Smith JH. The impact of introducing liquid based cytology into a routine screening laboratory. Cytopathology. 2006;17(supplement 1):24.

88. Beerman H, van Dorst EB, Kuenen-Boumeester V, Hogendoorn PC. Superior performance of liquid-based versus conventional cytology in a population-based cervical cancer screening program. Gynecol Oncol. 2009;112(3):572–6.
[PubMed]

89. Department of Health. Cancer Reform Strategy. 2007.

90. Patnick J, editor. NHSCSP annual review 2007. Sheffield: NHS Cancer Screening Programmes; 2008.

91. NHSCSP Workforce Survey Working Group. A survey of non-medical staff within the cervical screening programme 2002–2005. Sheffield: NHSCSP; 2006.

92. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12–9.
[PubMed]

93. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55(4):244–65.
[PubMed][PubMedCentral]

94. Bosch FX, Burchell AN, Schiffman M, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine. 2008;26(Suppl 10):K1–16.
[PubMed]

95. Gravitt PE, Coutlee F, Iftner T, et al. New technologies in cervical cancer screening. Vaccine. 2008;26(Suppl 10):K42–52.
[PubMed]

96. Cuzick J, Arbyn M, Sankaranarayanan R, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine. 2008;26(Suppl 10):K29–41.
[PubMed]

97. Arbyn M, Roelens J, Simoens C, et al. Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. Cochrane Database Syst Rev. 2013;3:CD008054.

98. Moss S, Gray A, Legood R, et al. Effect of testing for human papillomavirus as a triage during screening for cervical cancer: observational before and after study. BMJ. 2006;332(7533):83–5.

[PubMed][PubMedCentral]

99. Legood R, Gray A, Wolstenholme J, Moss S. Lifetime effects, costs, and cost effectiveness of testing for human papillomavirus to manage low grade cytological abnormalities: results of the NHS pilot studies. BMJ. 2006;332(7533):79–85.
[PubMed][PubMedCentral]

100. Kelly RS, Patnick J, Kitchener HC, Moss SM. HPV testing as a triage for borderline or mild dyskaryosis on cervical cytology: results from the sentinel sites study. Br J Cancer. 2011;105(7):983–8.
[PubMed][PubMedCentral]

101. Smith JH. The future of cervical screening in the UK. Diagn Histopathol. 2009;15(7):330–4.

102. Chan BK, Melnikow J, Slee CA, Arellanes R, Sawaya GF. Posttreatment human papillomavirus testing for recurrent cervical intraepithelial neoplasia: a systematic review. Am J Obstet Gynecol. 2009;200(4):422–9.
[PubMed][PubMedCentral]

103. Kitchener HC, Walker PG, Nelson L, et al. HPV testing as an adjunct to cytology in the follow up of women treated for cervical intraepithelial neoplasia. BJOG. 2008;115(8):1001–7.
[PubMed]

104. Husain OA. The history of automated cell scanners. In: Grohs HK, Husain OA, editors. Automated cervical cancer screening. New York: Igaku-Shoin; 1994. p. 3–14.

105. Biscotti CV, Dawson AE, Dziura B, et al. Assisted primary screening using the automated ThinPrep Imaging System. Am J Clin Pathol. 2005;123(2):281–7.
[PubMed]

106. Wilbur DC, Black-Schaffer WS, Luff RD, et al. The Becton Dickinson FocalPoint GS Imaging System: clinical trials demonstrate significantly improved sensitivity for the detection of important cervical lesions. Am J Clin Pathol. 2009;132(5):767–75.
[PubMed]

107. Broadstock M. Effectiveness and cost effectiveness of automated and semi-automated cervical screening devices: a systematic review of the literature. N Z Med J. 2001;114(1135):311–3.
[PubMed]

108. Willis PH, Barton P, Pearmain P, Bryan S, Hyde C. Cervical screening programmes: can automation help? Evidence from systematic reviews, an economic analysis and a simulation modelling exercise applied to the UK. Health Technol Assess. 2005;9:1–207.
[PubMed]

109. Kitchener HC, Blanks R, Dunn G, et al. Automation-assisted versus manual reading of cervical cytology (MAVARIC): a randomised controlled trial. Lancet Oncol. 2011;12(1):56–64.
[PubMed]

110. Anttila A, Pokhrel A, Kotaniemi-Talonen L, et al. Cervical cancer patterns with automation-assisted and conventional cytological screening: a randomized study. Int J Cancer. 2011;128(5):1204–12.
[PubMed]

111. Kitchener HC, Blanks R, Cubie H, et al. MAVARIC – a comparison of automation-assisted and manual cervical screening: a randomised controlled trial. Health Technol Assess. 2011;15(3):iii–xi, 1.

112. Public Health England. Human Papillomavirus (HPV) vaccination coverage in adolescent females in England: 2014/15. 1-12-2015. PHE. 8-8-2016.

113. Joura EA, Giuliano AR, Iversen OE, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015;372(8):711–23.
[PubMed]

114. Gardasil 9: new HPV vaccine approved in the European Union. 2015. 8-8-2016.

115. Franco EL, Cuzick J, Hildesheim A, de Sanjose S. Chapter 20: issues in planning cervical cancer screening in the era of HPV vaccination. Vaccine. 2006;24(Suppl 3):S171–7.

116. Brotherton JM, Fridman M, May CL, et al. Early effect of the HPV vaccination programme on cervical abnormalities in Victoria, Australia: an ecological study. Lancet. 2011;377(9783):2085–92.
[PubMed]

117. Pollock KG, Kavanagh K, Potts A, et al. Reduction of low- and high-grade cervical abnormalities associated with high uptake of the HPV bivalent vaccine in Scotland. Br J Cancer. 2014;111(9):1824–30.
[PubMed][PubMedCentral]

118. Palmer TJ, McFadden M, Pollock KG, et al. HPV immunisation and cervical screening–confirmation of changed performance of cytology as a screening test in immunised women: a retrospective population-based cohort study. Br J Cancer. 2016;114(5):582–9.
[PubMed][PubMedCentral]

119. Arbyn M, Anttila A, Jordan J, et al. European guidelines for quality assurance in cervical cancer screening. Second edition – summary document. Ann. Oncologia. 2010;21(3):448–58.

120. Wright Jr TC, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with abnormal cervical screening tests. J Low Genit Tract Dis. 2007;11(4):201–22.
[PubMed]

121. Naucler P, Ryd W, Tornberg S, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst. 2009;101(2):88–99.
[PubMed]

122. Cox JT, Castle PE, Behrens CM, et al. Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. Am J Obstet Gynecol. 2013;208(3):184.
[PubMed]

123. Denton KJ, Bergeron C, Klement P, et al. The sensitivity and specificity of p16(INK4a) cytology vs HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL pap cytology results. Am J Clin Pathol. 2010;134(1):12–21.
[PubMed]

124. Tambouret RH. Use of immunohistochemical staining for p16 in gynecological cytology. Cancer Cytopathol. 2016;124(9):611–2.
[PubMed]

125. Petry KU, Schmidt D, Scherbring S, et al. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. Gynecol Oncol. 2011;121(3):505–9.
[PubMed]

126. Ikenberg H, Bergeron C, Schmidt D, et al. Screening for cervical cancer precursors with p16/Ki-67 dual-stained cytology: results of the PALMS study. J Natl Cancer Inst. 2013;105(20):1550–7.
[PubMed][PubMedCentral]

127. Wentzensen N, Fetterman B, Castle PE, et al. p16/Ki-67 dual stain cytology for detection of cervical precancer in HPV-positive women. J Natl Cancer Inst. 2015;107(12):djv257.
[PubMed][PubMedCentral]

128. Hesselink AT, Heideman DA, Steenbergen RD, et al. Combined promoter methylation analysis of CADM1 and MAL: an objective triage tool for high-risk human papillomavirus DNA-positive women. Clin Cancer Res. 2011;17(8):2459–65.
[PubMed]

129. De Strooper LM, van Zummeren M, Steenbergen RD, et al. CADM1, MAL and miR124-2 methylation analysis in cervical scrapes to detect cervical and endometrial cancer. J Clin Pathol. 2014;67(12):1067–71.
[PubMed]

130. De Strooper LM, Meijer CJ, Berkhof J, et al. Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. Cancer Prev Res (Phila). 2014;7(12):1251–7.

131. Verhoef VM, van Kemenade FJ, Rozendaal L, et al. Follow-up of high-risk HPV positive women by combined cytology and bi-marker CADM1/MAL methylation analysis on cervical scrapes. Gynecol Oncol. 2015;137(1):55–9.
[PubMed]

132. Verhoef VM, Heideman DA, van Kemenade FJ, et al. Methylation marker analysis and HPV16/18 genotyping in high-risk HPV positive self-sampled specimens to identify women with high grade CIN or cervical cancer. Gynecol Oncol. 2014;135(1):58–63.
[PubMed]

133. De Strooper LM, Verhoef VM, Berkhof J, et al. Validation of the FAM19A4/mir124-2 DNA methylation test for both lavage- and brush-based self-samples to detect cervical (pre)cancer in HPV-positive women. Gynecol Oncol. 2016;141(2):341–7.
[PubMed][PubMedCentral]

134. Gravitt PE, Belinson JL, Salmeron J, Shah KV. Looking ahead: a case for human papillomavirus testing of self-sampled vaginal specimens as a cervical cancer screening strategy. Int J Cancer. 2011;129(3):517–27.
[PubMed][PubMedCentral]

135. Gravitt PE, Rositch AF. HPV self-testing and cervical cancer screening coverage. Lancet Oncol. 2014;15(2):128–9.
[PubMed]

136. Gok M, van Kemenade FJ, Heideman DA, et al. Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program. Int J Cancer. 2012;130(5):1128–35.
[PubMed]

137. Cervical Screening Programme: England, Statistics for 2014–15. http://www.hscic.gov.uk/pubs/cervical1415 . 2016. 8-8-2016.

138. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical

cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32.
[PubMed]

139. Kitchener HC, Almonte M, Gilham C, et al. ARTISTIC: a randomised trial of human papillomavirus (HPV) testing in primary cervical screening. Health Technol Assess. 2009;13(51):1–iv.
[PubMed]

140. Kitchener HC, Gilham C, Sargent A, et al. A comparison of HPV DNA testing and liquid based cytology over three rounds of primary cervical screening: extended follow up in the ARTISTIC trial. Eur J Cancer. 2011;47(6):864–71.
[PubMed]

141. Kitchener HC, Canfell K, Gilham C. The clinical effectiveness and cost-effectiveness of primary human papillomavirus cervical screening in England: extended follow-up of the ARTISTIC randomised trial cohort through three screening rounds. Health Technol Assess. 2014;18(23):1–196.

142. Herbert A, Johnson J, Patnick J. Achievable standards, benchmarks for reporting and criteria for evaluating cervical cytopathology. Cytopathology. 1995;6:301–3.
[PubMed]

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4. Surgical and Nonsurgical Management of Cervical Cancer: Current Practice and Future Directions

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Abstract
Cervical cancer is the second most common female cancer worldwide causing approximately 250,000 deaths each year. The standard of care for early-stage disease (FIGO IA and IB1) remains surgery. For more advanced disease, chemoradiotherapy is the treatment of choice. The challenges in treating cervical cancer are centered on improving survival and local control and minimizing treatment-related toxicity. This chapter discusses, stage by stage, the surgical and nonsurgical management of cervical cancer.

Keywords Radical hysterectomy – Trachelectomy – Chemoradiotherapy – Brachytherapy

Introduction

The principles of treating cervical cancer are to eliminate the primary tumor, assess for spread of the cancer, and treat appropriately. In the earliest stages of the disease, surgery alone suffices. Where there is spread to regional pelvic or para-aortic lymph

nodes, irradiation with concurrent chemotherapy is required. The presence of distant metastases requires a palliative approach and may include both radiation and chemotherapy depending on symptoms. This chapter will order the treatment in terms of stage using the internationally recognized FIGO system. Chapter 7 explains further the method of staging in early cervical carcinomas using measurements of the invasive lesion. This chapter will describe treatment of the standard cervical neoplasms of squamous cell carcinoma and adenocarcinoma, the pathology of which is described in Chaps. 7 and 8 respectively.

Stage IA Carcinoma of the Cervix

Stage IA1: Invasive Lesion ≤7 mm Wide and ≤3 mm Deep

Treatment of stage IA1 carcinoma of the cervix is local excision. The reasoning behind this is that the volume of tumor is sufficiently small that the risk of spread to the parametrial or cervical lymph nodes is less than 1% and thus no assessment for spread is required [1].

If the lesion has been diagnosed on a loop diathermy excision, the assessment of the tumor in a multidisciplinary meeting along with the pathologist is used to check that the margins are clear. If this is the case, no further treatment is required as the risk of residual disease is approximately 3% [2]. The small number of women with residual disease should be detected with follow-up. Follow-up is in the form of colposcopic examination and cervical smear tests. This would initially be on a three monthly basis. The length of follow-up can be as short as 3 years as the risk of recurrence is extremely low. If the margins of the excision are close or positive, then repeat excision is required [2].

If the patient has completed her family and no longer requires her fertility, a hysterectomy should be discussed, particularly if follow-up is difficult due to cervical stenosis. If the cervical canal is accessible, consideration is required of the risks of hysterectomy versus the extremely small risks of any recurrent disease.

Stage IA1 Plus Lymphovascular Space Invasion

The incidence of lymphovascular space invasion in very early cervical cancer may be as high as 15% [1]. Its significance is difficult to judge. The correlation between patients having metastatic disease in the lymph nodes and those having lymphovascular space invasion has been noted for many years [3]. However the positive predictive power of lymphovascular space invasion to detect which patients have lymph node metastasis is limited. Thus, a common practice is to assess the extent of lymphovascular space invasion in the specimen. If MDT evaluation confirms that more than two foci are present, then removal of the pelvic lymph nodes is required. This is typically in the form

of a laparoscopic pelvic lymph node dissection which would be added to the loop/cone biopsy, trachelectomy, or simple hysterectomy.

Stage IA2: Width ≤ 7 mm and Depth > 3 mm But ≤5 mm

The treatment of the central lesion is the same as for stage IA1. A cone biopsy or simple hysterectomy is acceptable. However, the risk of lymph node metastasis increases from less than 1% for stage IA1 to 3–6% for stage IA2 disease [4]. Thus, the pelvic lymph nodes should be removed by laparoscopic pelvic lymph node dissection.

The parametrium does not require assessment as the risk of parametrial involvement in a tumor of less than 1 cm diameter is 0.6% [5].

As for stage IA1, if fertility is not required, then a simple hysterectomy would be advised as the treatment.

Adenocarcinoma Versus Squamous Cell Carcinoma in Early Cervical Cancer

In the past it has been felt that adenocarcinoma should be treated differently from squamous cell carcinoma. The reasons for this were as follows: (i) the depth of the lesion is difficult to determine microscopically; (ii) skip lesions may be present; (iii) there was a lack of data for adenocarcinoma in comparison to squamous cell carcinoma; and (iv) follow-up was difficult for adenocarcinoma as colposcopy and cervical smears are unreliable.

More recent data have shown that these concerns are not enough to change management compared to squamous lesions. The rate of nodal metastasis and parametrial invasion for small lesions has been shown to be the same for adenocarcinoma and squamous cell carcinoma. A meta-analysis by Smith in 2002 showed high survival rate, low nodal metastatic rate, low recurrence rate, and low death rate in early adenocarcinoma [6].

In conclusion, cervical adenocarcinoma is treated as squamous cell carcinoma as there is no increased risk of lymph node metastasis and no increased risk of vault recurrence. Loop/cone biopsy is acceptable in women requiring fertility. However, follow-up is more difficult as the endocervix is not accessible, and smears do not detect glandular abnormalities effectively. In view of this, a hysterectomy is advised for women who no longer require their fertility.

Stage IB1 Carcinoma of the Cervix

Stage IB1: Depth >5 mm, Or Width >7 mm, But Tumour Less

Than 4 cm Diameter; or a Clinically Visible Lesion Confined to the Cervix

The measurements for stage IB1 carcinoma of the cervix describe a wide range of tumors from microscopic lesions to a macroscopic tumor 4 cm in diameter. Thus, the treatment is not uniform across this stage. This chapter divides the tumors into those with a diameter less than 1 cm, those with diameter 1–2 cm, tumors over 2 cm with low risk of metastatic disease, and finally tumors with a high risk of nodal metastatic disease.

The dimensions of the tumor are measured by magnetic resonance imaging (MRI). The use of examination under anesthetic with sigmoidoscopy and cystoscopy is still common, but MRI is more sensitive for assessing parametrial spread and more accurate for measuring the size of the tumor [7].

Stage IB1: Diameter Less Than 1 cm

These tumors are small with limited risk of spread. The risk of parametrial involvement in a tumor less than 1 cm is 0.6% [5]. The risk of spread to the pelvic lymph nodes is in the order of 15–20% for the whole stage IB1 [8]. For this low-risk group, it is likely to be in the range of 5–10%. Thus treatment for a woman requiring fertility can be resection of the central tumor with a loop/cone biopsy or simple trachelectomy plus laparoscopic lymph node dissection. A series of studies in this category show low recurrence rates and high fertility rates [9].

For women not requiring fertility, a type A radical hysterectomy (extrafascial hysterectomy) plus laparoscopic lymph node dissection is advised.

Stage IB1: 1–2 cm Diameter Tumor

Surgical treatment is advised for this tumor stage as the risk of metastatic disease to the lymph nodes is still low. If there is no metastatic disease, patients can be successfully treated with surgery as a single treatment modality limiting the extent of side effects of the treatment. The extent of the parametrial resection is being questioned. Traditionally, this size of tumor would have warranted at least some parametrial resection. More recently, it has been shown that tumors with no lymphovascular space invasion and a negative sentinel node have a very low parametrial involvement rate. In view of these factors, it has been proposed that parametrial resection is no longer required in tumors less than 2 cm. The SHAPE trial has been started in order to prospectively assess this issue in a randomized trial.

Until the results of SHAPE are known, the standard recommendation for treatment of a stage IB1 carcinoma with diameter 1–2 cm is surgical resection, including the parametrium, plus dissection of the pelvic lymph nodes. For women requiring fertility,

this can be in the form of a radical trachelectomy plus laparoscopic pelvic lymph node dissection. For women not requiring fertility, a type B radical hysterectomy plus laparoscopic pelvic lymph node dissection is recommended [10, 11]. The hysterectomy can be performed laparoscopically, with robotic assistance, vaginally or at open surgery.

Trachelectomy

The modern-day trachelectomy procedure was proposed by Daniel Dargent in 1992. It was originally proposed as a vaginal procedure, but the popularity of the abdominal/laparoscopic approach has increased. It is felt that the abdominal approach can provide a larger resection of the parametrium and thus potentially treat bigger tumors. However, there are fewer data on fertility from the abdominal approach. Case control studies show a similar oncological outcome to radical hysterectomy with recurrence rates of approximately 1–5% at 4 years [12].

875 vaginal procedures have been recorded with a total number of pregnancies of 382, 15% severe prematurity rate, 220 live births, recurrence rate 4%, and death rate 2%. Transabdominal procedures number 207 with 35 total pregnancies, 33% severe prematurity rate, 21 live births, 4% recurrence rate, and 2% death rate [13].

Other nonstandard uses of trachelectomy have been described in case reports. Trachelectomy in pregnancy has been reported and trachelectomy following neoadjuvant chemotherapy in patients with large higher-risk tumors [14, 15]. It should be noted that previous studies have shown that case selection is vital in the use of trachelectomy. The selection criteria have been for tumors with no lymphovascular space invasion, not grade 3 and not greater than 2 cm. In tumors less than 2 cm, the recurrence rate is reported at 1.2%. In tumors greater than 2 cm, the recurrence rate is 21% [16].

Stage IB1: 2–4 cm in Diameter

Surgical treatment is advised for this category of tumor, but consideration must be taken of the risk of postoperative adjuvant treatment being required. The aim of the pretreatment assessment is to ensure a single modality of treatment is used in order to minimize the posttreatment side effects.

Stage IB2 - IV Carcinoma of the Cervix

Non-surgical treatment is advised for larger (>4cm) tumors confined to the cervix, and for tumors that have spread beyond the cervix. This is discussed later in the chapter.

Reducing the Risks of Treatment

The risks of surgical treatment are lymphedema, reduced bladder emptying, vaginal shortening, altered bowel function, and early surgical complications such as hemorrhage, fistulae, or death. The risks from radiation are premature menopause with loss of fertility, increase in bowel frequency, altered bladder function, vaginal stenosis, lymphedema, and a small long-term risk of fistulae. Complications of either surgery or radiation alone can be minimized by meticulous technique, but some effects are significantly more severe and very difficult to avoid when both surgery and radiation are used. Vaginal stenosis and lymphedema are extremely common after combination treatments. These complications may be particularly debilitating in younger women.

Strategies can be used to reduce the risk of combination treatments and are listed below:

Strategy 1: Assess the Risk of Recurrence Prior to Undertaking Surgical Treatment

Delgado (1990) described a classification of tumors that estimated the risk of recurrence and thus the need for adjuvant radiation [17]. The paper describes surgical treatment for tumors where the operation has achieved clear margins and the lymph nodes showed no metastatic disease. As the tumor increases in risk, the chance of recurrence is increased, and thus the rationale for adjuvant radiation increases. The paper indicates that a tumor that is 4 cm in width, invades more than half the depth of the cervix and has lymphovascular space invasion requires adjuvant radiation despite a good surgical result due to a 30% risk of recurrence with surgery alone. This assessment can be performed prior to surgery to estimate the likely need for adjuvant treatment. In summary, surgery would be advised for tumors that are less than 4 cm in width, involve less than half the depth of the cervix, and do not have lymphovascular space invasion.

The survival for surgery versus radiation is similar [18] and discussed in more detail below. Since chemoradiation provides effective treatment for the central tumor as well as treating the surrounding lymph node regions, the rationale for its use in higher-risk tumors as primary treatment to avoid surgery is strong. Careful attention to radiological, clinical, and pathological findings enables the selection of such high-risk cases to be treated with chemoradiation.

Strategy 2: Lymph Node Assessment

The lymph nodes can be assessed surgically as the initial step in the treatment. If the lymph nodes are clear, then only 2% of patients will have parametrial metastatic disease [19]. Clear pelvic lymph nodes mean that chemoradiation should not be required as adjuvant treatment post-surgery.

Initial assessment of the pelvic lymph nodes can be in the form of a sentinel node procedure as opposed to a full pelvic lymph node dissection—see below.

Strategy 3: Radical Surgical Techniques

Centers that have moved further toward surgical treatments have advocated radical surgical resections using the embryonal compartments of the pelvis as a guide to the surgical procedure. Proponents of these radical surgical techniques stipulate that large tumors can be treated with surgery alone and do not require adjuvant radiation [20].

Future Directions in Surgical Treatments

Sentinel Node Surgery

Sentinel node surgery is an established technique in the treatment of breast cancer and is widely practiced in vulval cancer. Its use in cervical and endometrial cancer is less established, but there are numerous publications on the procedure. It would be fair to say that at present it is not accepted as standard practice, but as the number of publications showing the effectiveness increases, it is being adopted more widely in cervical cancer surgery.

Studies have shown that sentinel node technique is more sensitive in detecting metastatic disease to the pelvis than a standard pelvic lymph node dissection [21]. This may be because the pathologist's attention is guided toward the most relevant lymph nodes and an increased detection rate may be due to ultra-sectioning of a sentinel lymph node.

Algorithms, including sentinel node procedures, have been shown to be effective in case series, and the adoption of this technique is likely to increase with time [22, 23] .

Neoadjuvant Chemotherapy

The role of neoadjuvant chemotherapy before surgery has and continues to be much discussed. The use of neoadjuvant chemotherapy prior to radical trachelectomy in women requiring preservation of fertility has already been mentioned [14]. Six randomized controlled studies including 1078 women were evaluated as part of a Cochrane review of chemotherapy before hysterectomy [24]. In all of these studies, patients were allocated to receive a cisplatin-based chemotherapy regimen prior to surgery. There was a benefit for both progression-free and overall survival. However, three of the six studies were stopped early: one because the overall survival in the neoadjuvant chemotherapy arm was inferior to the surgical arm, one because there was a statistically significant benefit to the chemotherapy arm, and one because of the widespread use of additional radiotherapy. Additionally, although the use of

radiotherapy was balanced between the treatment groups, there was considerable variation between trials which may partly explain the differing results between the studies. For example, in one study, every patient received radiotherapy, while in the others, the proportion varied between 36% and 61%.

Overall, based on the available data, it appears that there may be a benefit for preoperative chemotherapy, but given the small number of studies and variation in the use of radiotherapy, further randomized controlled trials are needed before it should be routinely used.

Surgical Treatment of Locally Advanced and Recurrent Cervical Cancer

Central pelvic recurrent disease following chemoradiation has been treated surgically by exenteration for many years. This radical surgery can achieve cure in up to 60% of patients who have the optimal indications such as longer disease-free interval and smaller tumors [25, 26]. More recently, surgery has also been advocated for pelvic side wall recurrence. This is advocated for patients with or without prior radiation treatment. Encouraging survival statistics again up to 60% have been described [27].

Nonsurgical Management

Chemoradiotherapy

The simplicity of a single procedure with the advantage of preservation of ovarian function in younger women means that surgery remains the gold standard treatment for early disease. The most widely quoted data to support this come from a paper published in 1997 [18]. This randomly assigned 327 patients with stage IB or IIA disease to either radical hysterectomy or radical radiotherapy. Concomitant chemotherapy was not given as this study predates the National Cancer Institute (NCI) alert of 1999 recommending concurrent cisplatin chemoradiation. Overall survival and disease-free survival were equivalent, but it is noteworthy that two thirds of women in the surgical arm also received radiotherapy for what were considered to be adverse pathological features. Toxicity in the surgical group at 28% was more than double that seen in the radiotherapy arm, affirming that the combination of surgery and radiotherapy is more morbid than either alone and should be avoided.

For stage IB2 tumors, the incidence of microscopic lymph node metastasis is about 30%. If a surgical approach is chosen, there is a high probability of requiring additional pelvic chemoradiotherapy, and, with the increased morbidity of combined treatment, surgery is not recommended for tumors of stage IB2 [28]. Because of this, for women with FIGO IB2 disease and higher, chemoradiotherapy is the treatment of choice. The

addition of chemotherapy followed an unprecedented alert issued by the National Cancer Institute after the simultaneous publication of five studies recommending that cervical cancer be treated with concomitant chemoradiotherapy rather than radiotherapy alone.

Two subsequent meta-analyses of randomized trials confirmed improved overall survival and local control rates when concurrent cisplatin-containing chemotherapy is added to radiotherapy in the treatment of cervical cancer. The Cochrane Collaboration meta-analysis [29] included data from 19 trials, comprising 4580 patients, 12 of which used platinum-based chemotherapy. This showed a highly significant survival benefit with concomitant chemoradiation (hazard ratio = 0.71, $P < 0.00001$), which represented a 12% absolute benefit in survival. The addition of chemotherapy was shown, however, to increase acute hematological and gastrointestinal toxicity.

The second Canadian meta-analysis [30], based on eight randomized trials, exclusively examined platinum-based chemoradiation and estimated the survival benefit as 11%. Important issues were highlighted by these reviews that could have led to an overestimate of the benefit. For instance, many studies used different radiotherapy treatments in their standard arm, and many trials had a different definition of outcome. In order to overcome these issues, an updated analysis using individual patient data was carried out and published in 2010 [31].

This latest meta-analysis of 13 studies with 3128 women confirms the benefit of additional chemotherapy with an absolute benefit in survival of 6% and disease-free survival of 8%. It is interesting that the advantage of chemotherapy is not as great as had initially been thought and, of note, non-platinum-containing regimens, in particular those containing 5-fluorouracil (5FU) and/or mitomycin, showed similar benefit. This suggests that, for those patients, for example, with renal impairment, who are unable to receive platinum, alternative effective drugs could be considered.

Radiation

Radiotherapy has been an essential weapon in the armory of curative treatment for cervical cancer for over a century, and the combination of external beam together with intracavitary brachytherapy was, as early as the 1940s, showing 5-year survival figures of almost 50% [32] (Fig. 4.1).

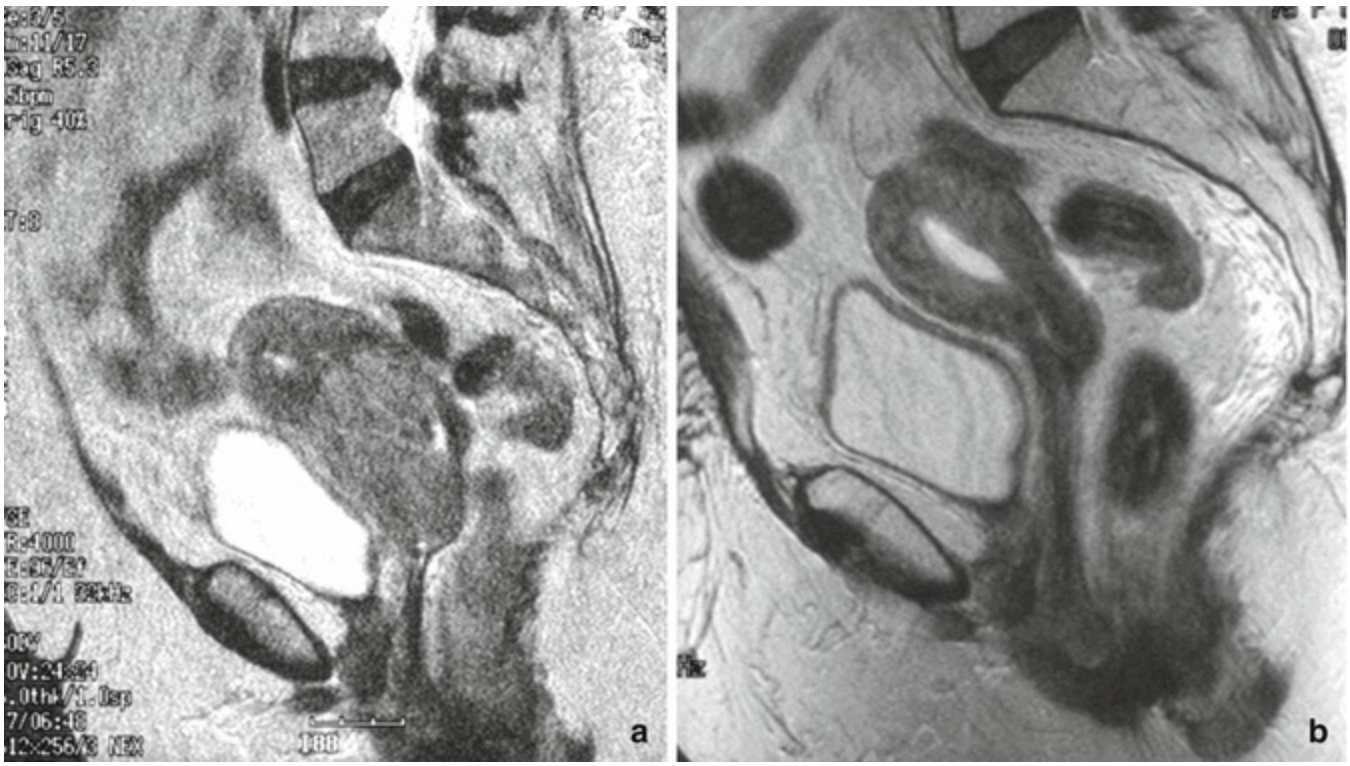

Fig. 4.1 MRI of pelvis showing a stage IIB cervical cancer (**a**) before and (**b**) 3 months after completion of radiotherapy

External beam radiotherapy routinely involves treating the primary tumor, sites of both actual and potential local spread to include the uterus and parametria, and pelvic lymph nodes. Extending radiation fields to encompass the para-aortic nodes is only considered where there is already pathological or radiological evidence of spread to that region or the common iliac chain.

The integration of CT imaging into the radiotherapy planning process has allowed the dose of radiation to match or conform to the outline of the target. This shaping of the radiation fields is known as conformal radiotherapy and would now be considered best practice when treating the pelvis. Intensity-modulated radiotherapy (IMRT) and volumetric arc therapy (VMAT) are extensions of this principle. By varying the intensity of the radiation beam, more accurate shaping, even around concavities, is possible. This means the high-dose area fits more precisely to the target volume, producing a concave shape both at the posterior aspect of the planning target volume, reducing dose to the rectum, and also anteriorly, curving around the lateral lymph node target volume while sparing more of the central bladder and bowel (Fig. 4.2).

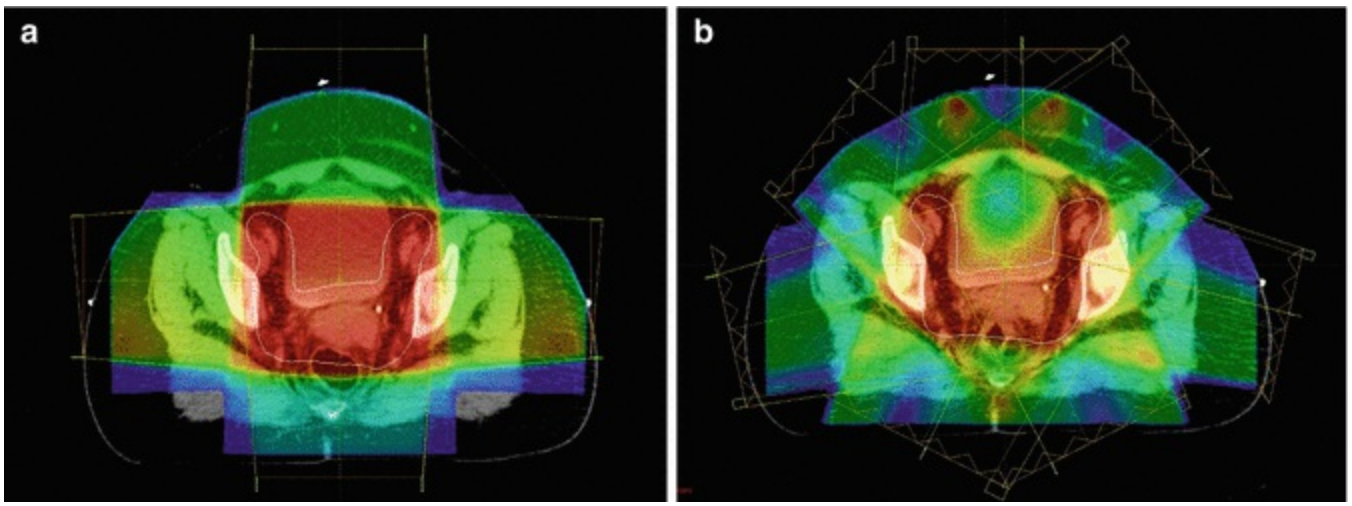

Fig. 4.2 Comparison of the distribution of radiation dose with conformal radiotherapy (**a**) versus intensity-modulated radiotherapy (**b**). The white line denotes the lymph node target. *Red* denotes maximal radiation dose. Color change from *yellow to green to blue* indicates decreasing dose. In Image **b** the high-dose region closely follows the target (*white outline*) sparing the centrally placed bladder from the higher dose

Radiotherapy planning studies have shown that radiation dose to sensitive structures such as the bladder and rectum, which lie in close proximity to the cervix, can be reduced with these techniques. This translates to a reduction in clinically significant acute and late toxicity by between 30% and 50% [33]. After further follow-up, late toxicity was reduced from 50% to 11% [34].

IMRT also allows allocation of different dose targets to discrete areas within the target volume, as well as enabling delivery of a boost to all sites of bulk disease. This may improve the therapeutic ratio by delivering a higher total dose to all of the macroscopic disease while maintaining standard doses to the areas of potential microscopic spread.

Neoadjuvant Chemotherapy and Radiotherapy

The issue of neoadjuvant chemotherapy prior to radiotherapy for cervical cancer remains unproven. Theoretical benefits of neoadjuvant chemotherapy include the eradication of micrometastases and reduction in tumor size prior to definitive treatment. A Cochrane meta-analysis published in 2004 by Tierney evaluated 2074 patients from 18 studies that compared neoadjuvant chemotherapy before radiotherapy with radiotherapy alone [35]. Combining all of the trials together showed no evidence of benefit for neoadjuvant chemotherapy, but there was a high level of statistical heterogeneity. Results were then reanalyzed according to how chemotherapy was delivered, and this showed a trend toward a survival advantage for more intense chemotherapy given either in cycles of less than 14 days or using cisplatin doses of greater than 25 mg/m^2. Giving cisplatin at lower doses or over longer intervals

appeared to be detrimental to outcome.

Interest in neoadjuvant chemotherapy prior to chemoradiotherapy has now been rekindled using dose-dense regimens. One such is the combination of carboplatin and paclitaxel given weekly for 6 weeks prior to standard cisplatin chemoradiotherapy which has shown a high response rate and good tolerability [36]. A phase 3 randomized controlled trial "INTERLACE" is currently underway to evaluate this approach.

Adjuvant Chemotherapy and Radiotherapy

An alternative, widely used approach in other tumor sites is to consider additional chemotherapy following definitive treatment with the aim of improving survival. This has so far been less successful in cervical cancer. There were encouraging results from a study of 515 patients randomized either to concurrent gemcitabine plus cisplatin and radiation followed by adjuvant gemcitabine and cisplatin or standard concurrent cisplatin chemoradiotherapy in patients with stage IIB to IVA carcinoma of the cervix [36]. Progression-free survival at 3 years was 74% in the experimental arm compared to 65% in the control group ($P = 0.029$). Overall survival and time to progressive disease were also significantly improved. But with grade 3/4 toxicity in the experimental group almost double that seen in the control group (87% versus 46%), and two treatment-related deaths, this novel regimen is unlikely to be widely used without modification and further evaluation.

The outback trial is an international randomized phase 3 trial of adjuvant carboplatin and paclitaxel following standard primary radical chemoradiotherapy underway. With an estimated 800 patients to be entered, it should help define the role of additional chemotherapy (https://clinicaltrials.gov/ct2/show/NCT01414608).

After surgery for early cervical cancer, certain histopathological features increase the risk of recurrence and reduce progression-free survival. These include positive pelvic lymph nodes, lymphovascular space invasion, parametrial involvement, positive margins, and tumor size of greater than 4 cm. With one or more of these features, the 5-year survival drops to between 50% and 70% [37]. It is well recognized that combining surgery and radiotherapy increases the acute and late morbidities associated with both modalities. A 2009 Cochrane review considered the role of adjuvant radiotherapy following surgery in early cervical cancer [38]. Only two trials (397 women) fulfilled the criteria for evaluation. They showed that while adjuvant radiotherapy reduced the risk of local recurrence by between 40% and 90%, it did not confer a statistically significant survival benefit. This highlights the importance of carefully selecting the initial primary treatment for an individual patient and of assessing the risks and benefits for each patient before considering the role of adjuvant radiotherapy.

Management of Recurrence and Metastatic Disease

For women with relapse after radiation, provided relapsed disease is confined to the central pelvis, the only potentially curative option is pelvic exenteration. However, this procedure carries with it a high morbidity and mortality, with less than 50% of patients surviving 5 years [39].

The prognosis for patients who develop recurrent disease and cannot be offered radical treatment of either salvage surgery or chemoradiotherapy remains poor, in the order of 6 months to 2 years. The only options are palliative chemotherapy or best supportive care.

There have been no randomized trials comparing chemotherapy to best supportive care in advanced cervical carcinoma. Cisplatin has been used for nearly three decades to treat recurrent and metastatic cancer and remains the mainstay of treatment. However, the short survival and low response rates to available treatments warrant innovative approaches.

Long et al. in GOG-0179, a randomized phase 3 trial, demonstrated for the first time a statistically significant survival advantage for combination chemotherapy over single-agent cisplatin in advanced cervical cancer [40]. Cisplatin was compared to cisplatin + topotecan and a methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) arm. The MVAC arm was closed due to four treatment-related deaths among 63 patients and was not included in the final analysis. Of the remaining patients, 146 received cisplatin and 147 cisplatin and topotecan. This trial demonstrated improved progression-free survival (PFS) and overall survival (OS) when compared with single-agent therapy, with median OS of 9.4 and 6.5 months ($P = 0.017$) and PFS of 4.6 and 2.9 months ($P = 0.014$).

The GOG then instituted a multi-arm trial in 2003 with four different platinum-based intravenous doublets containing topotecan, paclitaxel, vinorelbine, or gemcitabine. None of the experimental regimens, however, were found to be superior to the control arm of cisplatin plus paclitaxel [41].

The disappointing results with conventional chemotherapy agents have led to the incorporation of targeted agents with standard chemotherapy regimens. The American study GOG 240 compared the overall survival of 450 patients with stage IVB, recurrent, or persistent carcinoma of the cervix treated with paclitaxel in combination with cisplatin or topotecan with or without the vascular endothelial growth factor inhibitor bevacizumab. This has shown a statistically significant overall survival benefit with the addition of bevacizumab of 3.7 months (13.3 versus 17 months) [42].

Other agents have been looked at in phase 2 trials and show promise. For example, cediranib is an inhibitor of all three vascular endothelial growth factor receptor (VEGF-1, VEGF-2, VEGF-3) tyrosine kinases, thereby blocking VEGF signaling and angiogenesis. In a double-blind phase 2 study, the addition of cediranib to standard carboplatin and paclitaxel chemotherapy improved progression-free survival (8.1 versus 6.7 months) [43]. Although promising, this drug is not being pursued by the

pharmaceutical industry.

Conclusion Cervical cancer remains a significant worldwide problem. The better outcome seen with the introduction of chemoradiotherapy in 1999 has not been improved upon. The challenge is not only to look for ways to increase local control and survival rates but also to improve quality of life by minimizing morbidity of both surgical and nonsurgical treatment.

References

1. Ostor AG. Pandora's box or Ariadne's thread? Definition and prognostic significance of microinvasion in the uterine cervix. Squamous lesions. Pathol Annu. 1995;30(Pt 2):103–36.
[PubMed]

2. Roman LD, Felix JC, Muderspach LI, Agahjanian A, Qian D, Morrow CP. Risk of residual invasive disease in women with microinvasive squamous cancer in a conization specimen. Obstet Gynecol. 1997;90(5):759–64.
[Crossref][PubMed]

3. Kolstad P. Follow-up study of 232 patients with stage Ia1 and 411 patients with stage Ia2 squamous cell carcinoma of the cervix (microinvasive carcinoma). Gynecol Oncol. 1989;33(3):265–72.
[Crossref][PubMed]

4. Sevin BU, Nadji M, Averette HE, Hilsenbeck S, Smith D, Lampe B. Microinvasive carcinoma of the cervix. Cancer. 1992;70(8):2121–8.
[Crossref][PubMed]

5. Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol. 2002;84(1):145–9.
[Crossref][PubMed]

6. Smith HO, Qualls CR, Romero AA, Webb JC, Dorin MH, Padilla LA, et al. Is there a difference in survival for IA1 and IA2 adenocarcinoma of the uterine cervix? Gynecol Oncol. 2002;85(2):229–41.
[Crossref][PubMed]

7. Kim SH, Choi BI, Han JK, Kim HD, Lee HP, Kang SB, et al. Preoperative staging of uterine cervical carcinoma: comparison of CT and MRI in 99 patients. J Comput Assist Tomogr. 1993;17(4):633–40.
[Crossref][PubMed]

8. Hacker NF. Cervical cancer. In: Berek JS, Hacker NF, editors. Practical gynecologic oncology. Philadelphia: Lippincott Williams and Williams; 2000. p. 353.

9. Schmeler KM, Frumovitz M, Ramirez PT. Conservative management of early stage cervical cancer: is there a role for less radical surgery? Gynecol Oncol. 2011;120(3):321–5.
[Crossref][PubMed][PubMedCentral]

10. Querleu D, Morrow CP. Classification of radical hysterectomy. Lancet Oncol. 2008;9(3):297–303.
[Crossref][PubMed]

11. Rob L, Pluta M, Strnad P, Hrehorcak M, Chmel R, Skapa P, et al. A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. Gynecol Oncol. 2008;111(2 Suppl):S116–20.
[Crossref][PubMed]

12. Halaska M, Robova H, Pluta M, Rob L. The role of trachelectomy in cervical cancer. Ecancermedicalscience. 2015;9:506.
[Crossref][PubMed][PubMedCentral]

13. Shepherd JH, Shepherd ES. Fertility preservation in early cervical cancer. In: Patel HRH, Mould T, Joseph JV, Delaney CP, editors. Pelvic cancer surgery. London: Springer; 2015. p. 343–52.

14. Robova H, Rob L, Halaska MJ, Pluta M, Skapa P. Review of neoadjuvant chemotherapy and trachelectomy: which cervical cancer patients would be suitable for neoadjuvant chemotherapy followed by fertility-sparing surgery? Curr Oncol Rep. 2015;17(5):446.
[Crossref][PubMed]

15. Ungar L, Smith JR, Palfalvi L, Del Priore G. Abdominal radical trachelectomy during pregnancy to preserve pregnancy and fertility. Obstet Gynecol. 2006;108(3 Pt 2):811–4.
[Crossref][PubMed]

16. Hertel H, Kohler C, Grund D, Hillemanns P, Possover M, Michels W, et al. Radical vaginal trachelectomy (RVT) combined with laparoscopic pelvic lymphadenectomy: prospective multicenter study of 100 patients with early cervical cancer. Gynecol Oncol. 2006;103(2):506–11.
[Crossref][PubMed]

17. Delgado G, Bundy B, Zaino R, Sevin BU, Creasman WT, Major F. Prospective surgical-pathological study of disease-free interval in patients with stage IB squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1990;38(3):352–7.
[Crossref][PubMed]

18. Landoni F, Maneo A, Colombo A, Placa F, Milani R, Perego P, et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet. 1997;350(9077):535–40.
[Crossref][PubMed]

19. Benedetti-Panici P, Maneschi F, D'Andrea G, Cutillo G, Rabitti C, Congiu M, et al. Early cervical carcinoma: the natural history of lymph node involvement redefined on the basis of thorough parametrectomy and giant section study. Cancer. 2000;88(10):2267–74.
[Crossref][PubMed]

20. Ungar L, Palfalvi L, Tarnai L, Nechushkina V, Lintner B, Novak Z. Surgical treatment of stage IB cervical cancer. Int J Gynecol Cancer. 2012;22(9):1597–603.
[PubMed]

21. Gortzak-Uzan L, Jimenez W, Nofech-Mozes S, Ismiil N, Khalifa MA, Dube V, et al. Sentinel lymph node biopsy vs. pelvic lymphadenectomy in early stage cervical cancer: is it time to change the gold standard? Gynecol Oncol. 2010;116(1):28–32.
[Crossref][PubMed]

22. Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol. 2005;98(2):281–8.
[Crossref][PubMed]

23.

Rob L. Sentinel lymph node mapping in the management of cervical cancer. In: Patel HRH, Mould T, Joseph JV, Delaney CP, editors. Pelvic cancer surgery. London: Springer; 2015. p. 281–8.

24. Rydzewska L, Tierney J, Vale CL, Symonds PR. Neoadjuvant chemotherapy plus surgery versus surgery for cervical cancer. Cochrane Database Syst Rev. 2012;12:CD007406.
[PubMed]

25. Ferenschild FT, Vermaas M, Verhoef C, Ansink AC, Kirkels WJ, Eggermont AM, et al. Total pelvic exenteration for primary and recurrent malignancies. World J Surg. 2009;33(7):1502–8.
[Crossref][PubMed][PubMedCentral]

26. Yoo HJ, Lim MC, Seo SS, Kang S, Yoo CW, Kim JY, et al. Pelvic exenteration for recurrent cervical cancer: ten-year experience at National Cancer Center in Korea. J Gynecol Oncol. 2012;23(4):242–50.
[Crossref][PubMed][PubMedCentral]

27. Hockel M. Laterally extended endopelvic resection for locally advanced and recurrent cervical cancer. In: Patel HRH, Mould T, Joseph JV, Delaney CP, editors. Pelvic cancer surgery. London: Springer; 2105. p. 397–405.

28. Piver MS, Chung WS. Prognostic significance of cervical lesion size and pelvic node metastases in cervical carcinoma. Obstet Gynecol. 1975;46(5):507–10.
[PubMed]

29. Humber C, Tierney J, Symonds P, Collingwood M, Kirwan J, Williams C, et al. Chemotherapy for advanced, recurrent or metastatic endometrial carcinoma. Cochrane Database Syst Rev. 2005;3:CD003915.

30. Lukka H, Hirte H, Fyles A, Thomas G, Elit L, Johnston M, et al. Concurrent cisplatin-based chemotherapy plus radiotherapy for cervical cancer – a meta-analysis. Clin Oncol (R Coll Radiol). 2002;14(3):203–12.
[Crossref]

31. Chemoradiotherapy for Cervical Cancer Meta-analysis C. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: individual patient data meta-analysis. Cochrane Database Syst Rev. 2010;1:CD008285.

32. Phillips R. Supervoltage x-ray therapy. London: HK Lewis; 1944.

33. Mundt AJ, Lujan AE, Rotmensch J, Waggoner SE, Yamada SD, Fleming G, et al. Intensity-modulated whole pelvic radiotherapy in women with gynecologic malignancies. Int J Radiat Oncol Biol Phys. 2002;52(5):1330–7.
[Crossref][PubMed]

34. Mundt AJ, Mell LK, Roeske JC. Preliminary analysis of chronic gastrointestinal toxicity in gynecology patients treated with intensity-modulated whole pelvic radiation therapy. Int J Radiat Oncol Biol Phys. 2003;56(5):1354–60.
[Crossref][PubMed]

35. Tierney J. Neoadjuvant chemotherapy for locally advanced cervix cancer. Cochrane Database Syst Rev. 2004;2:CD001774.

36. McCormack M, Kadalayil L, Hackshaw A, Hall-Craggs MA, Symonds RP, Warwick V, et al. A phase II study of weekly neoadjuvant chemotherapy followed by radical chemoradiation for locally advanced cervical cancer. Br J Cancer. 2013;108(12):2464–9.
[Crossref][PubMed][PubMedCentral]

37. Peters 3rd WA, Liu PY, Barrett 2nd RJ, Stock RJ, Monk BJ, Berek JS, et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncol. 2000;18(8):1606–13.

[Crossref][PubMed]

38. Rogers L, Siu SS, Luesley D, Bryant A, Dickinson HO. Adjuvant radiotherapy and chemoradiation after surgery for cervical cancer. Cochrane Database Syst Rev. 2009;4:CD007583.

39. Chang HK, Lo KY, Chiang HS. Complications of urinary diversion after pelvic exenteration for gynecological malignancy. Int Urogynecol J Pelvic Floor Dysfunct. 2000;11(6):358–60.
[Crossref][PubMed]

40. Long 3rd HJ, Monk BJ, Huang HQ, Grendys Jr EC, McMeekin DS, Sorosky J, et al. Clinical results and quality of life analysis for the MVAC combination (methotrexate, vinblastine, doxorubicin, and cisplatin) in carcinoma of the uterine cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2006;100(3):537–43.
[Crossref][PubMed]

41. Monk BJ, Sill MW, McMeekin DS, Cohn DE, Ramondetta LM, Boardman CH, et al. Phase III trial of four cisplatin-containing doublet combinations in stage IVB, recurrent, or persistent cervical carcinoma: a Gynecologic Oncology Group study. J Clin Oncol. 2009;27(28):4649–55.
[Crossref][PubMed][PubMedCentral]

42. Tewari KS, Sill MW, Long 3rd HJ, Penson RT, Huang H, Ramondetta LM, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014;370(8):734–43.
[Crossref][PubMed][PubMedCentral]

43. Symonds RP, Gourley C, Davidson S, Carty K, McCartney E, Rai D, et al. Cediranib combined with carboplatin and paclitaxel in patients with metastatic or recurrent cervical cancer (CIRCCa): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Oncol. 2015;16(15):1515–24.
[Crossref][PubMed][PubMedCentral]

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5. Benign Lesions of the Cervix

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Abstract
A wide range of infections and other inflammatory disorders can involve the cervix. These can represent local cervical pathology or be a manifestation of systemic disease, for example vasculitis. Benign tumors and tumor-like lesions also occur in the cervix and form part of the differential diagnosis of intraepithelial, and sometimes invasive, lesions: recognition of their appearances is therefore important, particularly in the context of cervical screening programs. They can be subdivided into epithelial and mesenchymal lesions, with the epithelial lesions being further divided into squamous and glandular categories. This chapter focuses on benign squamous and glandular lesions and infections; neoplastic squamous and glandular lesions, mesenchymal lesions, and other cervical neoplasms are considered in other chapters.

Keywords Cervix – Infection – Inflammation – Benign – Squamous – Glandular

Introduction

A wide range of infections and other inflammatory disorders can involve the cervix. These can represent local cervical pathology or be a manifestation of systemic disease, for example vasculitis. Benign tumors and tumorlike lesions also occur in the cervix [1] and form part of the differential diagnosis of intraepithelial, and sometimes invasive, lesions: recognition of their appearances is therefore important, particularly in the context of cervical screening programs (see Chap. 3). They can be subdivided into

epithelial and mesenchymal lesions, with the epithelial lesions being further divided into squamous and glandular categories. Neoplastic squamous lesions of the cervix are considered in Chaps. 6 and 7, neoplastic glandular lesions in Chaps. 8 and 9, mesenchymal lesions in Chap. 10, and other cervical neoplasms in Chap. 11. This chapter therefore focuses on benign squamous and glandular lesions, and infections.

Benign Squamous Lesions

Squamous Metaplasia

Squamous metaplasia is a normal process in the uterine cervix and is discussed in detail in Chap. 1. It is always present in the adult cervix, and its diagnostic importance lies in its distinction from squamous intraepithelial lesions (SILs; see Chap. 6). This is also true colposcopically, where the distinction between intraepithelial lesions and squamous metaplasia can be difficult.

Squamous metaplasia occurs when the endocervical glandular epithelium is exposed to the vaginal environment, typically after puberty when the cervix grows and everts in response to hormones. It therefore represents a continuum, starting with the formation of a thin epithelium with features of squamous differentiation, progressing to a thicker epithelium which then matures to form an epithelium that morphologically resembles the native ectocervical nonkeratinizing squamous epithelium: these features are often referred to as immature and mature squamous metaplasia, respectively. Surface keratinization can occur, usually as parakeratosis, and this can cause diagnostic difficulty both colposcopically, where it produces a leukoplakic appearance, and histopathologically, where it raises the possibility of HPV-related changes. It is of note that squamous intraepithelial lesions (SILs) of the cervix can exhibit keratinization (see Chap. 6).

As this process develops, the squamocolumnar junction moves cranially, and the area between the original and current squamocolumnar junctions, which is lined by the metaplastic squamous epithelium, defines the region termed the transformation zone. The transformation zone therefore lies between the native ectocervical squamous and endocervical epithelia and is identified by the presence of squamous metaplasia (immature or mature) overlying endocervical glands. It is often inflamed and the squamous epithelium may therefore show reactive changes. These can be marked, particularly if there is ulceration, mimicking a SIL. Although the presence of prominent nucleolation of squamous cells can be a useful diagnostic feature in favor of metaplasia, the distinction between a thin SIL and immature squamous metaplasia can on occasion be very difficult [2, 3]. Immunostaining for the p16 protein can be extremely helpful in this situation, with block-type p16 positivity lending strong support to a high-grade SIL interpretation [4, 5] (and see Chap. 6).

Condyloma Acuminatum

Condyloma acuminatum is an exophytic papillary squamous lesion caused by productive HPV infection. The majority of condylomata acuminata are low-risk HPV infections (usually HPV 6 or HPV 11; see Chap. 2), but high-risk HPV infection can produce condylomatous lesions, and high-grade SIL can have a condylomatous architecture. The term condyloma acuminatum is reserved for exophytic productive HPV infections with the typical condylomatous architecture and at most mild atypia (Fig. 5.1). These lesions are therefore a subset of low-grade SIL (see Chap. 6). If features of high-grade SIL are present, the lesion should be categorized as such, and if features of HPV infection (such as koilocytosis, dyskeratosis, and multinucleation) are absent, the lesion should be categorized as a squamous cell papilloma (see below).

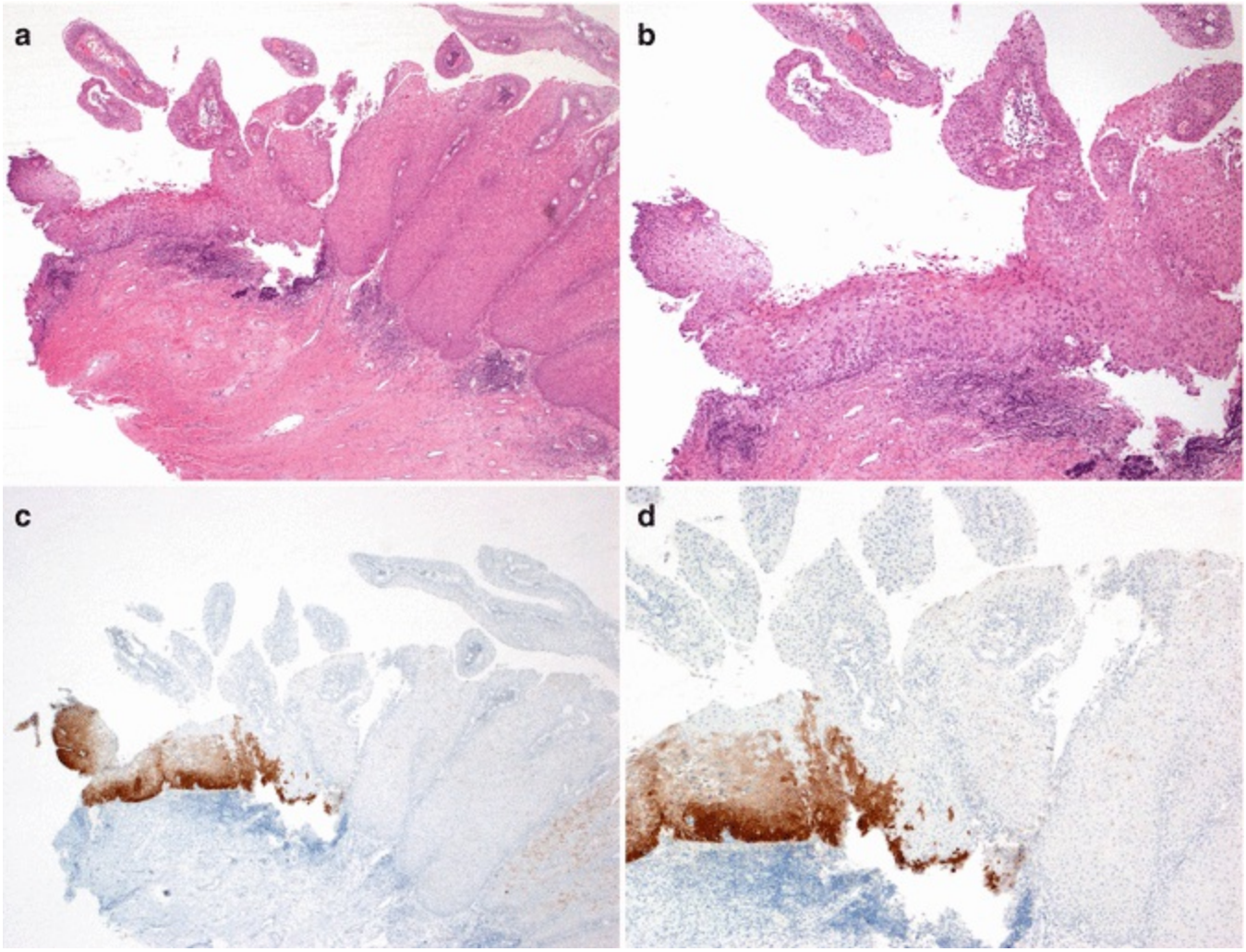


Fig. 5.1 This shows a flat low-grade squamous intraepithelial lesion (SIL) (left) contiguous with a condyloma acuminatum (right) at low (**a**) and medium (**b**) power together with the accompanying p16 immunostain (**c**, **d**). The flat LSIL is block positive for p16, consistent with high-risk HPV infection, whereas the condyloma acuminatum is p16 negative, in keeping with low-risk HPV infection. Note the sharp demarcation between the two lesions

Condylomata acuminata associated with low-risk HPV infection are p16 negative (Fig. 5.1). In this context, negativity is defined as the absence of block-type positivity, which in turn is defined as "continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upwards involving at least 1/3 of the epithelial thickness. The latter height restriction is somewhat arbitrary but adds specificity" according to the Lower Anogenital Squamous Terminology (LAST) recommendations [6]. Figure 5.1 shows the contrast between block-positive and block-negative p16 staining.

Squamous Papilloma

By definition, a squamous papilloma has a papillary structure, with a fibrovascular core lined by non-atypical squamous epithelium, and is not associated with HPV infection. These morphological features can be seen in association with HPV infection but, when HPV infection is present, a diagnosis of LSIL with a papillomatous or condylomatous pattern is preferred. Immunostaining for p16 and Ki67 can be very useful in identifying those papillomatous lesions associated with HPV infection, as Ki67 expression in the upper squamous epithelium is typically seen in HPV-driven lesions, including those that are associated with low-risk HPV infection and hence p16 negative (Fig. 5.2). Other differential diagnostic considerations include immature squamous metaplasia with a papillary pattern, which in turn may be associated with HPV infection [7].

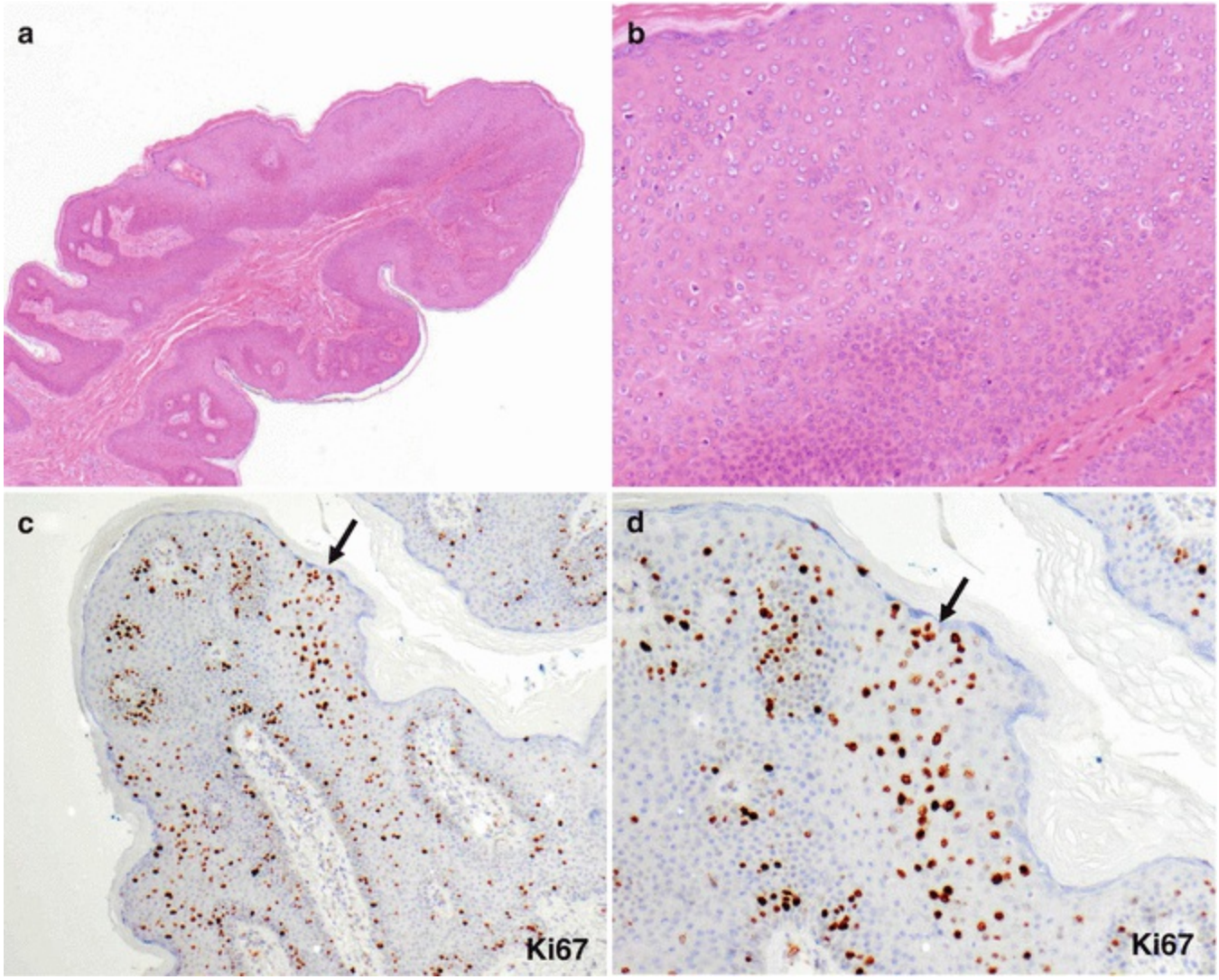


Fig. 5.2 "Squamous cell papilloma" (**a**) showing epithelial hyperplasia and single cell dyskeratosis but inconspicuous koilocytosis (**b**). Ki67 immunostaining shows both parabasal positivity and regional suprabasal positivity in keeping with activation in concert with HPV replication (**c, d**, *arrows*). This lesion was p16 negative, consistent with low-risk HPV infection. Given the features associated with HPV infection, this lesion is best categorized as a condyloma acuminatum (LSIL). This case is from the vulva but is included to illustrate the features of a more "papillomatous" condyloma acuminatum

Transitional Metaplasia

Transitional metaplasia is essentially a form of squamous metaplasia in which the lack of cytoplasmic maturation imparts a "transitional" appearance to the epithelium. This is reinforced by the presence of nuclear grooving. The low nucleus-cytoplasm ratio gives this lesion a hyperchromatic appearance at low power and, coupled with the lack of maturation of the epithelium, can lead to misdiagnosis as a high-grade SIL. Immunostaining for p16 is very helpful in resolving this differential diagnosis as transitional metaplasia is p16 negative; Ki67 positivity is also low, by contrast with high-grade SIL [8, 9].

Benign Glandular Lesions

Endocervical Polyp

Endocervical polyps comprise a fibrovascular core containing a variable number of endocervical glands, lined by benign endocervical epithelium. Squamous metaplasia, which is typically immature and may extend to involve endocervical glands, is common, as is microglandular hyperplasia, particularly in women taking hormone preparations (see below). These polyps may be identified incidentally, for example, when taking a cervical smear, but may also be associated with bleeding, particularly postcoitally, and/or discharge. Inflammation, with ulceration and reactive epithelial changes, is common, particularly at the tip of the polyp. Although common and generally benign, endocervical polyps should be examined carefully microscopically, as glandular neoplasia can occasionally involve the glands and SILs can involve the metaplastic squamous epithelium. Cervical sarcomas can also present as cervical polyps (see Chap. 10).

Müllerian Papilloma

This is a specific lesion found in children, most commonly between the ages of 2 and 5 years but with an age range of 1–9 years. This rare entity is considered to be of Müllerian origin, occurs in the upper vagina and cervix as a friable polypoid lesion up to 2 cm in diameter, and presents with vaginal bleeding or discharge [10]. By contrast with the more common endocervical polyp, Müllerian papilloma has branching fibrous papillae. The lining epithelium is benign and cuboidal to columnar but may show metaplastic changes. Local recurrence may occur if incompletely excised, but this lesion is considered to be benign. When considering a diagnosis of Müllerian papilloma, it is important to include Müllerian adenosarcoma and embryonal rhabdomyosarcoma in the differential diagnosis (see Chap. 10).

Nabothian Cysts

Nabothian cysts are distended but otherwise normal endocervical glands. The lining epithelium is often attenuated and may show reactive changes. Most are asymptomatic and come to clinical attention incidentally. Deep cysts may enlarge the cervix and produce a suspicious appearance (see the section below on "Endocervicosis"). Most lesions are asymptomatic, but they can be associated with chronic cervicitis and mucous discharge. In cases of deep wall Nabothian cysts, the cervix can become enlarged and clinically suspicious of a malignant process.

Tunnel Clusters

These common aggregates of benign endocervical glands, which generally form lobular structures, without (type A clusters) or with (type B clusters) cystic change, are typically identified incidentally on microscopic examination of the cervix [11]. When cystic, they can lead to a macroscopic abnormality.

Lobular Endocervical Glandular Hyperplasia (LEGH)

This entity is composed of lobular aggregates of endocervical glands showing gastric (pyloric type) metaplasia and may be a precursor of gastric-type adenocarcinoma (a spectrum of lesions including minimal deviation adenocarcinoma of mucinous type). It is discussed in detail in Chaps. 8 and 9.

Diffuse Laminar Endocervical Hyperplasia

This is composed of benign but crowded endocervical glands that form a band-like structure beneath the endocervical surface. There is typically an inflammatory infiltrate beneath the glandular aggregates. This entity is discussed further and illustrated in Chap. 8.

Mesonephric Remnants and Hyperplasia

Remnants of the mesonephric (Wolffian) duct are a not uncommon incidental microscopic finding in cervical specimens, particular hysterectomies (Fig. 5.3). These remnants are typically found in the outer cervical wall in a location consistent with the embryological position of the mesonephric duct but can on occasion be present in the inner cervical wall and can communicate with the endocervical canal. A combination of small tubular structures containing PAS-positive eosinophilic material surrounding larger duct-like structures is often present. These structures are lined by predominantly cuboidal epithelial cells [12]. Mesonephric hyperplasia may be lobular or diffuse, the latter on occasion raising the possibility of invasive carcinoma [13]. However, there is minimal atypia and Ki67 labeling is low. Recent studies have identified nuclear GATA3 immunoreactivity to be typical of mesonephric lesions [14, 15], and this can be helpful in difficult cases. Mesonephric carcinoma is discussed in Chap. 11.

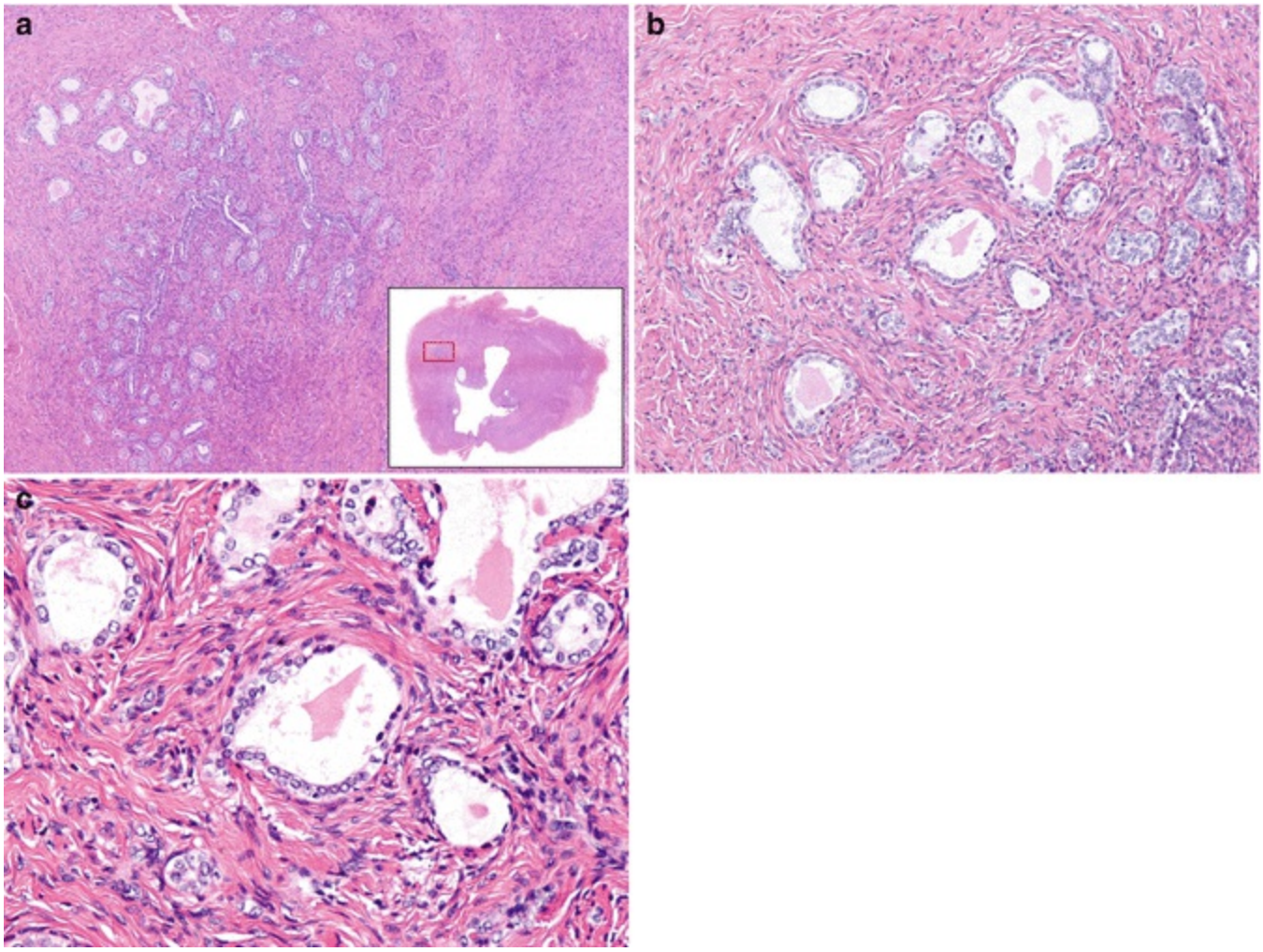


Fig. 5.3 Transverse (axial) section of the cervix from a hysterectomy showing a cluster of small glandular structures within the wall of the cervix (**a**). These are lined by cuboidal epithelial cells and contain eosinophilic material (**b, c**). The appearances are typical of mesonephric duct remnants

Arias-Stella Reaction

An Arias-Stella reaction may affect the endocervical glandular epithelium in the same way it affects endometrial glands [16]. This produces a potentially worrisome appearance, with enlargement of glandular epithelial cells with hyperchromatic nuclei (Fig. 5.4). The cytoplasm may be clear or show oxyphilic change. Its importance is in its distinction from clear cell or usual type adenocarcinoma of the cervix. An important clue to the diagnosis is a history of current pregnancy or hormone therapy, with which this change is associated. It is a benign hormone-related alteration with no malignant potential.

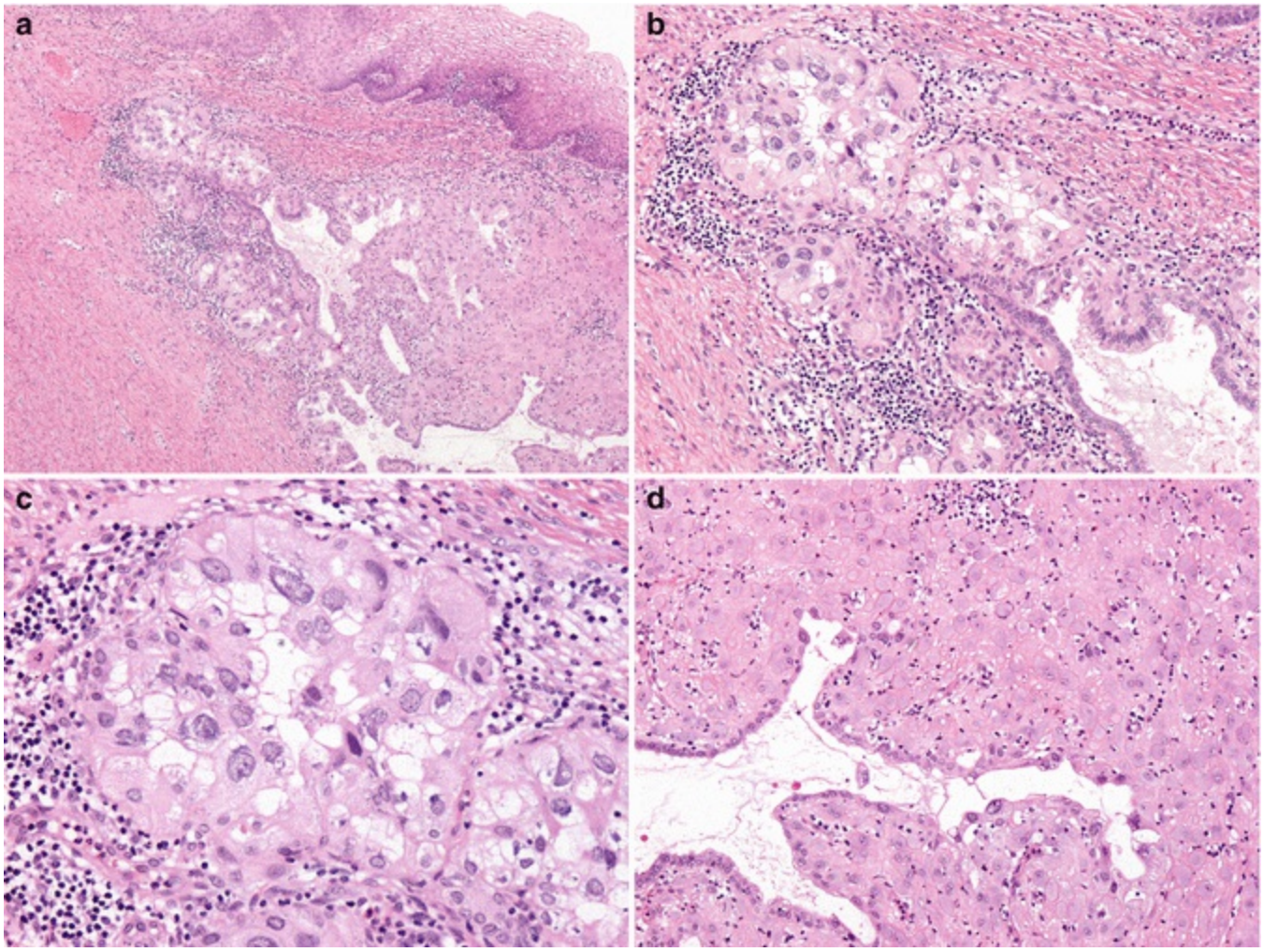


Fig. 5.4 Loop excision was performed during pregnancy following a biopsy diagnosis of superficially invasive squamous cell carcinoma of the cervix. Focally, the endocervical glands showed marked nuclear pleomorphism (**a**), which raised concern for adenocarcinoma in situ (**b**, **c**). However, the morphological features, in the context of pregnancy and an adjacent prominent decidual reaction (**d**), led to a diagnosis of Arias-Stella reaction

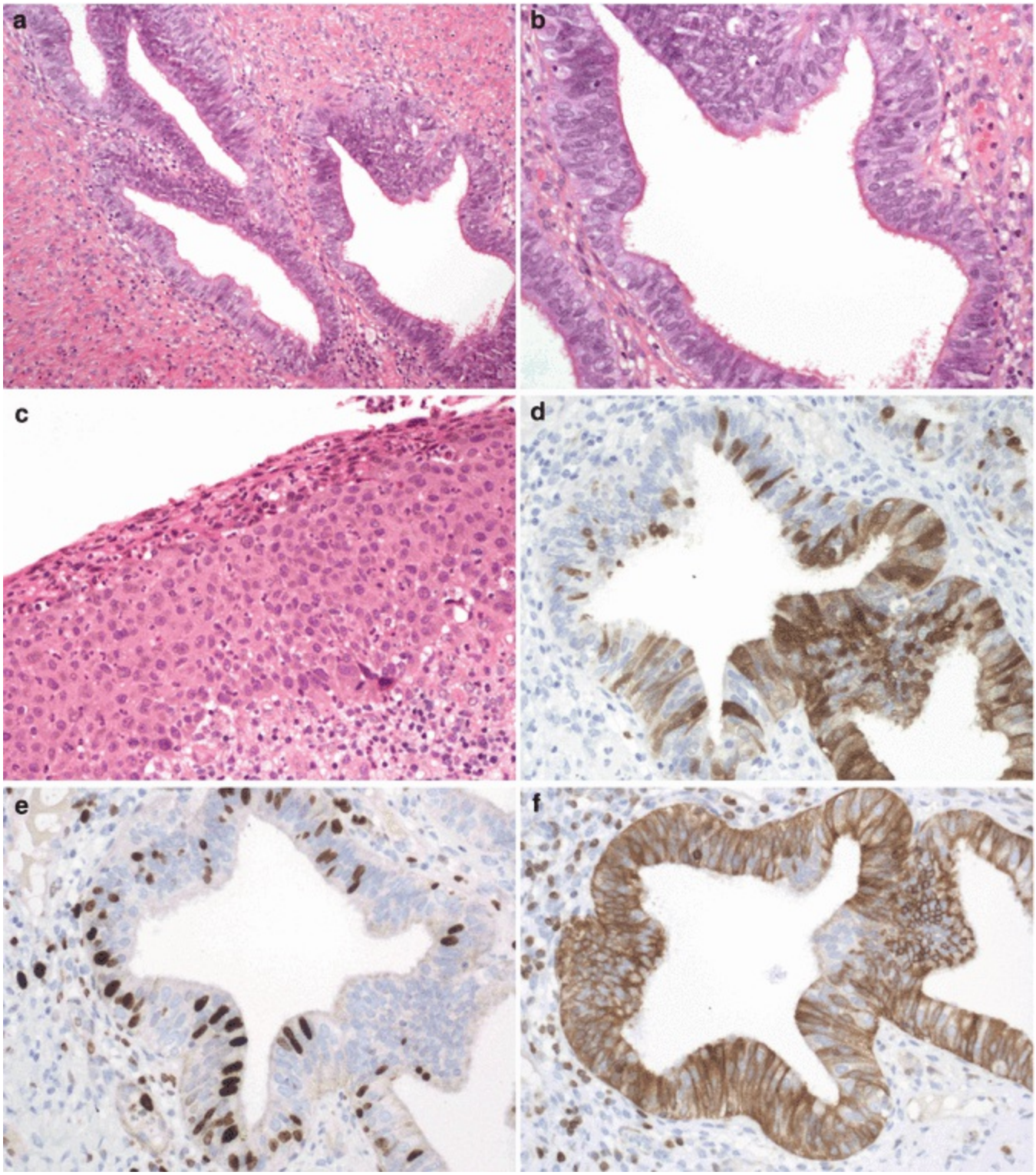
Endocervicosis

Endocervicosis refers to the presence of endocervical glands in the outer half of the uterine wall [17]. This usually produces a mass, or cyst, involving the cervical wall, typically anteriorly. The epithelium lining the cystic spaces is benign. Mucin extravasation with an accompanying stromal reaction may be seen. There may be a history of antecedent Caesarian section.

Tuboendometrioid Metaplasia

Tuboendometrioid metaplasia of the endocervical glands, in which the lining epithelium resembles the tubal (ciliated) or endometrioid (non-ciliated) epithelium, is common, particularly where there is a history of cervical surgery. This may on occasion present diagnostic difficulty [18] as, when inflamed, it can mimic adenocarcinoma in situ.

Immunohistochemistry for p16, Bcl2, and Ki67 can be helpful in resolving this differential diagnosis, with tuboendometrioid metaplasia and endometriosis typically showing patchy p16 positivity, Bcl2 positivity, and a relatively low Ki67 labeling index [19] (Fig. 5.5). Adenocarcinoma in situ is usually diffusely and strongly p16 positive and Bcl2 negative and has a high Ki67 labeling index (see Chap. 8).



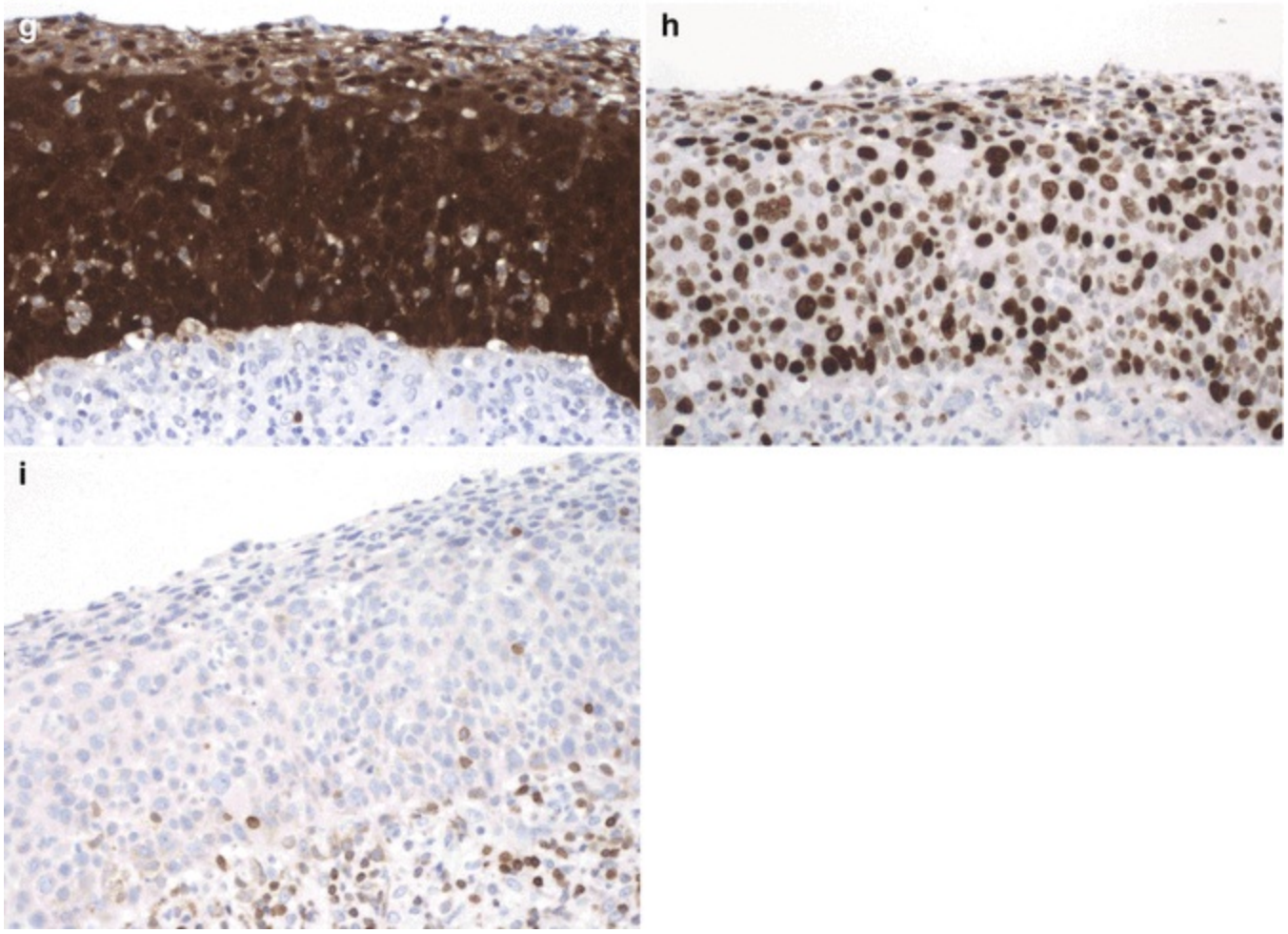


Fig. 5.5 Loop excision was performed following a biopsy diagnosis of high-grade SIL. This showed abnormal glands (**a**, **b**) in addition to residual high-grade SIL (**c**). Morphologically, the bland cytology and prominent ciliation indicate tuboendometrioid metaplasia, confirmed by immunohistochemistry which shows patchy p16 positivity (**d**), a relatively low Ki67 labeling index, (**e**) and diffuse Bcl2 positivity (**f**). This contrasts with the staining pattern in the adjacent high-grade SIL, which shows block-type p16 positivity (**g**), a high Ki67 labeling index, (**h**) and no epithelial positivity for Bcl2 (**i**)

Endometriosis

Cervical endometriosis refers to the presence of endometrial-type glands and associated stroma in the cervix. It may affect the endocervical canal and subjacent stroma, in which case it is often associated with previous cervical surgery, for example cone biopsy or loop excision (superficial endometriosis), or it may affect the deep cervical wall, particularly posteriorly, when it is associated with pelvic endometriosis (deep endometriosis). Superficial endometriosis is often associated with tuboendometrioid metaplasia of surrounding glands.

Ectopic Prostate Tissue

Prostate tissue may be identified in the cervix [20].

Microglandular Hyperplasia

Microglandular hyperplasia is a common finding in cervical biopsies and cervical polyps, particularly in association with progestogen therapy and pregnancy. It produces closely packed glands with subnuclear vacuolation, the latter being a helpful feature in reaching the correct diagnosis. When architecturally complex, inflamed, or presenting with more unusual solid or trabecular patterns, this lesion can cause diagnostic confusion with adenocarcinoma [11, 21].

Infections

Cervical HPV infection is extremely common and is discussed in detail in Chaps. 2, 3, and 6. Other viral infections that can be present in specimens from the cervix include herpes simplex virus (HSV) infection, Epstein-Barr virus (EBV) infection, cytomegalovirus (CMV) infection, and molluscum contagiosum. HSV produces characteristic epithelial changes on cervical smears. A non-exhaustive list of cervical infections is given in Table 5.1.

Table 5.1 Infections of the cervix

	References
Human papillomavirus (HPV)	See Chap. 2
Herpes simplex virus (HSV)	[22]
Epstein-Barr virus (EBV)	[23]
Molluscum contagiosum	[24]
Syphilis	[25]
Chlamydia and lymphogranuloma venereum	[26]
Granuloma inguinale	[27]
Mycoplasma	[28]
Chancroid	[29]
Tuberculosis	[30]
Trichomoniasis	[31]
Amoebiasis	[32]
Schistosomiasis	[33]

References

1. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO classification of tumours of female reproductive

organs. 4th ed. Lyon: IARC Press; 2014.

2. Geng L, Connolly DC, Isacson C, Ronnett BM, Cho KR. Atypical immature metaplasia (AIM) of the cervix: is it related to high-grade squamous intraepithelial lesion (HSIL)? Hum Pathol. 1999;30:345–51.
[Crossref][PubMed]

3. Park JJ, Genest DR, Sun D, Crum CP. Atypical immature metaplastic-like proliferations of the cervix: diagnostic reproducibility and viral (HPV) correlates. Hum Pathol. 1999;30:1161–5.
[Crossref][PubMed]

4. Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade BJ, Sun D, Duensing S, Sheets EE, Munger K, Crum CP. Ki-67, cyclin E, and p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Pathol. 2001;25:884–91.
[Crossref][PubMed]

5. Klaes R, Benner A, Friedrich T, Ridder R, Herrington S, Jenkins D, Kurman RJ, Schmidt D, Stoler M, von Knebel Doeberitz M. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. Am J Surg Pathol. 2002;26:1389–99.
[Crossref][PubMed]

6. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC. The lower anogenital squamous terminology standardization project for HPV-associated lesions. Int J Gynecol Pathol. 2012;16:205–42.

7. Trivijitsilp P, Mosher R, Sheets EE, Sun D, Crum CP. Papillary immature metaplasia (immature condyloma) of the cervix: a clinicopathologic analysis and comparison with papillary squamous carcinoma. Hum Pathol. 1998;29:641–8.
[Crossref][PubMed]

8. Harnden P, Kennedy W, Andrew AC, Southgate J. Immunophenotype of transitional metaplasia of the uterine cervix. Int J Gynecol Pathol. 1999;18:125–9.
[Crossref][PubMed]

9. Weir MM, Bell DA, Young RH. Transitional cell metaplasia of the uterine cervix and vagina: an underrecognized lesion that may be confused with high-grade dysplasia. A report of 59 cases. Am J Surg Pathol. 1997;21:510–7.
[Crossref][PubMed]

10. Lane BR, Ross JH, Hart WR, Kay R. Mullerian papilloma of the cervix in a child with multiple renal cysts. Urology. 2005;65:388.
[Crossref][PubMed]

11. Nucci MR. Symposium part III: tumor-like glandular lesions of the uterine cervix. Int J Gynecol Pathol. 2002;21:347–59.
[Crossref][PubMed]

12. Ferry JA, Scully RE. Mesonephric remnants, hyperplasia, and neoplasia in the uterine cervix. A study of 49 cases. Am J Surg Pathol. 1990;14:1100–11.
[Crossref][PubMed]

13. Seidman JD, Tavassoli FA. Mesonephric hyperplasia of the uterine cervix: a clinical pathologic study of 51 cases. Int J Gynecol Pathol. 1995;15:293–9.
[Crossref]

14.

Howitt BE, Emori MM, Drapkin R, Gaspar C, Barletta JA, Nucci MR, McCluggage WG, Oliva E, Hirsch MS. GATA3 is a sensitive and specific marker of benign and malignant mesonephric lesions in the lower female genital tract. Am J Surg Pathol. 2015;39:1411–9.
[Crossref][PubMed]

15. Roma AA, Royal A, Yang B. Differential expression patterns of GATA3 in uterine mesonephric and nonmesonephric lesions. Int J Gynecol Pathol. 2015;34:480–6.
[Crossref][PubMed]

16. Nucci MR, Young RH. Arias-Stella reaction of the endocervix: a report of 18 cases with emphasis on its varied histology and differential diagnosis. Am J Surg Pathol. 2004;28:608–12.
[Crossref][PubMed]

17. Young RH, Clement PB. Endocervicosis involving the uterine cervix: a report of four cases of a benign process that may be confused with deeply invasive endocervical adenocarcinoma. Int J Gynecol Pathol. 2000;19:322–8.
[Crossref][PubMed]

18. Oliva E, Clement PB, Young RH. Tubal and tubo-endometrioid metaplasia of the uterine cervix. Unemphasised features that may cause problems in differential diagnosis: a report of 25 cases. Am J Clin Pathol. 1995;103:618–23.
[Crossref][PubMed]

19. El-Ghobashy AA, Shaaban AM, Innes J, Prime W, Herrington CS. Differential expression of cyclin-dependent kinase inhibitors and apoptosis-related proteins in endocervical lesions. Eur J Cancer. 2007;43:2011–8.
[Crossref][PubMed]

20. McCluggage WG, Ganesan R, Hirschowitz L, Miller K, Rollason TP. Ectopic prostate tissue in the uterine cervix and vagina: report of a series with a detailed immunohistochemical analysis. Am J Surg Pathol. 2006;30:209–15.
[Crossref][PubMed]

21. Nucci MR. Pseudoneoplastic glandular lesions of the uterine cervix: a selective review. Int J Gynecol Pathol. 2014;33:330–8.
[Crossref][PubMed]

22. Tomkins A, White C, Higgins SP. Primary herpes simplex virus infection mimicking cervical cancer. Br Med J Case Rep. 2015; pii: bcr2015210194.

23. Andersson-Ellstrom A, Bergstrom T, Svennerholm B, Milsom I. Epstein-Barr virus DNA in the uterine cervix of teenage girls. Acta Obstet Gynecol Scand. 1997;76:779–83.
[Crossref][PubMed]

24. Lang TU, Michelow P, Khalbuss WE, Monaco SE, Pantanowitz L. Molluscum contagiosum of the cervix. Diagn Cytopathol. 2012;40:615–6.
[Crossref][PubMed]

25. Iwasaka T, Ikeda N, Sugimori H. Secondary syphilis with extensive cervical lesion. Asia Oceania J Obstet Gynaecol. 1987;13:311–4.
[Crossref][PubMed]

26. Nonato DR, Alves RR, Ribeiro AA, Saddi VA, Segati KD, Almeida KP, de Lima YA, D'Alessandro WB, Rabelo-Santos SH. Prevalence and factors associated with coinfection of human papillomavirus and Chlamydia trachomatis in adolescents and young women. Am J Obstet Gynecol. 2016;215:753.e1–9.

27. Bassa AG, Hoosen AA, Moodley J, Bramdev A. Granuloma inguinale (donovanosis) in women. An analysis of 61 cases from Durban, South Africa. Sex Transm Dis. 1993;20:164–7.
[Crossref][PubMed]

28. Ross JDC, Jensen JS. Mycoplasma genitalium as a sexually transmitted infection: implications for screening, testing and treatment. Sex Transm Infect. 2006;82:269–71.
[Crossref][PubMed][PubMedCentral]

29. Lewis DA. Chancroid: clinical manifestations, diagnosis and management. Sex Transm Infect. 2003;79:68–71.
[Crossref][PubMed][PubMedCentral]

30. Mandato VD, Sacchetti F, Costagliola L, La Sala GB. Primary tuberculosis of the uterine cervix: keep it in mind. J Low Genit Tract Dis. 2014;18:E29–33.
[Crossref][PubMed]

31. Lusk MJ, Garden FL, Rawlinson WD, Naing ZW, Cumming RG, Konecny P. Cervicitis aetiology and case definition: a study in Australian women attending sexually transmitted infection clinics. Sex Transm Infect. 2016;92:175–81.
[Crossref][PubMed]

32. Ahuja A, Bhardwaj M. Cervical amoebiasis mimicking cervical carcinoma: a rare presentation of a common infection. J Infect Public Health. 2016;9:516–8.
[Crossref][PubMed]

33. Chen W, Flynn EA, Shreefter MJ, Blagg NA. Schistosomiasis: an unusual finding of the cervix. Obstet Gynecol. 2012;119:473–5.
[Crossref]

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6. Cervical Squamous Intraepithelial Lesions

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Abstract
Cervical squamous intraepithelial lesions [also known as cervical intraepithelial neoplasias (CINs), cervical dysplasias] impact a significant proportion of the population and are among the most commonly encountered diagnoses for the cytopathologist and surgical pathologist. Screening programs to detect and eradicate the subset of these lesions that are precancerous remain among the most impressive success stories in preventative medicine. Although many aspects of cervical neoplasia are familiar to all pathologists, this is a perpetually dynamic field due to changes in both technology and prevalence, leading to ongoing refinement of patient management. Recent decades have seen a rapid expansion in our understanding of cervical carcinogenesis leading to the development of new diagnostic biomarkers, novel testing assays, and effective vaccines. Screening strategies have also undergone renovation in response to these developments. This chapter reviews cervical squamous intraepithelial neoplasia from its earliest historical characterizations to its most up-to-date molecular underpinnings. It covers terminology, epidemiology, and screening techniques. Cytological and histological features are reviewed, as are morphological mimics. Finally, the molecular basis for disease is discussed with attention to potential immunohistochemical and molecular biomarkers.

Keywords Cervical squamous intraepithelial lesions – Cervix – Squamous intraepithelial lesion – Human papillomavirus

History and Terminology

Uterine cervical squamous intraepithelial lesions represent the model for intraepithelial neoplasia throughout the body. Their existence was initially recognized in the early 1900s, when histologic descriptors such as "surface carcinoma," "intraepithelial carcinoma," and "carcinoma in situ (CIS)" were applied to lesions that bore the cytologic features of malignancy, but that had yet to invade through the basement membrane. These diagnoses corresponded with a fundamental clinical decision branch point: a diagnosis of CIS provoked a hysterectomy, while the uterus and cervix were retained in cases that failed to meet CIS criteria. The mid-twentieth century saw the recognition of lesions with less marked severity than CIS, ultimately leading to the application of the term "dysplasia" by Reagan and colleagues, who went on to usher in gradations of mild, moderate, and severe [1]. Although the diagnostic interface between CIS and severe dysplasia remained somewhat murky, the former continued to provoke complete hysterectomy, while the latter was more often treated with conization.

The next significant development in the characterization of squamous intraepithelial lesions came with Koss and Dufree's description of koilocytes in 1956 [2]. They derived this term from the Greek word for cave due to the "cave-like" vacuoles that surrounded the enlarged nuclei of these cells and noted the morphological homology between these cells and the mild dysplasia depicted in Reagan's system. It took another two decades for this morphology to be ascribed to human papillomavirus infection by Miesels and Fortin, with confirmatory electron microscopy studies performed shortly thereafter [3–6].

At this point, cervical dysplasias were not necessarily recognized as precursors for invasive carcinoma. This concept was championed by Richart in 1969, who suggested that the continuum of mild, moderate, and severe dysplasia all imparted some risk of progression to carcinoma [7, 8]. Recognition of this shared risk led to a nomenclature change to the cervical intraepithelial neoplasia (CIN) system, with mild dysplasia equating with CIN1, moderate dysplasia CIN2, and severe dysplasia and CIS being collapsed into CIN3. This conceptual shift was accompanied by a shift in treatment practices, with local resection supplanting hysterectomy for all grades of CIN, including those formerly termed CIS. The end of the twentieth century saw the advent of molecular biology and an explosion in our understanding of cervical carcinogenesis and its relationship to human papillomavirus. Notable breakthroughs included the first demonstration of high-risk HPV DNA in cervical cancer cell lines by Boshart et al. while working in the laboratory of Harald zur Hausen, who was awarded the Nobel Prize in 2008 for the relationship of HPV to cervical carcinogenesis. Subsequently, Crum and colleagues were the first to identify HPV 16 in cervical intraepithelial neoplasia [9, 10].

As molecular evidence mounted and was carefully correlated with epidemiological

studies, it became clear that CIN1 (e.g., mild dysplasia, usually with koilocytes) represented the histological correlate for productive HPV infection, while CIN2/CIN3/CIS was identified as a morphological indication of HPV oncogene-induced cell transformation. CIN1 lesions were further shown to regress in the majority of instances, whereas CIN2/CIN3/CIS lesions showed much higher rates of persistence and progression. This understanding led to the return of a binary managerial approach to cervical pathology: CIN1 lesions were considered a low-grade squamous intraepithelial lesion (LSIL) and managed with observation, whereas CIN2/CIN3/CIS lesions were combined as high-grade squamous intraepithelial lesions (HSIL) and warranted resection [11]. These grades correlate with risk of progression to cancer.

This two-tiered risk schema informed the Bethesda Classification System for Cervical Cytology, first introduced in 1988 and refined three times, most recently in 2014. In 2012, the Lower Anogenital Squamous Terminology (LAST) project further advocated for the use of LSIL/HSIL terminology not only in the uterine cervix but also elsewhere in the male and female genital tracts, as did the fourth edition of the World Health Organization's text on gynecological neoplasia [12, 13].

Epidemiology and Risk Factors

HPV is ubiquitous in human populations, with the majority of cervical infections passing without detectable clinical sequelae. It is estimated that up to 80% of women in their early 20s will experience at least a transient HPV infection, and this decade also represents the peak of microscopically detected cervical squamous intraepithelial lesions. The incidence of cervical squamous lesions goes on to parallel infection epidemiology throughout the ensuing decades, falling off to roughly 5% of women in their 50s. Risks factors for infection include earlier age of sexual activity, increased number of sexual partners, immunosuppression, coexistence of other sexually transmitted diseases, and smoking [13, 14].

There are >40 of HPV types that infect the cervix, but the majority of cases can be attributed to 13–15 high-risk types and 4–6 low-risk types. Historically, high-risk types 16 and 18 and low-risk types 6 and 11 have predominated; however, this distribution is likely to change in time following the advent of HPV immunization which targets these types. It is notable that clinically validated HPV testing platforms typically cover only 13–15 subtypes in total; therefore, their sensitivity for infection is imperfect by design. This deliberate limitation on sensitivity is due to the need for screening assays to maximize the sensitivity and specificity for precancer risk as opposed to viral detection. For more detail on the epidemiology of high-risk HPV infection, please refer to Chap. 2.

Specimen Types

Cervical squamous neoplasia is typically first encountered in a cytology sample, with surgical biopsy representing the initial method of collection only in rare cases with grossly visible lesions. A diagnosis of squamous intraepithelial lesion by cytology prompts a range of interventions depending upon the degree of dysplasia and the patient's age, HPV status, and gravidity; the most up-do-date management guidelines in the United States are available through the American Society for Colposcopy and Cervical Pathology (ASCCP). Follow-up actions include watchful waiting and cervical biopsy, with the latter sometimes prompting a larger excision [either loop electrosurgical excision (LEEP) or cone biopsy] or, occasionally, hysterectomy.

Cytological Specimens ("Pap Smears")

The term "Pap smear" is used colloquially to describe all manner of cervical cytology samplings. A more apt generic classification for any cytological sampling of the cervix is "Pap test," as this term allows for a variety of collection methods and still retains the homage to Dr. George Papanicolaou, the anatomist who first reported malignant cells in gynecological samplings. Technically, a "Pap *smear*" refers to a direct conventional smear collected from the cervix, typically using a specialized spatula alone or in tandem with an endocervical sampling device (historically a cotton-tipped applicator but today more often a patented "broom" or "brush"). The collected material is then transferred directly to a glass slide and smeared out. Conventional smears typically contain between 50,000 and 300,000 cells with a minimum of 8000–12,000 well-visualized cells required for specimen adequacy. Although under optimal conditions sensitivity for CIN can be robust, it remains imperfect, and DNA analysis has shown that the cells represented on a direct smear constitute a small minority (as little as 5%) of the total cells removed from the patient, with practitioner skill/technique, anatomy, and technical preparation considerably influencing specimen sensitivity [15]. Furthermore, interpretation can be hindered significantly by excessive smear thickness, obscuring blood, and variable fixation.

Given the propensity for such variables to confound conventional smear interpretation, many cytopathologists prefer more standardized preparation methods whenever they are available, and improvements in laboratory technology have made direct smears uncommon in many modern pathology practices. Indeed, in the United States, most of the cervical cytology specimens we now encounter are thin-layer liquid-based preparations. When compared to conventional smears, liquid-based specimens have equivalent or better sampling sensitivity, more uniform fixation and staining, and decreased background [15]. The most commonly employed techniques are ThinPrep (Hologic) and SurePath (BD).

ThinPrep samples are collected using either a broom-type device or a plastic spatula in combination with an endocervical brush. The sampling apparatus is then

swished and stored in a vial containing methanol-based preservative solution which lyses red blood cells. Collection vials are loaded into a patented instrument which disperses cells and collects them on a 20 mm polycarbonate filter, which is then transferred onto a glass slide. Generally only a small proportion of the specimen is used to create a ThinPrep slide; therefore, residual material remains available for molecular diagnostic testing, preparation of additional slides, and/or cell block preparation. Several studies have shown improved detection of dysplasia in ThinPrep slides when compared to conventional smears [16].

In contrast to ThinPrep, SurePath uses an ethanol-based preservative, and the collection device is snipped off and included in the vial. The specimen is vortexed and syringed through a small opening to disaggregate large cell clumps. Next, it is centrifuged through a density gradient which eliminates red blood cells and some white blood cells. The centrifuged pellet is then resuspended and centrifuged again. Finally, the centrifuge tube is transferred to a staining instrument which samples the pellet and settles the cells onto a cationic polyelectrolyte-coated slide. Although some studies have shown improved detection of LSIL by SurePath as compared to conventional smears, significant differences in the detection of HSIL have not been demonstrated [17, 18].

Cervical Biopsy

Surgical biopsies are typically small, unorientated portions of tissue. While most often directed at visible lesions, they may also be either random or systematic quadrant samplings. The sensitivity of sampling may be significantly influenced by operator skill and patient anatomy, and the absence of a lesion on biopsy does little to assuage concern prompted by positive cytology. Indeed, while colposcopic biopsy has long been erroneously considered the diagnostic "gold standard" by many clinicians, it may miss roughly 25% of cytologically detected intraepithelial lesions [19, 20]. This makes sense, as surgical biopsies collect only a small focus of the epithelium, whereas cytological samples include cellular representations of much broader areas. This issue of sampling discrepancy is also of significance in discussions regarding the utility (and limitations) of prognostic markers in cervical specimens, which is further discussed here under the section "Biomarkers" heading of this chapter.

Cone Biopsy

These excisional specimens encompass the transformation zone and serve the dual purposes of diagnosis and therapy. They are performed using a scalpel (hence the term "cold-knife cone"). Specimen size varies considerably based on the cervical anatomy, surgical technique, and patient's age and interest in preserving fertility but typically measures around 1.5 cm in width by 1.0 cm in depth.

Loop Electrosurgical Excision Procedure (LEEP)

LEEP (also known as loop excision of the transformation zone (LETZ)) has largely supplanted traditional conization as the excisional method of choice for cervical dysplasia. It is similar to a traditional or "cold-knife" cone, but instead of an unheated scalpel, it relies on a hot wire-shaped loop or similar device carrying an electrical current to slice and simultaneously cauterize the cervical tissue.

Hysterectomy

Hysterectomy usually involves the *en bloc* resection of the uterus and cervix, although the specimen type varies somewhat based on the underlying pathology. Radical hysterectomy with pelvic lymph node resection is the intervention of choice for the treatment of invasive cervical cancer in patients who are surgical candidates (typically those with disease stage ≤IB). Patients with higher stage disease are often treated using neoadjuvant chemotherapy and/or radiation. SILs may be encountered overlying and/or adjacent to areas of invasion in patients with squamous cell carcinoma. SIL can also be seen without associated invasive malignancy as the decision to perform hysterectomy may be spurred by recalcitrant SIL, particularly when childbearing is not at issue. Finally, SIL may be an incidental finding in hysterectomies performed for benign or non-cervical neoplastic reasons.

Gross/Colposcopic Features

With the exception of exophytic lesions such as condyloma acuminatum, cervical SILs are not typically visible to the naked eye. They may be clinically identified using colposcopy and the sequential application of normal saline, 3–5% dilute glacial acetic acid, and in some cases Lugol's iodine. Lesions may be demarcated from the background normal cervix based on the color tone/intensity of acetowhite areas, the margins and surface contour of acetowhite areas, and vascular features and color changes following acetic acid and/or iodine application.

The initial application of normal saline aids in the identification of vasculature and demarcates the transition zone borders. Subsequent application of acetic acid highlights intraepithelial lesions: LSILs are typically thin, smooth acetowhite lesions with irregular but well-demarcated margins, whereas HSILs are more often thick and dense with an irregular surface and raised or rolled margins and abnormal vascular patterns.

In the final (but for some optional) step, iodine solution is taken up by glycogen-rich normal squamous and mature metaplastic cells but not by columnar cells or intraepithelial lesions, further delineating cervical intraepithelial neoplasia from the background cervix. Directed biopsies can then be collected using information gleaned from this colposcopic examination. That said, colposcopic impressions are imperfect

correlates for microscopic findings; therefore, some discordance between the grossly anticipated grade and the ultimate diagnosis is not surprising.

Morphological Manifestations

Cervical dysplasia manifests microscopically either as LSIL (CIN1) or HSIL (CIN2/CIN3). These two basic morphological patterns reflect the status of HPV infection in the involved tissues. Although some assume that CIN1, CIN2, and CIN3 represent a continuous spectrum, linear progression through each level of dysplasia does not necessarily occur in all cases: varying degrees of dysplasia may occur in tandem with progression of LSIL to HSIL within the same biopsy, or they can occur independently as not all cases of LSIL are obligate precursors to HSIL and a "direct-to-HSIL" pathway is thought to exist.

Normal Squamous Epithelium

To understand the morphological appearances of cervical neoplasia, it is important to appreciate the maturation pattern of normal squamous epithelium. Normal anatomy is discussed in detail in Chap. 1 of this text and will be reviewed only briefly here. Squamous mucosa lines the ectocervix with transition to the glandular epithelium in the endocervix (Fig. 6.1). In normal squamous mucosa, cell division is restricted to basal and parabasal cells. These proliferative basal layers are responsible for populating the full thickness of the epithelium, with maturation and functional refinement (but not cell division) occurring as the cells become more superficial. Maturation manifests in a decrease in nuclear size and an increase in cytoplasmic volume, often with acquisition of cytoplasmic glycogen. The maturing cytoplasm stains differentially on ThinPrep-based cytology specimens with intermediate cells derived from the middle of the epithelium bearing pale blue cytoplasm and the most superficial and mature cells bearing abundant "orangophilic" cytoplasm. On H&E-stained slides, maturation manifests in decreasing nuclear size with concomitant increase in eosinophilic, keratin-enriched cytoplasm. Perinuclear clearing is often present due to the presence of cytoplasmic glycogen and should not be mistaken for koilocytic halos.

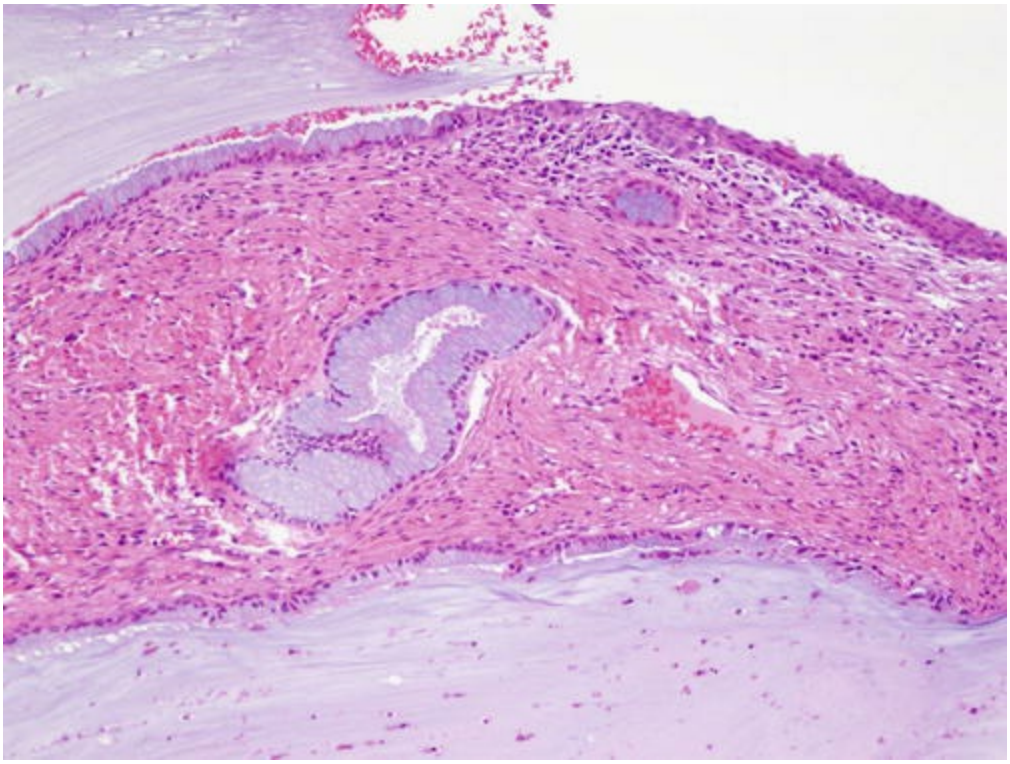

Fig. 6.1 The cervical transition zone, demonstrating a convergence of normal squamous and glandular mucosa

The degree of maturation is influenced by the amount of estrogen available either endogenously or exogenously. Complete maturation can be expected in epithelia from post-menarchal, premenopausal women who are not pregnant or lactating and are not using any hormonal contraceptives. Varying degrees of immaturity or atrophy may be seen approaching and after menopause, during pregnancy and lactation, and in the setting of hormonal contraceptives. The atrophic cervix is characterized by a predominance of basal, parabasal, and occasionally intermediate cells without a significant contribution of superficial cells.

LSIL/CIN1

In LSIL, HPV infects all levels of the epithelium; however, productive viral gene expression is restricted to those cells that have begun to mature. In the suprabasal zone, viral gene expression is restricted exclusively to the early viral genes. Further up in the epithelium, all viral genes are induced, and ultimately viral DNA is synthesized, leading to the production and assembly of virions in the most superficial cell layers. LSIL includes both flat lesions and exophytic lesions conventionally referred to as condylomata. Some pathologists have attempted to divide LSIL into lesions showing koilocytosis without atypia, condylomata, and CIN1, but these distinctions do not appear biologically relevant or clinically reproducible and are not recommended by LAST criteria [12, 19]. LSIL can be due to either high-risk or low-risk HPV types, with

high-risk HPV accounting for the majority (80–85%) of cases involving the cervix [21].

LSIL Histology

LSIL is morphologically characterized by koilocytic atypia. Koilocytes contain enlarged and irregular nuclei, sometimes with binucleation, surrounded by a region of cytoplasmic clearing (Fig. 6.2). The area of cleared cytoplasm shows an irregular edge (a "ribbon-like" or "calligraphy pen" border due to its vacillating thickness). The nuclear chromatin is coarse and irregular with either no nucleoli or only tiny, indistinct nucleoli.

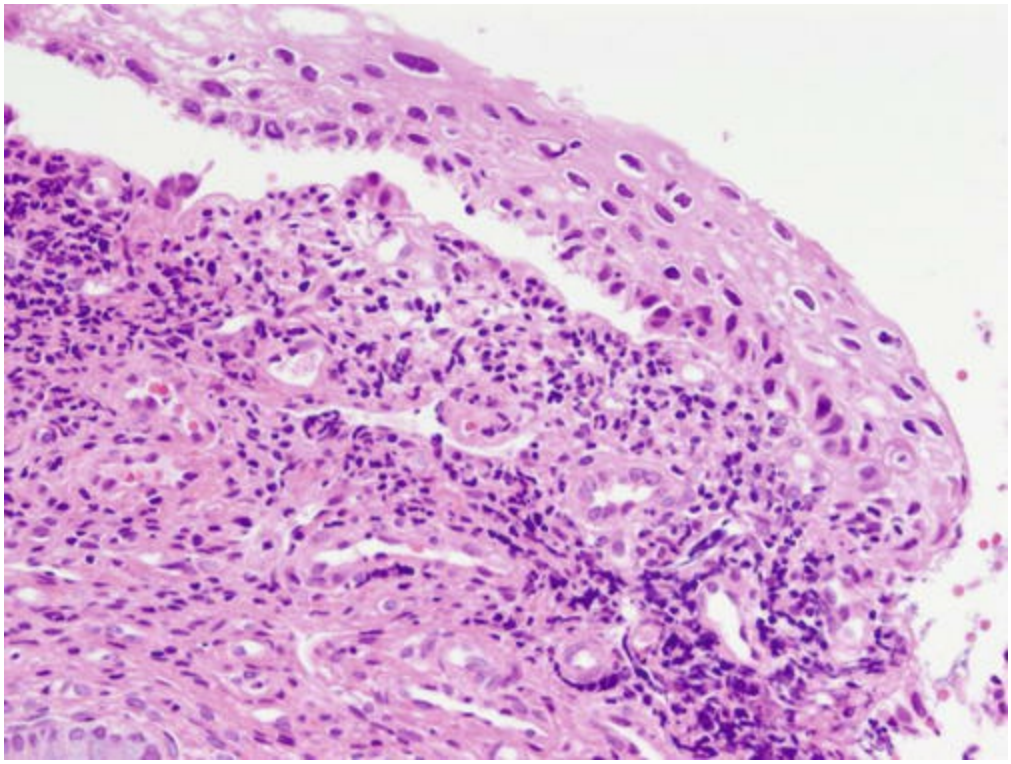

Fig. 6.2 LSIL histology. Koilocytic atypia extends into the superficial epithelium, and occasional binucleate cells are seen. Nuclear enlargement is marked, often exceeding the nuclear size of basal cells; however, cytoplasm remains abundant

Histological samples of LSIL may show a proliferation of basal-like cells with occasional mitoses, but this proliferation is limited to the lower third of the epithelium. Although simplified explanations of LSIL often imply that the cytological atypia ends there, it is critical to note that full-thickness atypia is indeed present in LSIL: enlarged, hyperchromatic, and irregular nuclei percolate throughout the upper portions of the epithelium. Indeed, that is why cytological specimens, which typically sample only the topmost layers of the epithelium, remain such an effective screen for LSIL. However, in contrast to HSIL, the surface atypia of LSIL is koilocytic in nature, with retention of abundant cytoplasm and a relatively low/normal nucleus/cytoplasm (N:C) ratio. Indeed,

the largest and most morphologically striking nuclei seen in HPV infections are typically identified in LSIL rather than HSIL, but unlike in HSIL these nuclei are seen in conjunction with considerable cytoplasm.

LSIL Cytology

The koilocytes observed on histological sections of LSIL can also be appreciated on cytological samples, with the aforementioned morphological features (perinuclear halos with thick, irregular borders, enlarged and irregular nuclei, binucleation, etc.) translating reliably across preparation types (e.g., ThinPrep, SurePath, conventional smears) (Fig. 6.3). Of particular significance in the assessment of cytological specimens is the relatively large size of LSIL cells when compared to HSIL cells. The smallest LSIL nuclei are 3× the size of an intermediate cell nucleus, but often they far exceed this. The large, eye-catching koilocytes of LSIL can occasionally distract from the smaller, more subtle cells (so-called "no-see-ums" or, alarmingly, "litigation cells") of a concurrent HSIL. The co-occurrence of LSIL and HSIL in such cases illustrates that sometimes HPV infection manifests as a spectrum of dysplasia, with some cases of LSIL either progressing to or occurring in tandem with HSIL. One must therefore be cautious not to jump to a diagnosis of LSIL when koilocytes are evident, instead pausing to carefully evaluate the background for coincident HSIL.

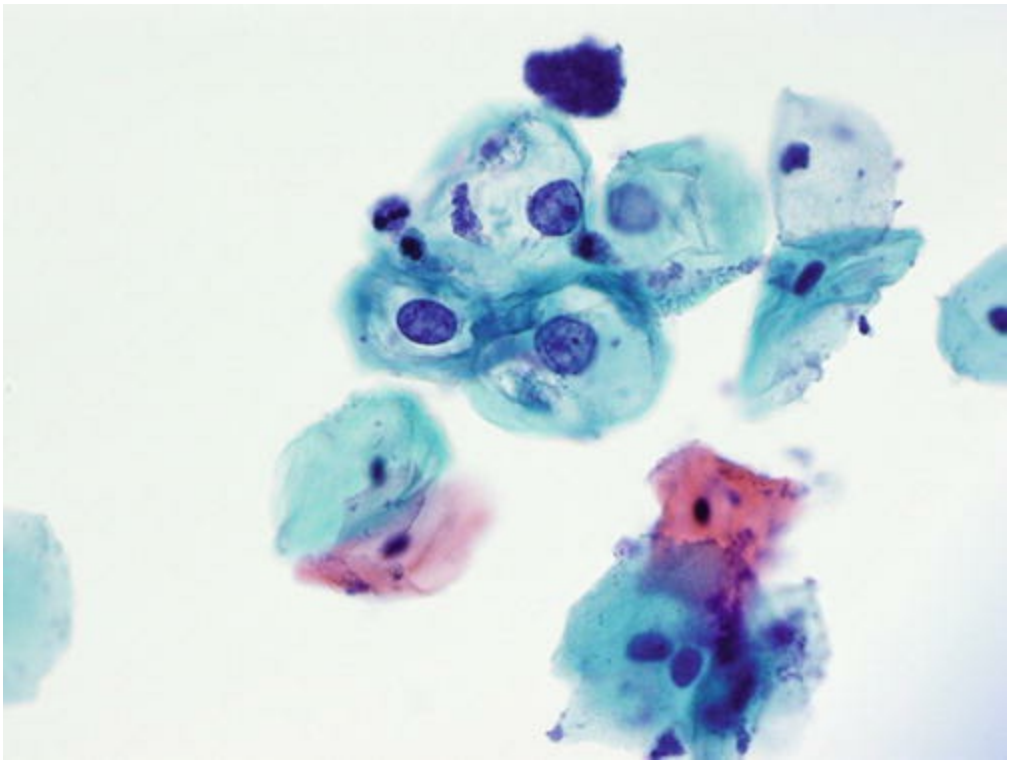

Fig. 6.3 LSIL cytology. A cluster of four koilocytes is present at the center of the image. These cells show nuclear enlargement ≥3× the background intermediate cells. Nuclear chromatin is coarse with focal irregularity of the nuclear membrane. The perinuclear halos have thick, irregular "calligraphy pen"-type borders

HSIL: CIN2

In straightforward cases LSIL is readily and reproducibly classified, but many cases present diagnostic difficulties. In particular, the CIN1/CIN2 interface can be challenging.

CIN2 Histology

On histological sections, the finding of "higher-riding" (e.g., in the middle third of the epithelium) atypical high N:C ratio cells and mitotic activity in the middle of the epithelium raises concern for moderate dysplasia, or CIN2, which represents the lower end of the HSIL spectrum and an area of significant diagnostic difficulty (Fig. 6.4). This difficulty is highlighted by the fact that CIN2 has the lowest interobserver reproducibility of all cervical diagnoses [19]. A common explanation for the overdiagnosis of CIN2 is the tendency of some observers to overinterpret high-riding nuclear atypia without attention to the associated cytoplasm. It is therefore worth reemphasizing: full-thickness nuclear atypia is allowable and expected for CIN I. It is the proliferation of high N:C ratio, basal-like cells, often with associated mitotic figures, into the middle or upper third of the epithelium that warrants consideration for a HSIL diagnosis. The more extensive the expansion of the cellular component, the more certain the diagnosis of HSIL. Yet, assessing the level of atypia and proliferative activity can also be complicated by tangential sectioning and epithelial sloughing; therefore a confident diagnosis of CIN2 requires clear visualization of the epithelium from base to surface. If orientation is suboptimal but CIN2 remains on the differential, level sections should be considered. If ambiguity remains, a diagnosis of "dysplasia" can be rendered and accompanied by a note explaining the issue. However, such equivocal, ungraded reads can generate understandable frustration for our clinical colleagues and patients and should therefore be used very sparingly.

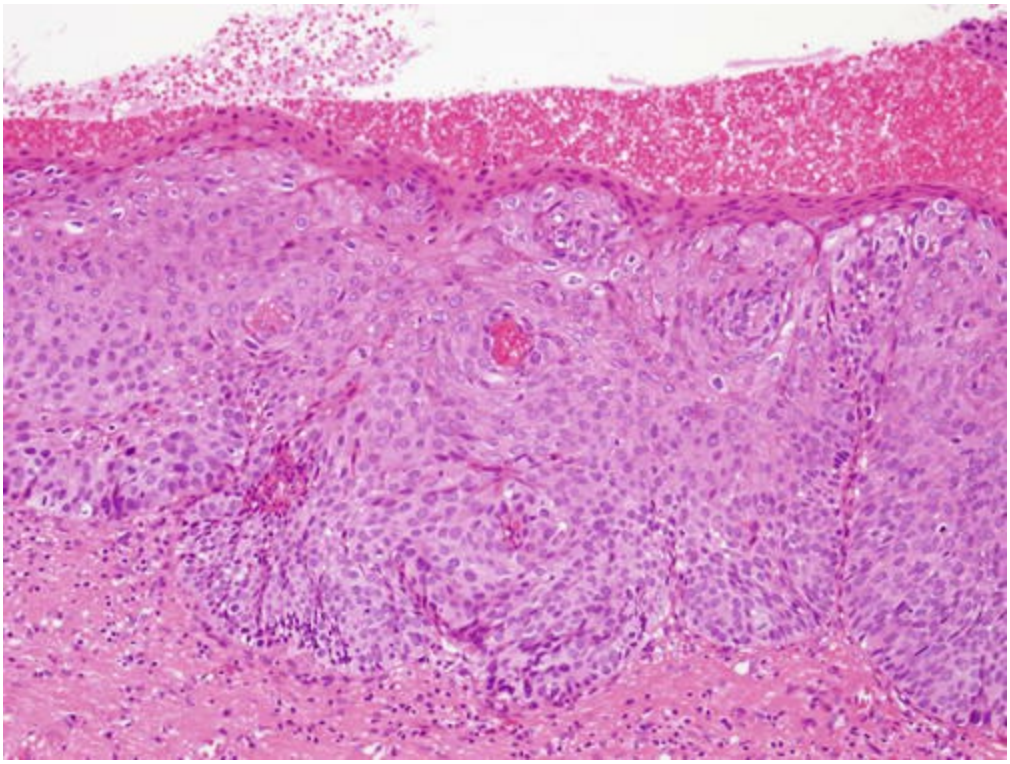

Fig. 6.4 HSIL (CIN2) histology. This case shows basaloid cells and mitotic activity percolating through the middle of the epithelium; however, the surface retains some degree of maturation with some preservation of the N:C ratio. This particular case of CIN2 sits closer to the CIN2/CIN3 interface as compared to the CIN1/CIN2 interface, with the limited degree of retained maturation preventing classification as "severe" dysplasia

Given the poor interobserver reproducibility for CIN2 the frustration generated by equivocal reads, and the importance of managing HSIL, LAST recommends the use of ancillary studies (specifically, p16 immunohistochemistry) for all cases of suspected CIN2 [12]. Recent work suggests that enlisting p16 downgrades roughly 1/3 of CIN2 lesions, preventing unnecessary follow-up in a significant subset of patients [22]. p16 interpretation is further discussed in the "Biomarkers" section of this chapter. It is also notable that LAST emphasizes that it is the distinction between LSIL and HSIL that guides management. Separating CIN2 from CIN3 even with biomarkers is only relevant in ASCCP guidelines for the management of women under age 25 with small lesions. Hence in many laboratories, CIN2 and CIN3 are combined as HSIL (CIN2–CIN3) for diagnostic purposes.

CIN2 Cytology

Likewise, it is not necessary to differentiate between CIN2 and CIN3 on cytological specimens; however, some pathologists elect to do so. CIN2 cells sampled on Pap tests generally demonstrate more abundant cytoplasm than their CIN3 counterparts, which are almost entirely bereft of the cytoplasm (Fig. 6.5). However, their N:C ratio exceeds what is allowable for LSIL, even though the nuclei may be smaller than those of LSIL.

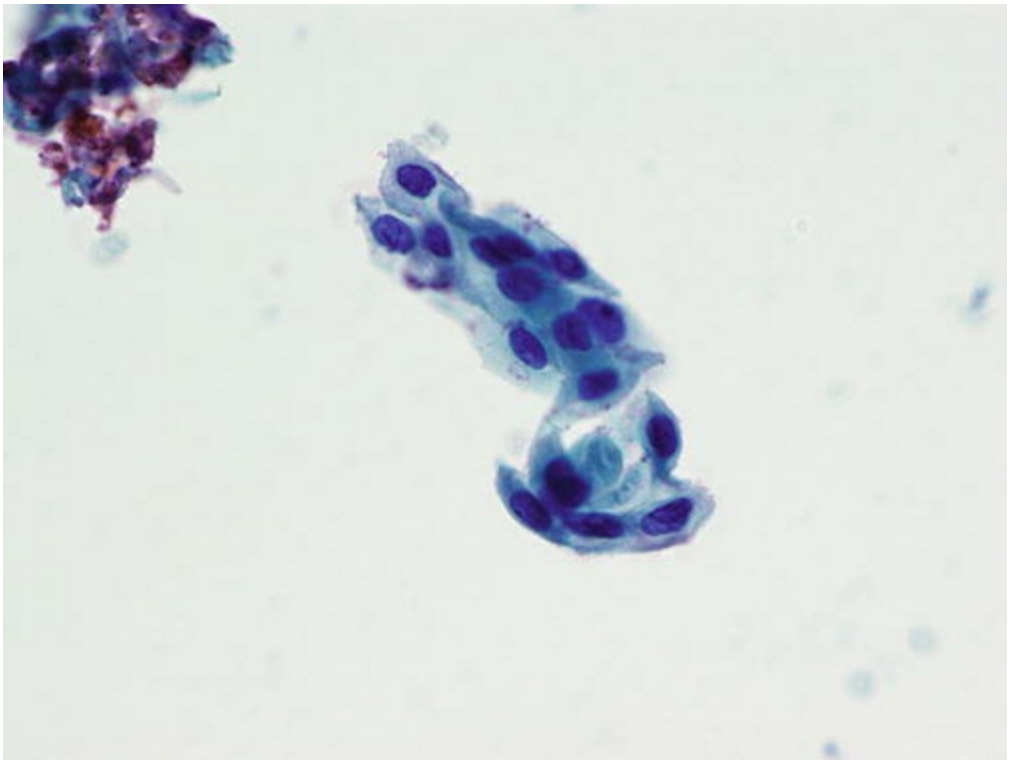

Fig. 6.5 HSIL (CIN2) cytology. This cluster of HSIL cells demonstrates nuclear enlargement and hyperchromasia with irregular nuclear borders. The nuclear size is less impressive than is typical of LSIL. While it is not necessary to subdivide CIN2 and CIN3 on cytology, the N:C ratio seen here is lower than is expected for severe dysplasia; therefore, classification as "HSIL" with the qualifier "moderate dysplasia" is acceptable

HSIL: CIN3

CIN3 represents the highest grade of dysplasia and denotes near or complete repopulation of the epithelium by a clonal expansion of proliferative, high N:C ratio basaloid cells. As previously mentioned, historic attempts to segregate CIN3 from CIS proved poorly reproducible and biologically inconsequential; therefore, the latter term has been discarded and these cases collapsed into CIN3. Often the constituent cells of CIN3 bear nuclei that are smaller and less strikingly pleomorphic than the cells observed in LSIL and are instead marked by their monotony.

CIN3 Histology

On histological sections, this monotony translates into an epithelium that essentially shows no distinction between the superficial and basal layers: in the most classic cases, one could imagine flipping it over entirely, so that the superficial cells lined the base and vice versa, without changing the appearance (Fig. 6.6). Mitotic figures are commonly present and extend into the upper third of the epithelium (Fig. 6.7). This impressive appearance leads to relatively high interobserver reproducibility in the diagnosis of CIN3 when compared to CIN2, although benign mimics such as squamous

metaplasia and atrophy can lead to diagnostic difficulty. It is critical to note that while there is remarkable monotony within a CIN3 lesion, that lesion should differ significantly from the background squamous epithelium. The presence of a morphologically distinct clonal population is a hallmark of dysplasia. If the entire epithelium is lined by relatively uniform basaloid, high N:C ratio cells, the differential of atrophy should be considered.

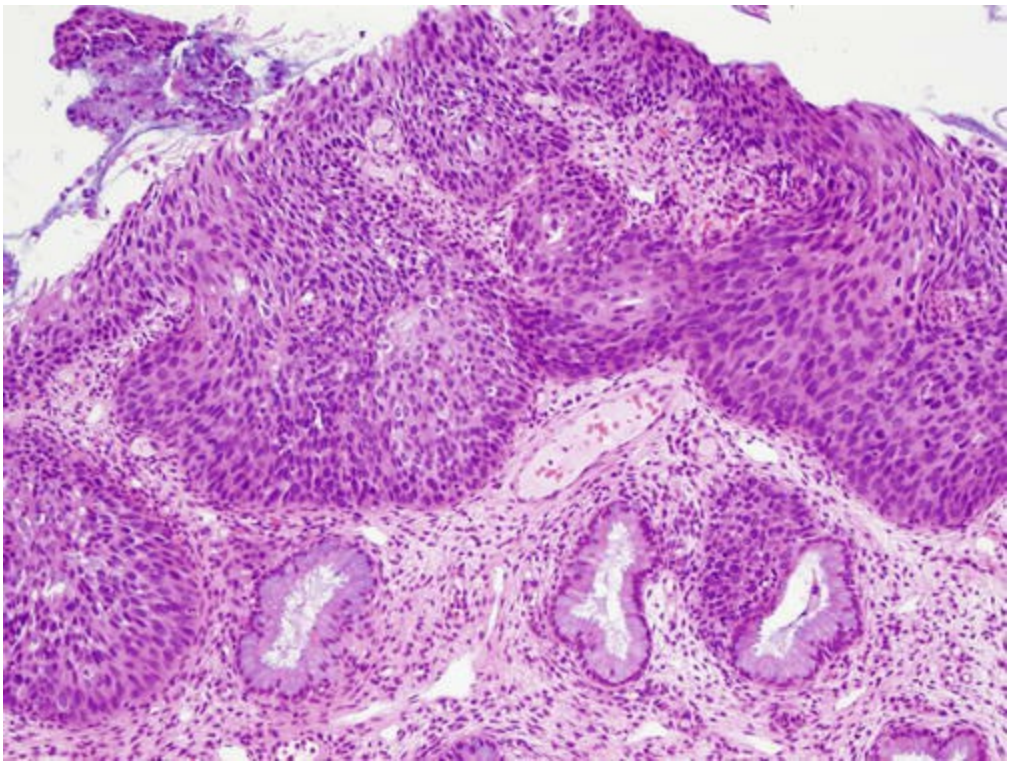

Fig. 6.6 HSIL (CIN3) histology. The epithelium is completely repopulated by basaloid cells with nuclear enlargement, hyperchromasia, and irregular nuclear outlines. Cytoplasm is scant and uniformly distributed across the thickness of the epithelium. Mitotic figures are readily identifiable and extend to the surface

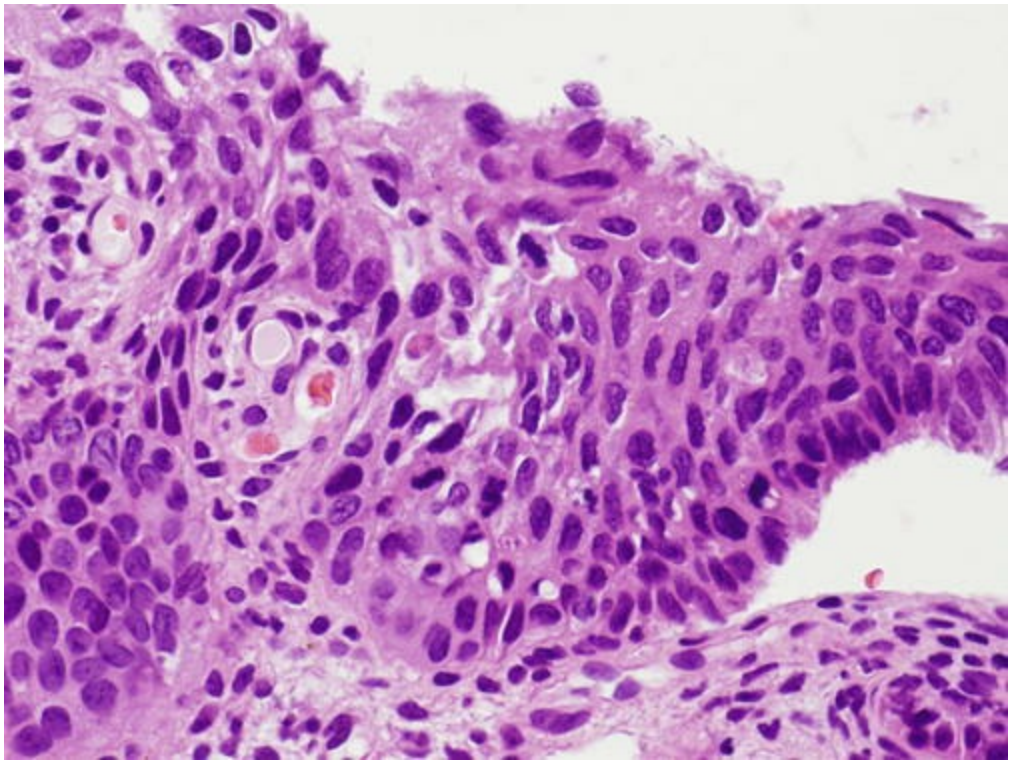

Fig. 6.7 HSIL (CIN3) mitotic figures. This high-power view case demonstrates the abundant, high-riding mitotic figures that can be appreciated in HSIL (CIN3)

CIN3 Cytology

CIN3 cells can be subtle on cytological preparations, in large part due to their relatively small size. Although two to three times larger than intermediate cell nuclei, unlike LSIL cells they are not often much bigger than that (Fig. 6.8). Furthermore, they typically constitute a relatively small proportion of total cellularity and are not apparent on quick or low-power review. Missed HSIL is therefore unsurprisingly a common source of diagnostic misadventure for cytopathologists, leading to the name “litigation cells.” As previously emphasized, HSIL can occur in tandem with LSIL, and a diagnosis of LSIL is premature without careful review for accompanying HSIL.

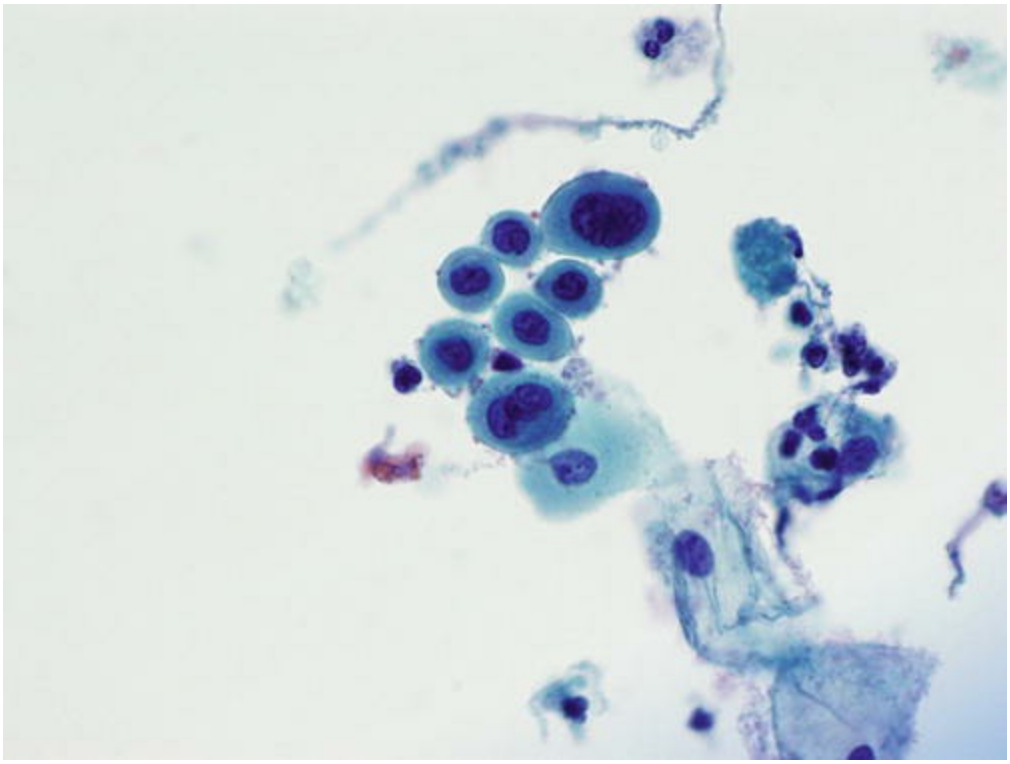

Fig. 6.8 HSIL (CIN3) cytology. This loose aggregate of cells shows nuclear hyperchromasia, notched and irregular nuclear borders, and scant cytoplasm. Notably, most of the cells in the cluster are quite small and could easily be overlooked in isolation; only one of the cells in the group has a nucleus that significantly exceeds the dimensions of the background intermediate cells

HSIL Variants

Thin HSIL

Thin HSIL is an immature squamous epithelial lesion typically measuring fewer than ten cells in thickness. These lesions may be difficult to differentiate from atrophy or repair, and their diagnosis can be aided by the use of ancillary biomarkers such as p16.

Keratinizing HSIL

Keratinizing HSIL has an atypical surface layer bearing dyskeratotic and pleomorphic nuclei. These lesions are often encountered in the ectocervix and are reminiscent of the dysplasia seen in high-risk HPV-related cancers outside the cervix, such as the vulva and penis. Care must be taken not to underestimate these lesions as LSIL based on the presence of moderate to abundant keratin-rich cytoplasm. Keratinizing HSIL can also be found in lesions which are clinically condylomas, and in these instances, the HSIL dictates the prognosis.

Papillary HSIL

Papillary HSIL (e.g., “noninvasive papillary squamo-transitional carcinoma” or “papillary squamous carcinoma in situ”) is reminiscent of urothelial neoplasia. It is a diagnosis contingent on complete excision with exclusion of stromal invasion.

Endocervical Gland Extension

Any grade of squamous dysplasia can show endocervical gland extension (Fig. 6.9). Distinguishing such glandular extension from invasive carcinoma is usually straightforward but can occasionally lead to diagnostic difficulty. Architecturally, endocervical gland extension should retain the distribution pattern of the native glands; therefore, “outlier” squamous nests which are (in a well-orientated section) significantly deeper than the normal endocervical glands should make one pause. The borders of squamous nests are smooth in endocervical gland extension and coordinate with confinement to the preexisting gland; therefore, jagged and irregular nest borders provoke concern. Finally, nests populated exclusively by high N:C ratio, basaloid cells are reassuring for endocervical gland extension by HSIL rather than invasive carcinoma because invasive cancers typically demonstrate paradoxical maturation with accumulation of acidophilic cytoplasm and occasional keratin pearl formation. Paradoxical maturation is not, however, sufficient for a diagnosis of invasion as this can occasionally be seen in nests of endocervical extension which remain clearly confined by the basement membrane.

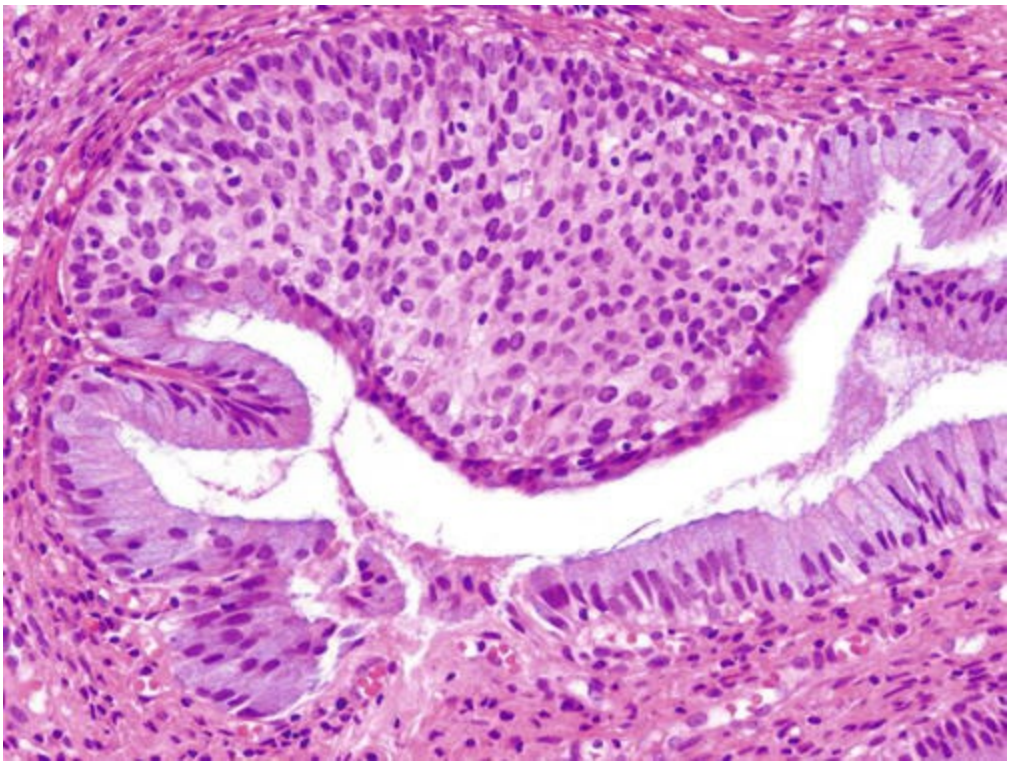

Fig. 6.9 Endocervical gland extension. This endocervical gland has been partially replaced by a basaloid population of

mitotically active squamous cells, consistent with involvement by HSIL. Notably, the native endocervical cells are benign without features suggestive of adenocarcinoma in situ. The borders of the squamous population remain smooth and even, raising no concern for invasion

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Atypical Squamous Cells (ASC and ASC-H)

A proportion of cytological samples (ideally around 5%) will be classified as atypical squamous cells of uncertain significance (ASC) because they show some findings which provoke concern for LSIL (such as nuclear enlargement and cytoplasmic halos) but which fail to meet diagnostic criteria for SIL. A much smaller subset (typically 10% of the 5% ASC cases) will be classified as "atypical squamous cells, cannot rule out HSIL" (ASC-H) when the morphological differential includes HSIL [19, 23].

It is well established that a significant proportion of ASC and (less often) ASC-H diagnoses will be attributable to benign microscopic mimics such as reactive changes, squamous metaplasia, and atrophy. Such benign changes are more likely to underlie atypia among older women as the proportion of ASC cases attributable to biopsy-confirmed dysplasia is much lower in postmenopausal (17%) when compared to premenopausal women (46%) [24]. The classification of a subset of benign cases as ASC/ASC-H should not be considered erroneous, as the goal of a screening test is to maximize sensitivity with acceptance that a proportion of negative cases will be identified. In contrast, in tissue biopsies similar equivocation is to be discouraged especially with the use of biomarkers.

HPV co-testing or reflex testing results are currently enlisted on cytological samples to triage ASC cases, with HPV-positive ASC treated as tantamount to an LSIL diagnosis in most cases. Indeed, ASCCP guidelines have taken a risk factor-based approach to combining HPV testing with cytology for better management stratification. HPV testing is further discussed in the "Biomarkers" section.

Microscopic Mimics

There are several important mimics to consider in the assessment of cervical dysplasia, including reactive atypia, basal cell hyperplasia, squamous metaplasia, and atrophy. These mimics are discussed in detail in the preceding chapter from this text but will also be addressed briefly here.

Reactive Changes

Reactive atypia is frequently encountered and causes considerable diagnostic difficulty. Reactive changes can present problems on Pap specimens, biopsies, and excisions (particularly when these interventions are temporally close to one another and therefore subject to post-procedural responses). In general, the presence of an acute inflammatory

background can cause considerable nuclear atypia in any of these specimens, and its presence should cause one to retreat from (or at least pause very carefully before rendering) a diagnosis of dysplasia.

On cytology, reactive features include a streaming appearance to cellular clusters with "pulled-out," elongated cytoplasm with tapered edges. This so-called "school-of-fish" appearance is reassuring as to the group's reactive nature. Attention to nuclear detail is critical for distinguishing reactive from dysplastic changes on both cytology and histology. Prominent nucleoli can provide reassurance as to the benign nature of a benign reactive proliferation, particularly when the surrounding chromatin is smooth and even and all nuclei are similar (e.g., pleomorphism and anisocytosis are minimal). Rounded nuclear borders also favor a benign diagnosis, whereas sharp nuclear contours and clefting are more typical of dysplasia. Assessments of nuclear irregularity must also account for nuclear size. Inflammatory and physiological vacuoles can also prompt misdiagnosis of LSIL in benign biopsies when nuclear size is ignored. Traditional teaching about cervical dysplasia places tremendous emphasis on the irregular, so-called "rasinoid" nuclei seen in koilocytes; however, it is important to note that small, irregular, darkly staining nuclei commonly reside in glycogen pools of the normal cervical epithelium. However, when nuclei are small (<2× the size of an intermediate cell nucleus), irregularity and mild hyperchromasia are not alarming. Currently available biomarkers are of little utility in the distinction of LSIL from reactive lesions in histological sections. HPV RNA ISH shows some early promise for addressing this differential diagnosis, but more studies are needed to elucidate its potential value. Available ancillary studies are further discussed in the "Biomarkers" section.

Basal Cell Hyperplasia

Basal cell hyperplasia is typified by a thickened basal/parabasal zone with preserved maturation in the overlying epithelium. Because it results in basaloid, higher N:C ratio cells within the middle of the epithelium, it may be mistaken for HSIL. Because this proliferation ascends higher in the epithelium, it may be sampled on cytology specimens. As with reactive proliferations, attention to nuclear features is key for excluding dysplasia and confirming the benign nature of this proliferation. The nuclei remain oval and euchromatic without appreciable mitotic activity.

Squamous Metaplasia

Squamous metaplasia is a normal physiological process characterized by the nonneoplastic transformation of endocervical cells into a squamous phenotype. It can provoke concern for dysplasia because the nuclei of squamous metaplastic cells are invariably larger than those of the native squamous epithelium. Squamous metaplasia causes diagnostic difficulty on both cytology and histology samples and may be mature

or immature, depending upon the amount of accumulated cytoplasm.

Mature squamous metaplasia may provoke concern for LSIL because the cells show nuclear enlargement with relatively abundant cytoplasm. Of note, the cytoplasm in mature squamous metaplastic cells is denser than is seen in the squamous epithelium, and cytoplasmic borders are often well demarcated. Often angulated cytoplasmic tails and projections are present. Despite their enlargement the nuclei retain smooth borders and even nuclear chromatin, mitigating against a dysplasia diagnosis. Importantly, p16 immunostaining has no role in the distinction of LSIL from mature squamous metaplasia [25, 26].

Immature squamous metaplasia is characterized by cells with nuclear enlargement and a paucity of cytoplasm. It may therefore raise concern for HSIL but lacks the nuclear atypia and cellular crowding characteristic of dysplasia. Mitoses may be observed on histologic specimens but should be restricted to the lower one-third of the epithelium. p16 is of value in this differential, as is further discussed in the "Biomarkers" section [25, 27].

Atrophy

Atrophy is among the principal differential diagnoses for HSIL lesions, as it is characterized by a relatively uniform population of basal- and parabasal-type cells with high nuclear/cytoplasmic ratios. The uniformity of this population across the sampled epithelium is of critical significance, as HSIL lesions typically have a clonally distinct appearance when compared to the background. This becomes evident in the evaluation of atrophic cytology specimens: at first glance, the reviewer may be struck by an abundance of single cells with high N:C ratios and therefore tempted to categorize them as HSIL; however, further review of the slide reveals that virtually all cells share the observed changes. This uniformity provides reassurance as to the benign, atrophic nature of the specimen. Atrophic cytology samples often contain so-called blue blobs, which are somewhat amorphous bodies though to represent degenerating cells. Though eye-catching and potentially suggestive of HSIL cells, high-power review reveals an absence of distinct nuclear and cytoplasmic structure, thereby exonerating them.

Atrophic epithelium can also be concerning on hematoxylin and eosin-stained sections, where it manifests as an attenuated lining composed of basaloid cells. As in cytological preparations, the paucity of cytoplasm may raise concern for high-grade dysplasia; however, the ubiquity of the change provides reassurance. Nevertheless, atrophy is at the top of the differential diagnosis for HSIL, and differentiating these two entities may be difficult, particularly when sloughing limits assessment of the background and full thickness of the epithelium. Comfort with benign atrophy can be obtained by studying the cervices of hysterectomy specimens collected from postmenopausal women without history of cervical disease. Ancillary molecular and

immunohistochemistry studies can be enlisted in difficult cases (see "Biomarkers" section for further details).

Squamous Cell Carcinoma

While covered in more detail in Chap. 8, squamous cell carcinoma (SCC) warrants brief discussion here for direct comparison with SIL. On histological sections the differentiation of cervical squamous dysplasia from invasive squamous cell carcinoma is usually straightforward but can be quite difficult in cases with only superficial invasion. Tangential sectioning of a tongue of dysplastic epithelium must be excluded in cases with suspected invasion which show only rare infiltrative-appearing squamous nests lying close to the surface. The presence of a stromal desmoplastic or inflammatory response provides helpful support for a diagnosis of invasion when present (Figs. 6.10 and 6.11).

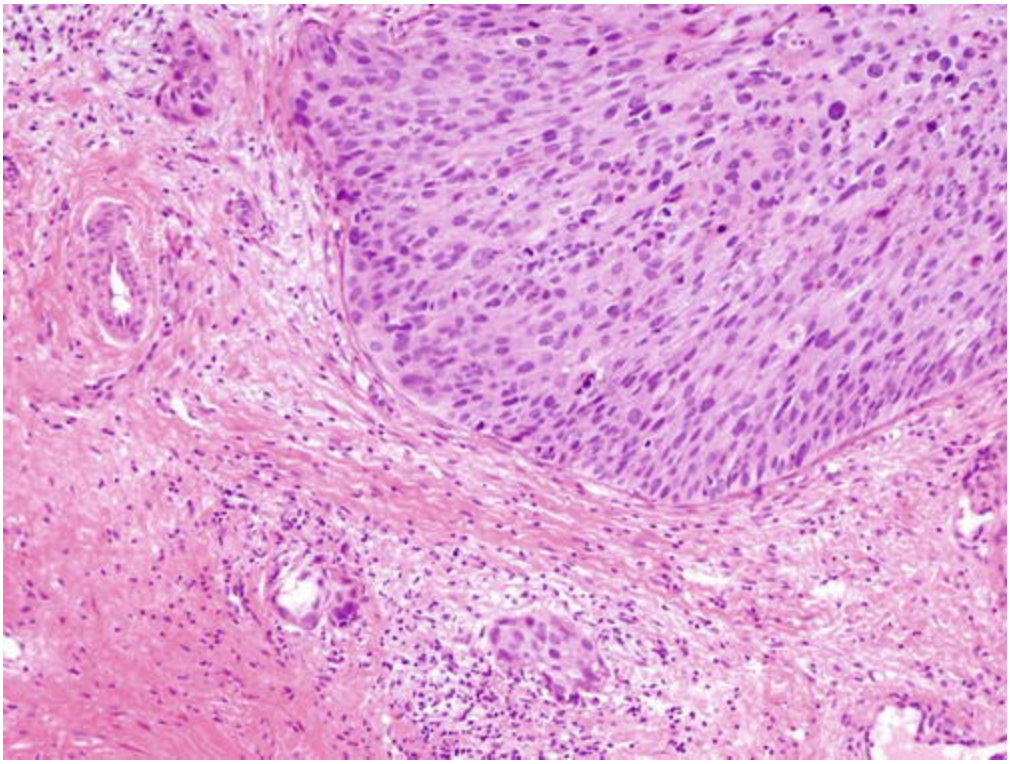

Fig. 6.10 HSIL with superficially invasive squamous cell carcinoma. This specimen showed extensive HSIL (CIN3) with endocervical gland extension, as demonstrated by the large, blunt-edged nest in the upper right-hand side of the image. However, several foci of jagged, irregular squamous nests are seen underlying the in situ lesion

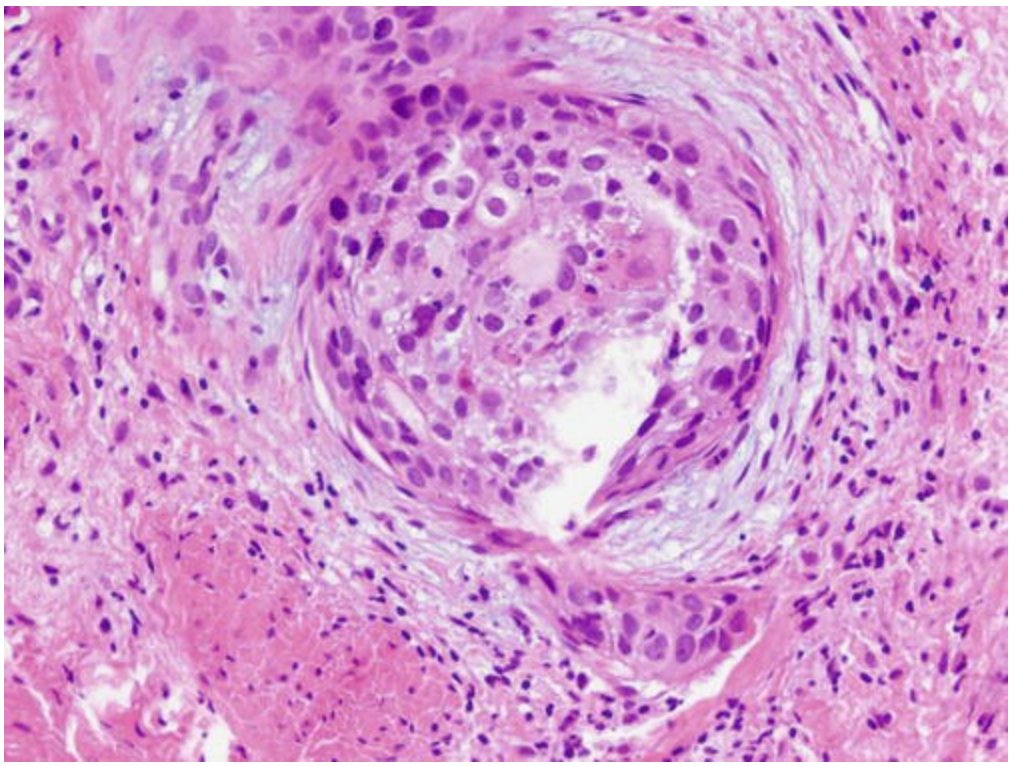

Fig. 6.11 Superficially invasive squamous cell carcinoma. This high-power image of a different area from the case depicted in Fig. 6.10 shows a squamous nest with surrounding desmoplasia and a chronic inflammatory cell infiltrate, supporting the presence of invasion

Occasional cases with dysplasia with extensive endocervical gland extension may also present difficulty. The presence of squamous nests with jagged, irregular borders raises considerable concern for invasion, as do nests located outside of the normal pattern of endocervical glands. Paradoxical squamous maturation with accumulation of eosinophilic cytoplasm and keratin pearl formation is a worrisome feature suggestive of progression and, although not independently confirmatory of invasion, should prompt a careful search for invasion elsewhere in the lesion.

Cytological samples of invasive squamous cell carcinoma are characterized by markedly atypical cells arranged in a background of tumor diathesis. The constituent cells of conventional squamous cell cancers typically show abundant keratinous cytoplasm, a feature known as paradoxical maturation. Often this cytoplasm is elongated and tapered, with individual "tadpole cells" showing very prominent cytoplasmic projections. Notably, these features have considerable overlap with reactive changes; therefore, the chief differential for SCC on cytology is actually reactive change, rather than SIL. However, the degree of nuclear atypia in SCC far exceeds what is allowable in reactive changes, and the cell-to-cell variation is more marked. Furthermore, reactive processes should not show individual atypical cells. Finally, chromatin irregularity and prominent nucleoli are common in cancer but not in SIL.

Because SCC often has a relatively low N:C ratio, LSIL must also be on the

differential. Nuclear atypia is of limited utility in resolving this differential because LSIL is permitted to have marked nuclear atypia, often exceeding the pleomorphism and enlargement of its high-grade counterparts. The background tumor diathesis is critical for distinguishing LSIL from SCC; however, it is important to note that liquid-based preparations will considerably reduce the background inflammation and debris and failure to appreciate a muted diathesis may lead to mis-categorization of a well-differentiated conventional squamous carcinoma as LSIL.

Differentiating SCC from HSIL can be problematic when the invasive component is predominantly basaloid. In cases of basaloid SCC, the volume of atypical high N:C ratio cells may be among the most diagnostically useful features of the specimen, since HSIL cells are usually relatively rare. Diathesis remains critical for SCC diagnosis, but may not be apparent in liquid-based samples. In these cases a diagnosis of "At least HSIL, suspicious for invasive squamous cell carcinoma" should be considered.

Biomarkers

A variety of immunohistochemical markers, in situ hybridization platforms, and molecular assays have been utilized as adjuncts in the diagnosis of squamous intraepithelial neoplasia. These biomarkers are applied both for diagnosis (e.g., to confirm the presence of HPV infection and/or the presence/degree of dysplasia) and prognostic purposes (e.g., predicting the likelihood that a lesion will progress). The diagnostic utility of a variety of biomarkers has been well established; prognostically useful biomarkers, however, are more difficult to come by. These biomarkers can be applied to both cytological and histological samples, with varying strengths and applicability in each setting.

Immunohistochemistry

p16

p16 immunohistochemistry is the most widely enlisted biomarker in the uterine cervix. p16 protein is thought to accumulate in the setting of transcriptionally active HPV infection as a response to unchecked proliferation due to the viral E7 oncoprotein's interference with the tumor suppressor protein retinoblastoma (pRb). It is therefore important to note that high-risk HPV does not interfere directly with p16, nor is p16 mutated in these cancers: rather, p16 overexpression serves as a proxy for downstream molecular changes. As such, p16 should not be considered a globally specific marker of HPV infection; indeed, this tumor suppressor protein can be overexpressed in a variety of non-HPV-related malignancies such as myometrial leiomyosarcoma. However, it has strong specificity for the presence of a high-risk HPV-driven lesion in the appropriate anatomic context (mainly, the mucosa of the anogenital tract and the oropharynx).

p16's utility in anogenital locations is chiefly in the diagnosis of high-grade dysplasia. It is most often performed on tissue sections but has occasional (and potentially growing) utility in cytology preparations [28]. Although some early studies suggested that p16 may be able to differentiate between LSIL and its benign/reactive mimics that has not borne out in subsequent works, which showed unreliable p16 marking in histologically unequivocal CIN1 and frequent patchy, blush-like staining in morphologically benign epithelia [25] (Fig. 6.12). Such focal, predominantly cytoplasmic staining should be considered negative. In contrast, the presence of confluent groups (e.g., >5–6 cell thickness) of cervical epithelial cells with nuclear and/or nuclear and cytoplasmic p16 positivity has robust sensitivity for HSIL and can aid both in upgrading cases at the CIN1/CIN2 interface and confirming dysplasia in cases of possible HSIL with a differential of atrophy or metaplasia [22, 27] (Figs. 6.13 and 6.14). The LAST recommendations therefore advocate the use of p16 immunohistochemistry in all cases of suspected CIN2 as well as cases with a differential diagnosis of CIN3 vs. benign (atrophy, squamous metaplasia, etc.) [12]. The LAST report defines p16 positivity as "continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upwards involving at least 1/3 of the epithelial thickness. The latter height restriction is somewhat arbitrary but adds specificity." This type of positivity is often referred to as "block type." Note that cytoplasmic staining alone is considered negative.

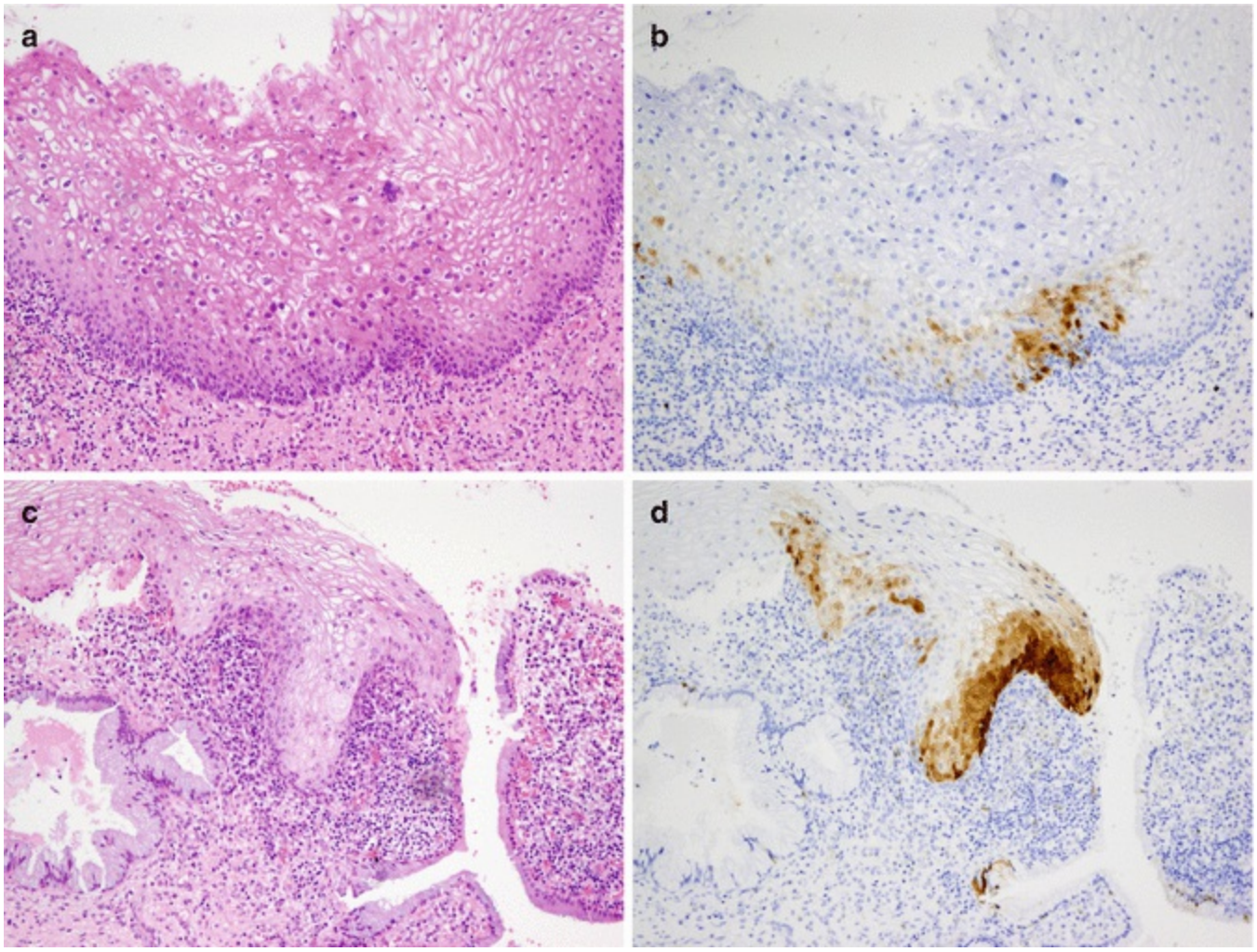

Fig. 6.12 p16 in LSIL. LSIL shows variable staining with p16, with some cases showing completely negative or only blush-like occasional staining (which is considered negative) ((**a**, **b**), H&E and p16, respectively) and others showing strong, block-like positivity extending into the middle epithelium ((**c**, **d**), H&E and p16, respectively)

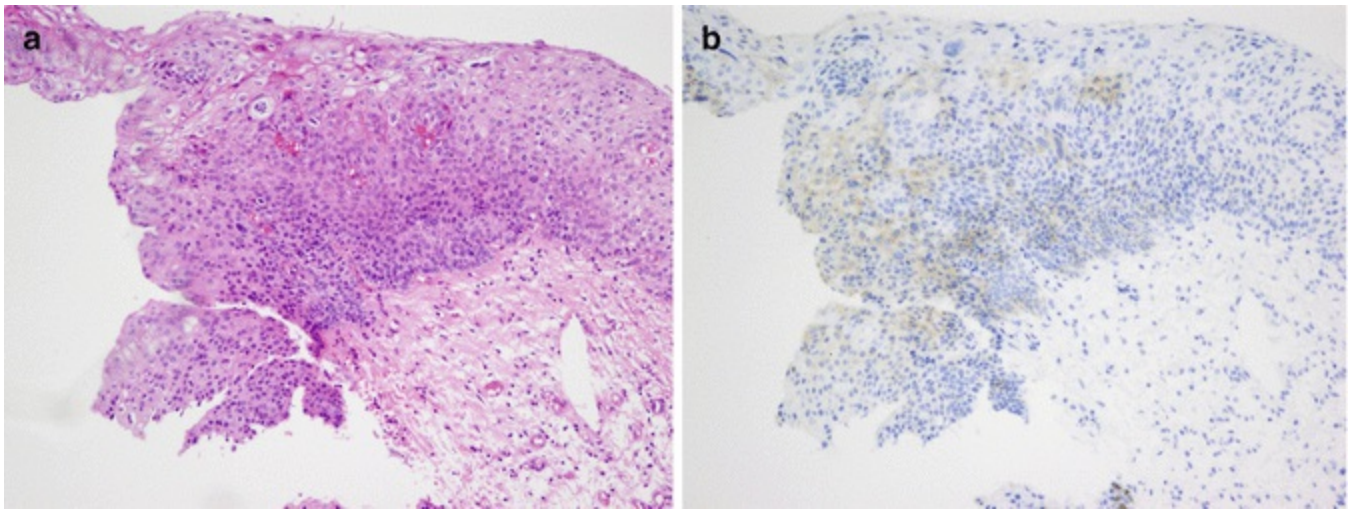

Fig. 6.13 p16 in CIN1 vs. CIN2. p16 immunostaining is recommended by LAST [12] in cases at the CIN1/CIN2 interface, but is not always a perfect stratifier. This challenging case shows predominantly koilocytic atypia more

suggestive of LSIL but some higher-riding atypical mitotic figures concerning for HSIL (**a**). However, p16 is negative with only blush-like cytoplasmic positivity (**b**), a pattern that would argue against HSIL. Of note, unequivocal p16 positivity was present elsewhere in this case; therefore, a diagnosis of HSIL was ultimately rendered

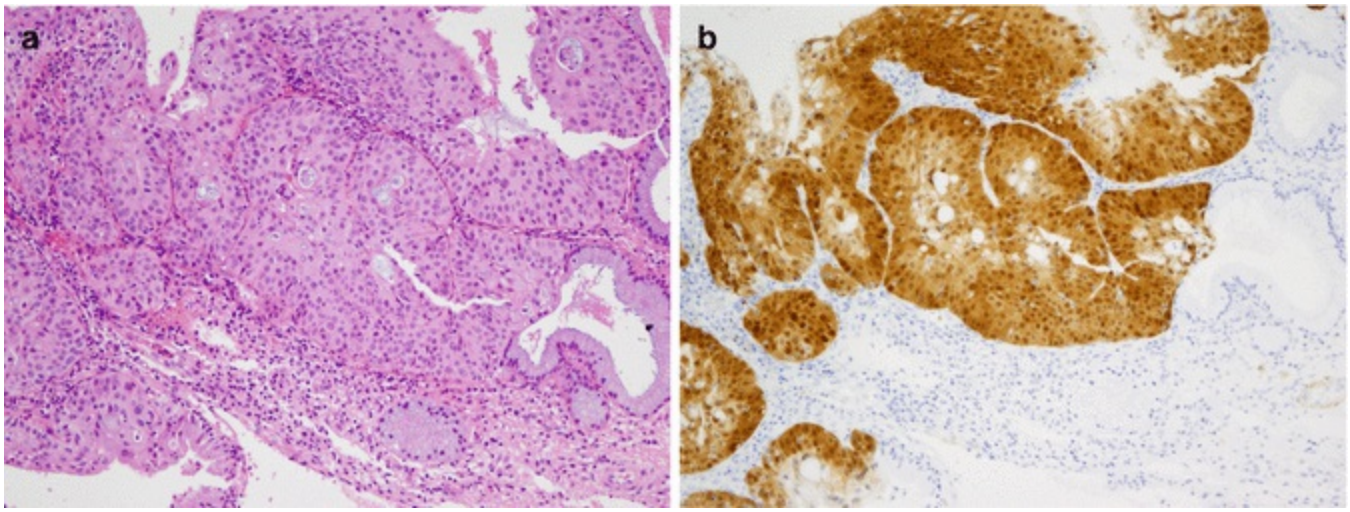

Fig. 6.14 p16 in HSIL vs. reactive/metaplastic epithelium. p16 immunostaining is also of value for confirming HSIL in cases with a differential diagnosis of reactive, metaplastic, or atrophic changes. Although very mitotically active, this HSIL case has a somewhat metaplastic appearance with more abundant cytoplasm than is typical of dysplasia, as well as the very well-demarcated cytoplasmic edges characteristic of dysplasia (**a**). Furthermore, the background is inflammatory, and nucleoli are focally prominent, invoking the possibility of a reactive process. However, the strong diffuse nuclear p16 positivity extending throughout the epithelium permits unequivocal classification as HSIL (**b**)

Although the diagnostic value of p16 immunohistochemistry in the uterine cervix is well established in these scenarios (e.g., diagnosis of CIN2 and CIN3 vs. mimics), p16 falters when it comes to prognostication. In recent years there has been considerable interest in identifying biomarkers that can predict which LSIL lesions will progress to HSIL. This small subset of cases (roughly 10% of LSIL) presents considerable managerial difficulty as it necessitates close clinical follow-up and has prompted consideration of excision for all LSIL, despite the fact that the vast majority of these cases will resolve spontaneously without intervention.

Although initial studies suggested that p16 immunostaining may have a role in flagging the subset of LSIL cases that will progress to HSIL, these studies had incomplete disease ascertainment and poor control for interpretive variability, which is known to be considerable at the CIN1/CIN2 interface [19, 29, 30]. Subsequent works—including a recent study of LSIL/CIN1 expert-adjudicated cases—have deflated this promise, and at this point p16 is not considered a reliable prognostic marker in LSIL cases [22, 26, 31, 32].

ProExC

ProExC has been investigated as another immunohistochemical marker of cervical

intraepithelial lesions. This immunomarker contains antibodies against topoisomerase II alpha and minichromosome maintenance 2 proteins, both of which can be overexpressed in a variety of neoplasms including cervical dysplasias and carcinomas. It showed initial promise as a useful adjunct to p16 staining in tissue sections of difficult squamous cervical lesions [33–35] but has in practice failed to provide significant added value to p16. That said, it can be enlisted in cases with equivocal morphology and p16 results.

Ki67

The proliferative marker Ki67 has also been enlisted as an ancillary test in the diagnosis of cervical neoplasia [36]. It has some value in limited diagnostic samples, where its negative predictive value can prevent unnecessary colposcopy [37], and may be useful in conjunction with p16 for morphologically equivocal cases. It has no clear utility, however, in the triage of morphologic CIN1 cases [38].

CK7 and Other SCJ Markers

Cytokeratin 7 (CK7) has appeared more recently as a putative prognostic marker in tissue sections of cervical intraepithelial neoplasia; its role in cytological specimens has not been investigated. CK7 is thought to mark a biologically distinct squamocolumnar junctional (SCJ) cell population which represents the putative origin of HSIL [39, 40]. Herfs, Crum, and colleagues have done meticulous work on the presence and behavior of this cellular subset and drew on their findings to position CK7 as a potential stratifier for LSIL cases, with CK7 marking the subset more likely to progress to HSIL. The prognostic significance of CK7 has borne out in preliminary studies from our laboratory but only after rigorous definition of what constitutes CK7 positivity ("diffuse" staining only, i.e., block-like positivity with a thickness of at least five to six contiguous cells) [41]. Larger studies are required before this biomarker should be enlisted in the clinical setting, but it holds initial promise. A variety of other markers (including CK17, p63, and MMP7) have also been posited as SCJ markers, but CK7 is thus far the most thoroughly validated of this group [42, 43].

In Situ Hybridization (ISH)

HPV DNA ISH

Theoretically all CIN should be HPV positive; therefore, in situ hybridization (ISH) for HPV DNA is an appealing adjunct for the diagnosis of these lesions. However, HPV DNA ISH has historically shown robust specificity but less-than-optimal sensitivity for the detection of high-risk HPV, with poor detection in cases with low viral copy numbers [34]. It also performed inferiorly to p16 in differentiating HSIL vs. benign

mimics [27]. No doubt these defects in sensitivity can be attributed to a variety of technical factors such as limited probe types and other analytical variables. Nevertheless, HPV DNA ISH had value as an adjunct to H&E in improving the specificity, particularly in the minimization of false-positive CIN1 results [44].

HPV RNA ISH

As noted above DNA ISH is an imperfect proxy for clinically significant infection, that is, transcriptionally active HPV. An HPV RNA ISH assay that can be performed on formalin-fixed, paraffin-embedded tissue has therefore been much sought after in this field. The last 2 years have seen the emergence of such assays, and early investigations of their sensitivity and specificity have shown great promise. Other studies of HPV E6/E7 RNA ISH patterns may be able to effectively stratify CIN [45]. Further validation of HPV RNA ISH assays is needed prior to their enlistment in the routine practice setting, and these efforts should be expedited given the clinical need for better assays to assign HPV as an etiological agent for a given pathological sample.

HPV Molecular Assays in Solution

HPV detection by PCR or other similar methods are the only biomarker techniques that can be applied to patient material which is suspended in solution; all the previously discussed IHC and ISH biomarkers are applied directly to tissue/cells of interest, allowing correlation between morphology and biomarker positivity, maximizing specificity. While PCR-based assays are most common, other solution-based assays are also available such as HPV RNA and Hybrid capture.

PCR and other amplification assays can maximize sensitivity by detecting HPV even in the absence of cytological/histological changes. Its specificity is therefore potentially quite low, as HPV infection often occurs transiently and without associated neoplastic transformation. This makes HPV PCR an attractive primary screening test for Pap specimens where it has been approved by the US Food and Drug Administration (FDA) as an initial testing modality, with reflex to cytology when positive. This algorithm remains somewhat controversial, and there is ongoing discussion about the use of HPV PCR as a lone primary screen versus up-front co-testing with HPV PCR and cytology [46].

Genetic Features

The oncogenic mechanism of high-risk HPV is well established and discussed in great detail elsewhere. Briefly, the viral oncogenes *E6* and *E7* become upregulated with integration into the host genome or through other genetic mechanisms. Their protein products interfere with the tumor suppressor proteins p53 and retinoblastoma (pRb),

leading to unchecked cellular proliferation. These inciting changes lead to an accumulation of genetic abnormalities with increasing numbers and severity of alterations with worsening dysplasia grade. For instance, the relative genetic instability of HSIL is demonstrated by the fact that LSIL lesions are typically DNA stable with euploid or polyploid nuclei, whereas HSILs typically exhibit aneuploidy [47]. HPV DNA integration is detected more often in HSIL than LSIL and is thought to be critical for progression to invasive cancer, with high-grade and invasive lesions often showing multiple integration events [48]. HSILs also more often show genetic anomalies characteristic of cancer, such as 1p and 3q chromosome abnormalities, when compared to LSIL [47]. *C-myc* copy numbers are increased in HSILs and indicate a low probability of dysplastic regression among LSILs [44, 49, 50].

There is little clinically useful information related to host genetic variables and LSIL. Although there is no strong evidence for a heritable susceptibility to HSIL, some investigations have suggested HLA variation, and *TP53* codon 72 polymorphisms might play a role in rendering patients vulnerable to high-grade dysplasia [51–54].

Clinical Course and Management

Screening and management practices are discussed in detail in Chaps. 3 and 4 of this text, and the most up-to-date US guidelines for the management of cervical squamous dysplasia can be obtained from the ASCCP. However, some nuances of these practices warrant discussion here within the context of the behavior of squamous intraepithelial lesions and the diagnostic and prognostic uncertainty that sometimes attends their diagnosis.

In general and in keeping with biological behavior, the management of LSIL allows considerable room for "watchful waiting," whereas HSIL management is more aggressive. Prognosis of all dysplasia grades can be negatively influenced by factors such as smoking and immunosuppression, with particularly high rates of persistence among patients with human immunodeficiency virus (HIV).

LSIL Behavior/Management

Current clinical management of LSIL is driven largely by the small percentage (~10%) of cases that "progress" to HSIL [55–57]. The vast majority of LSILs will resolve without excisional intervention within approximately 12 months, although it is notable that teasing out true regression from removal due to "therapeutic biopsy" can be difficult. However, with the exception of very young women, it is not acceptable to leave LSIL patients unmonitored for long durations because of the subset who will go on to develop HSIL lesions. It is unclear whether these represent true progression vs. initially unsampled lesions, and it bears reinforcing that an LSIL surgical biopsy result

should never simply negate a HSIL or ASC-H cytology result. As discussed in the "Biomarkers" section, there is considerable interest in the identification of ancillary tools to aid in the prognostic stratification of LSILs, but as of yet clinically validated markers are lacking.

HSIL Behavior/Management

The management of HSIL is guided by its relatively high propensity to eventually progress to invasive cancer. While HSIL more often occurs in older patients when compared to LSIL, this does not necessarily mean that HSIL invariably takes a long time to develop. HSIL has been documented within 1–2 years of initial HPV infection among adolescents, suggesting that it can manifest relatively rapidly [58]. HSIL is estimated to progress to invasive carcinoma at a rate of 0.5–1.0% per year, with invasive cancers diagnosed up to two decades later—and, on average, one decade later—than in situ lesions [59]. Although a large proportion of untreated HSIL are likely to progress to invasive cancer, HSIL nevertheless represents a non-obligate precursor to invasive cancer, with reported regression rates ranging from 30% to 50%. However, these numbers may be considerably inflated by the potential for therapeutic biopsy in a large proportion of these presumably "regressed" cases, as well as contamination by CIN1 cases misclassified as CIN2.

Another somewhat controversial issue in the management of HSIL is whether or not CIN2 and CIN3 should ever be treated differently. Although our understanding of their biological underpinnings and behavior supports the collapse of CIN2 and CIN3 into a single managerial category (HSIL), differentiation between moderate and severe dysplasia sometimes becomes important in women with HSIL who are willing to accept some risk of progression in order to maintain fertility. There is evidence that LEEP increases rates of miscarriage and preterm labor (although these risks are lower than was previously thought); therefore, one could argue for less aggressive excision in women who wish to maximize childbearing potential [60, 61]. In such cases, less aggressive interventions may be allowable for a diagnosis of CIN2. However, such differential management for CIN2 and CIN3 must be performed with considerable caution and awareness of the considerable interpretive variability that attends the classification of these lesions.

References

1. Reagan JW, Hamonic MJ. The cellular pathology in carcinoma in situ; a cytohistopathological correlation. Cancer. 1956;9(2):385–402.
[Crossref][PubMed]

2. Koss LG, Durfee GR. Unusual patterns of squamous epithelium of the uterine cervix: cytologic and pathologic

study of koilocytotic atypia. Ann N Y Acad Sci. 1956;63(6):1245–61.
[Crossref][PubMed]

3. Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. cytologic patterns. Acta Cytol. 1976;20(6):505–9.
[PubMed]

4. Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix. II. cytologic, colposcopic and histopathologic study. Acta Cytol. 1977;21(3):379–90.
[PubMed]

5. Della Torre G, Pilotti S, de Palo G, Rilke F. Viral particles in cervical condylomatous lesions. Tumori. 1978;64(5):549–53.
[PubMed]

6. Hills E, Laverty CR. Electron microscopic detection of papilloma virus particles in selected koilocytotic cells in a routine cervical smear. Acta Cytol. 1979;23(1):53–6.
[PubMed]

7. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol. 1969;105(3):386–93.

8. Richart RM. A theory of cervical carcinogenesis. Obstet Gynecol Surv. 1969;24(7 Pt 2):874–9.
[Crossref][PubMed]

9. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, Zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 1984;3(5):1151–7.
[PubMed][PubMedCentral]

10. Crum CP, Ikenberg H, Richart RM, Gissman L. Human papillomavirus type 16 and early cervical neoplasia. N Engl J Med. 1984;310(14):880–3.

11. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30(Suppl 5):F55–70.

12. Darragh TM, Colgan TJ, Thomas Cox J, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the college of american pathologists and the american society for colposcopy and cervical pathology. Int J Gynecol Pathol. 2013;32(1):76–115.

13. Kurman RJ. International Agency for Research on Cancer, World Health Organization, WHO classification of tumours of female reproductive organs. 4th ed. Lyon: International Agency for Research on Cancer; 2014. p. 307.

14. Saslow D, Solomon D, Lawson HW, et al. American cancer society, american society for colposcopy and cervical pathology, and american society for clinical pathology screening guidelines for the prevention and early detection of cervical cancer. Am J Clin Pathol. 2012;137(4):516–42.

15. Peyton CL, Schiffman M, Lorincz AT, et al. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J Clin Microbiol. 1998;36(11):3248–54.
[PubMed][PubMedCentral]

16. Siebers AG, Klinkhamer PJ, Grefte JM, et al. Comparison of liquid-based cytology with conventional cytology for

detection of cervical cancer precursors: a randomized controlled trial. JAMA. 2009;302(16):1757–64.

17. Bishop JW, Bigner SH, Colgan TJ, et al. Multicenter masked evaluation of AutoCyte PREP thin layers with matched conventional smears. including initial biopsy results. Acta Cytol. 1998;42(1):189–97. [Crossref][PubMed]

18. Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J. Liquid compared with conventional cervical cytology: a systematic review and meta-analysis. Obstet Gynecol. 2008;111(1):167–77.

19. Stoler MH, Schiffman M, Atypical Squamous Cells of Undetermined Significance-Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. JAMA. 2001;285(11):1500–5.

20. Cinel A, Oselladore M, Insacco E, Minucci D. The accuracy of colposcopically directed biopsy in the diagnosis of cervical intraepithelial neoplasia. Eur J Gynaecol Oncol. 1990;11(6):433–7. [PubMed]

21. Guido RS, Jeronimo J, Schiffman M, Solomon D, ALTS Group. The distribution of neoplasia arising on the cervix: results from the ALTS trial. Am J Obstet Gynecol. 2005;193(4):1331–7.

22. Maniar KP, Sanchez B, Paintal A, Gursel DB, Nayar R. Role of the biomarker p16 in downgrading -IN 2 diagnoses and predicting higher-grade lesions. Am J Surg Pathol. 2015;39(12):1708–18.

23. Nayar R, Wilbur D. The bethesda system for reporting cervical cytology. Definitions, criteria, and explanatory notes. 3rd ed. Cham: Springer; 2015.

24. Symmans F, Mechanic L, MacConnell P, DaSilva K, Stricker B, Nuovo GJ. Correlation of cervical cytology and human papillomavirus DNA detection in postmenopausal women. Int J Gynecol Pathol. 1992;11(3):204–9. [Crossref][PubMed]

25. Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR, Stoler MH. Using biomarkers as objective standards in the diagnosis of cervical biopsies. Am J Surg Pathol. 2010;34(8):1077–87.

26. Mills AM, Paquette C, Castle PE, Stoler MH. Risk stratification by p16 immunostaining of CIN1 biopsies: a retrospective study of patients from the quadrivalent HPV vaccine trials. Am J Surg Pathol. 2015;39(5):611–7.

27. Kong CS, Balzer BL, Troxell ML, Patterson BK, Longacre TA. p16INK4A immunohistochemistry is superior to HPV in situ hybridization for the detection of high-risk HPV in atypical squamous metaplasia. Am J Surg Pathol. 2007;31(1):33–43.

28. Morgan TK, Berlin M. Immunocytochemical analysis of the cervical pap smear. Methods Mol Biol. 2015;1249:203–12.

29. Razmpoosh M, Sansregret A, Oligny LL, et al. Assessment of correlation between p16INK4a staining, specific subtype of human papillomavirus, and progression of LSIL/CIN1 lesions: first comparative study. Am J Clin Pathol. 2014;142(1):104–10.

30. Carozzi F, Gillio-Tos A, Confortini M, et al. Risk of high-grade cervical intraepithelial neoplasia during follow-up in HPV-positive women according to baseline p16-INK4A results: a prospective analysis of a nested substudy of the NTCC randomised controlled trial. Lancet Oncol. 2013;14(2):168–76.

31. Pacchiarotti A, Ferrari F, Bellardini P, et al. Prognostic value of p16-INK4A protein in women with negative or CIN1 histology result: a follow-up study. Int J Cancer. 2014;134(4):897–904.

32. Sagasta A, Castillo P, Saco A, et al. P16 staining has limited value in predicting the outcome of histological low-grade squamous intraepithelial lesions of the cervix. Mod Pathol. 2016;29(1):51–9.

33. Badr RE, Walts AE, Chung F, Bose SBD, ProEx C. A sensitive and specific marker of HPV-associated squamous lesions of the cervix. Am J Surg Pathol. 2008;32(6):899–906.

34. Guo M, Baruch AC, Silva EG, et al. Efficacy of p16 and ProExC immunostaining in the detection of high-grade cervical intraepithelial neoplasia and cervical carcinoma. Am J Clin Pathol. 2011;135(2):212–20.

35. Sanati S, Huettner P, Ylagan LR. Role of ProExC: a novel immunoperoxidase marker in the evaluation of dysplastic squamous and glandular lesions in cervical specimens. Int J Gynecol Pathol. 2010;29(1):79–87.

36. Cabibi D, Giovannelli L, Martorana A, et al. Predictive role of histological features and Ki67 pattern on high-risk HPV presence in atypical cervical lesions. Histopathology. 2007;51(5):713–6.

37. Lee S, Sabourin J, Gage J, Franko A, Nation JG, Duggan MA. Squamous intraepithelial lesions in cervical tissue samples of limited adequacy and insufficient for grading as low or high grade: outcome, clinico-pathological correlates, and predictive role of p16INK4a and Ki67 biomarker staining. J Low Genit Tract Dis. 2015;19(1):35–45.
[Crossref][PubMed]

38. Kisser A, Zechmeister-Koss I. A systematic review of p16/ki-67 immuno-testing for triage of low grade cervical cytology. BJOG. 2015;122(1):64–70.

39. Herfs M, Yamamoto Y, Laury A, et al. A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci U S A. 2012;109(26):10516–21.

40. Herfs M, Parra-Herran C, Howitt BE, et al. Cervical squamocolumnar junction-specific markers define distinct, clinically relevant subsets of low-grade squamous intraepithelial lesions. Am J Surg Pathol. 2013;37(9):1311–8.

41. Paquette C, Mills AM, Stoler MH. Predictive value of cytokeratin 7 immunohistochemistry in cervical low-grade squamous intraepithelial lesion as a marker for risk of progression to a high-grade lesion. Am J Surg Pathol. 2016;40(2):236–43.

42. Selvi K, Badhe BA, Papa D, Ganesh RN. Role of p16, CK17, p63, and human papillomavirus in diagnosis of cervical intraepithelial neoplasia and distinction from its mimics. Int J Surg Pathol. 2014;22(3):221–30

43. Regauer S, Reich O. CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). Histopathology. 2007;50(5):629–35.

44. Zhang W, Kapadia M, Sugarman M, et al. Adjunctive HPV in-situ hybridization (ISH) assay as an aid in the diagnosis of cervical intraepithelial neoplasia in cervical tissue specimens: an analytical and functional characterization. Int J Gynecol Pathol. 2012;31(6):588–95.

45. Evans MF, Peng Z, Clark KM, et al. HPV E6/E7 RNA in situ hybridization signal patterns as biomarkers of three-tier cervical intraepithelial neoplasia grade. PLoS One. 2014;9(3):e91142.

46. Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol. 2015;136(2):178–82.

47. Hopman AH, Smedts F, Dignef W, et al. Transition of high-grade cervical intraepithelial neoplasia to micro-invasive carcinoma is characterized by integration of HPV 16/18 and numerical chromosome abnormalities. J

Pathol. 2004;202(1):23–33.

48. Lu X, Lin Q, Lin M, et al. Multiple-integrations of HPV16 genome and altered transcription of viral oncogenes and cellular genes are associated with the development of cervical cancer. PLoS One. 2014;9(7):e97588.

49. Zhu D, Jiang XH, Jiang YH, et al. Amplification and overexpression of TP63 and MYC as biomarkers for transition of cervical intraepithelial neoplasia to cervical cancer. Int J Gynecol Cancer. 2014;24(4):643–8.

50. Kubler K, Heinenberg S, Rudlowski C, et al. C-myc copy number gain is a powerful prognosticator of disease outcome in cervical dysplasia. Oncotarget. 2015;6(2):825–35.

51. Hu B, Tao N, Zeng F, et al. A risk evaluation model of cervical cancer based on etiology and human leukocyte antigen allele susceptibility. Int J Infect Dis. 2014;28:8–12.

52. Matsumoto K, Maeda H, Oki A, et al. Human leukocyte antigen class II DRB1*1302 allele protects against cervical cancer: at which step of multistage carcinogenesis? Cancer Sci. 2015;106(10):1448–54.

53. Klug SJ, Wilmotte R, Santos C, et al. TP53 polymorphism, HPV infection, and risk of cervical cancer. Cancer Epidemiol Biomarkers Prev. 2001;10(9):1009–12.
[PubMed]

54. Laprano TD, Lemos EH, Cunha LM, Junior JE, de SousaTeles RA, Rabenhorst SH. Association of TP53 codon 72 and intron 3 16-bp ins/del polymorphisms with cervical cancer risk. Tumour Biol. 2014;35(8):7435–40.

55. Katki HA, Gage JC, Schiffman M, et al. Follow-up testing after colposcopy: five-year risk of CIN 2+ after a colposcopic diagnosis of CIN 1 or less. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S69–77.

56. Cox JT, Schiffman M, Solomon D, ASCUS-LSIL Triage Study (ALTS) Group. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol. 2003;188(6):1406–12.

57. Bansal N, Wright JD, Cohen CJ, Herzog TJ. Natural history of established low grade cervical intraepithelial (CIN 1) lesions. Anticancer Res. 2008;28(3B):1763–6.
[PubMed]

58. Schiller JT, Castellsague X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine. 2012;30(Suppl 5):F123–38.

59. McCredie MR, Sharples KJ, Paul C, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol. 2008;9(5):425–34.

60. Miller ES, Sakowicz A, Grobman WA. The association between cervical dysplasia, a short cervix, and preterm birth. Am J Obstet Gynecol. 2015;213(4):543.e1–4.

61. Ciavattini A, Clemente N, Delli Carpini G, et al. Loop electrosurgical excision procedure and risk of miscarriage. Fertil Steril. 2015;103(4):1043–8.

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7. Squamous Cell Carcinoma of the Cervix

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Abstract
Squamous cell carcinomas constitute the vast majority of all cervical cancers. Unlike vulval squamous carcinomas, these are almost invariably human papillomavirus (HPV) mediated, and the incidence worldwide varies widely, largely depending on the presence of a universal screening programme for detecting precancerous changes and/or genitally transmitted high-risk HPV. Although a range of morphologically defined "subtypes" is listed in the WHO classification, these generally have no bearing on management or behavior; similarly there is no universally accepted grading system with direct prognostic relevance. It is far more important to diagnose these tumors accurately, as there are a number of mimics with clinical implications. Most important of all is to assign the correct clinicopathological stage, an exercise directly dependent on pathological assessment; at the earlier stages, generally in screen-detected lesions, the challenge is to measure lesions accurately and reproducibly so the patient is appropriately managed and neither over- nor undertreated. In this regard it is important to clearly delineate those cases of invasive cancer that can be managed conservatively based on current evidence. At the higher stages, the pathologist must determine whether there is microscopic parametrial or vaginal involvement not detected on clinical examination, in addition to the presence and extent of nodal involvement, and the careful assessment of radical surgical specimens including exenterations for the status and clearance of margins. These aspects will be discussed in this chapter.

Keywords Squamous cell carcinoma – Cervix – Squamous – Carcinoma – Measurement – Prognosis – Stage

Definition

Squamous cell carcinoma (SCC) of the cervix is defined by the WHO (2014) as an invasive epithelial tumor composed of squamous cells of varying degrees of differentiation.

Etiology

The vast majority of cervical SCC is HPV-related. However, non-HPV-related cervical SCC is reported [12]. Although cervical adenocarcinomas are two- to threefold more likely to be HPV negative, 2.9–13% of squamous carcinomas are reported to be HPV negative [21, 95]. This is relevant as, in common with other disease sites such as the head and neck and vulva, non-HPV-related cervical carcinomas show reduced disease-specific survival [95].

Clinical Features

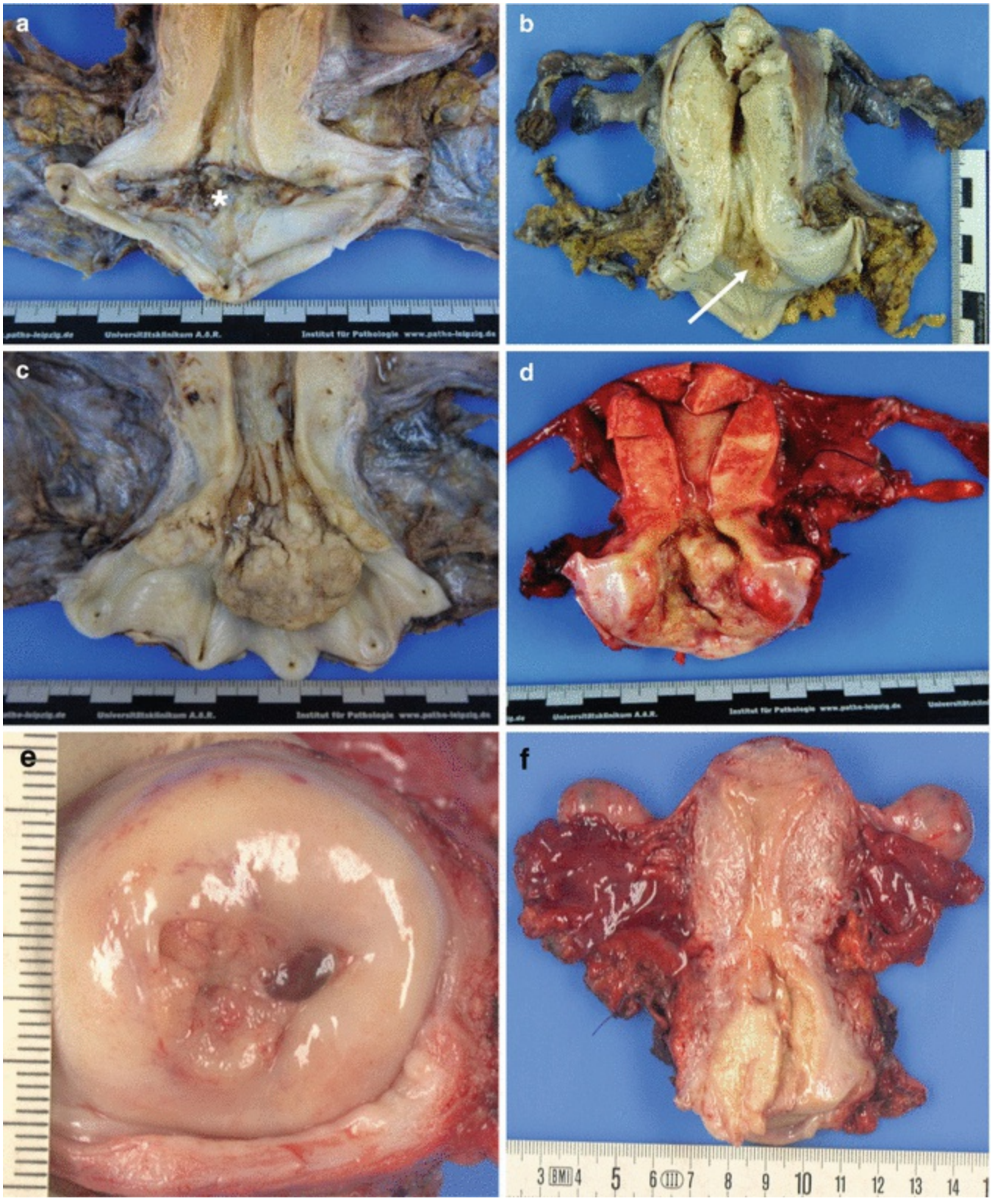
The introduction of screening dramatically reduced the incidence and mortality of cervical cancer [67], with further decline likely as a result of HPV vaccination strategies. In the UK about 1:135 women develop cervical cancer [9], and more than 85% of these are SCC [32, 91, 103]. Preinvasive and early invasive disease are diagnosed through screening, as these are asymptomatic.

Depending on tumor stage, cervical carcinoma may present with:

- Abnormal Pap smear (and colposcopic findings)
- Bleeding disturbances and vaginal discharge
- Renal obstruction
- Pain
- Features of lymphedema

There is no difference in presentation between different histological types. More than 60% of patients present with carcinomas confined to the uterine cervix (FIGO stage IB) or locally advanced disease with vaginal (FIGO IIA) or parametrial/mesometrial involvement (FIGO IIB; [32, 91]). In these patients, vaginal bleeding is the most common symptom, occurring as intermenstrual or postcoital bleeding. Visible tumors at gynecological examination present as exophytic, endophytic, or polypoid lesions (Fig.

7.1). Exophytic tumors with or without ulceration are more common than endophytic, barrel-shaped tumors.



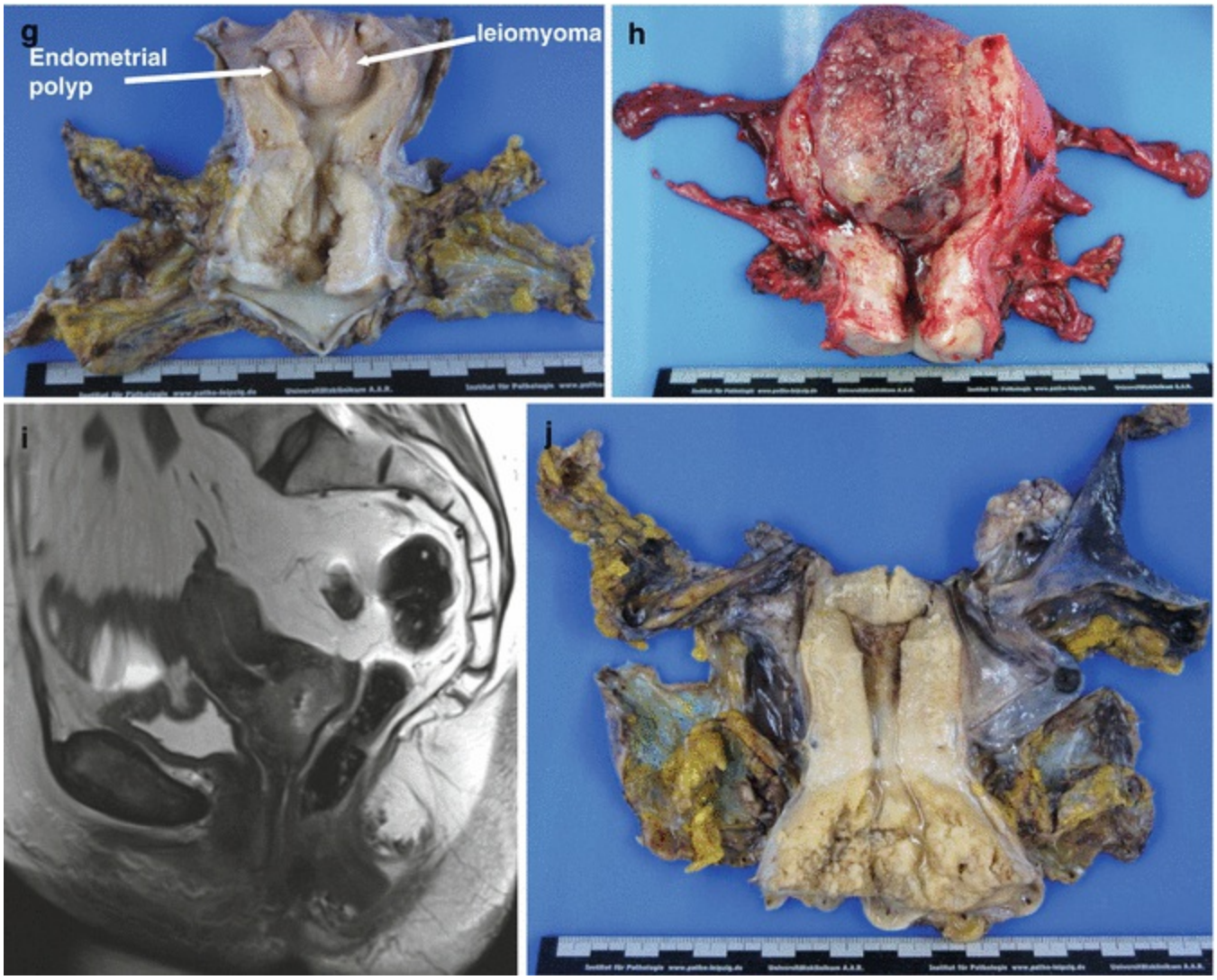


Fig. 7.1 Macroscopic features of squamous cell carcinoma of the uterine cervix (most specimens are opened at the 12 o'clock position with scissors). (**a**) Radical hysterectomy after previous conization procedure. Note the ulceration within the cervix (*asterisk*). There is no visible tumor, so the ecto- and endocervix should be completely processed. (**b**) Radical hysterectomy (total mesometrial resection (TMMR) technique) with a small ulcerated tumor between 3 and 10 o'clock. (**c**) Large polypoid exophytic carcinoma with circumferential growth. (**d**) Large ulcerated carcinoma involving the whole circumference of the cervix. (**e**) Ectocervix with a small polypoid and ulcerated carcinoma between 6 and 12 o'clock. (**f**) Frontal cut of a radical hysterectomy (same case as pictured in (**e**) confined to the cervix. (**g**) Radical hysterectomy (TMMR technique) with a large mostly endophytic growth tumor (*barrel-shaped*) with smooth outer appearance of the ectocervix. (**h**) Radical hysterectomy (TMMR technique) in pregnancy and small carcinoma confined to the cervix. (**i**) Magnetic resonance imaging (MRI) of a large cervical SCC (Courtesy of Dr. Gudrun Borte, University Hospital of Leipzig). (**j**) Radical hysterectomy (TMMR technique) from the same patient pictured in (**i**) with a bulky ulcerated carcinoma

Regardless of screening strategies, about one fourth of patients present with advanced stage disease (FIGO ≥IIIA; [32, 91]). In this cohort, bleeding disturbances may become more continuous and may be accompanied by malodorous discharge. Because of its embryonal development, cervical cancer preferentially grows within the parametria/mesometria [36], which causes obstruction of the ureter by narrowing the

lumen or direct infiltration resulting in flank pain or even renal failure. Infiltration of the pelvic tissues may provoke pain, frequently within the pelvis or leg, caused by organ obstruction, local inflammatory response, and infiltration of the perineurium or lumbosacral nerve ganglia. Additionally, pedal edema may occur due to lymphatic and venous obstruction and may indicate pelvic lymph node involvement.

Macroscopic Appearances (Fig. 7.1)

SCC may be located in the ecto- or endocervix. The principal macroscopic appearance is that of an exophytic or endophytic growth, with or without superficial ulceration. A primarily endocervical localization may produce a barrel-shaped appearance. Deep stromal invasion results in a hard consistency. In locally advanced cases, infiltration of the resected vaginal cuff may be present, and involvement of the adjacent para-/mesometrial tissue may be palpable. Macroscopically, there are no differences between the different histological types (adenocarcinoma versus squamous cell versus neuroendocrine carcinomas).

Microscopic Appearances

Histopathological Tumor Types

There are several variants of SCC of the uterine cervix [103, 113], some of which cause diagnostic challenges or show prognostic importance as discussed under individual subheadings below. The WHO classification defines the following subtypes of SCC [108]:

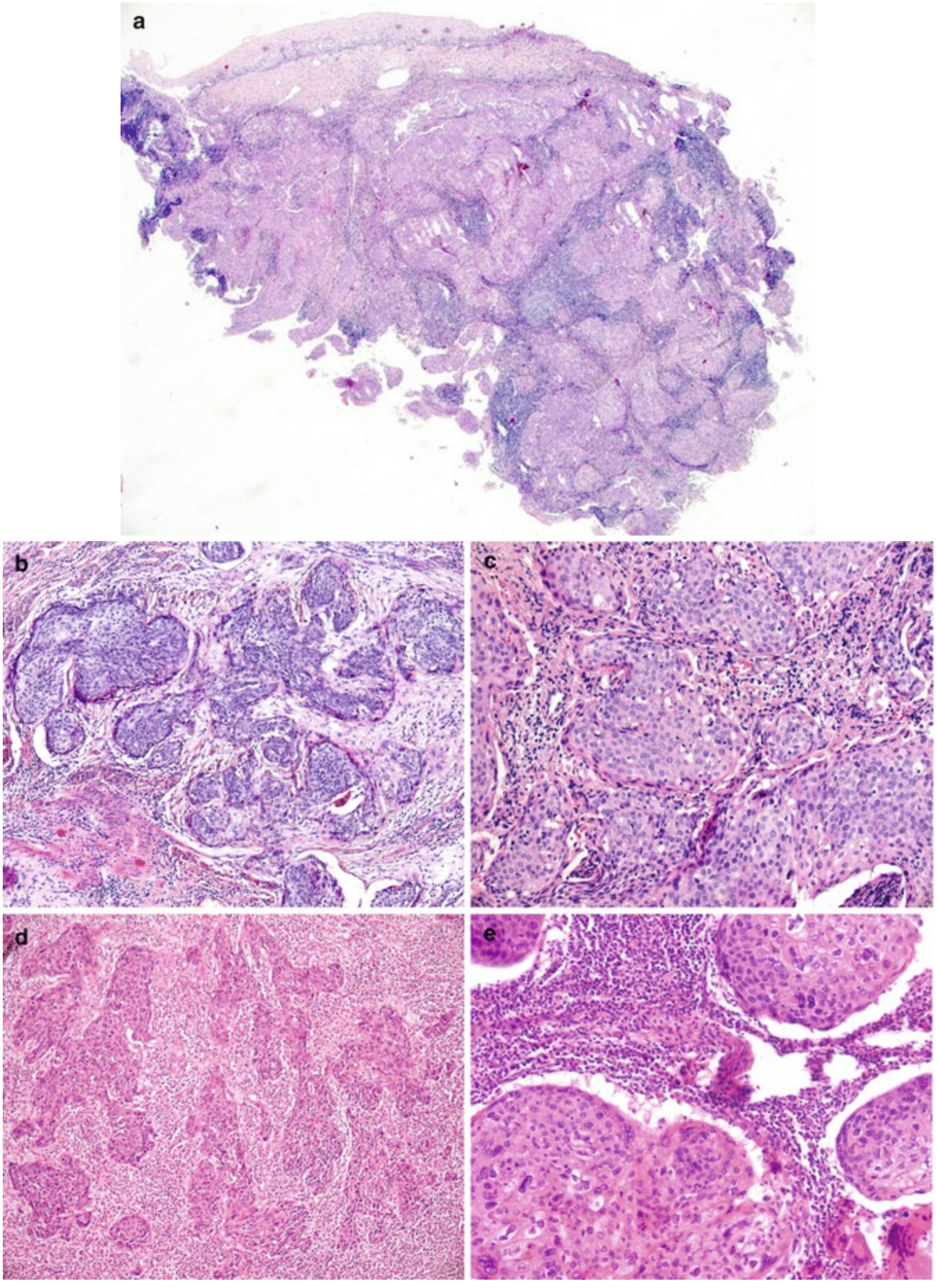
- Keratinizing SCC
- Non-keratinizing SCC
- Papillary SCC
- Basaloid SCC
- Warty-type SCC
- Verrucous SCC
- Squamotransitional SCC
- Papillary SCC
- Lymphoepithelioma-like SCC

The International Collaboration on Cancer Reporting (ICCR) recommends that subtyping of cervical SCC is not required, as this does not influence treatment decisions

[50].

Non-keratinizing SCC

Non-keratinizing SCCs (7.2) are the most common, composed of polygonal squamous cells without keratinization [108]. The cells grow in anastomosing cords or nests with a round, angulated, or spiky appearance. The cells are oval to polygonal, often with an eosinophilic cytoplasm; cell borders may be indistinct and are sometimes prominent with intercellular bridges. The nuclei may be uniform with a coarse and granular chromatin with or without nucleoli but may display considerable pleomorphism. The mitotic count is variable. As seen in other carcinomas and in adenocarcinoma of the uterine cervix [96], there exist different patterns of invasion [40, 47] which may represent different degrees of tumor cell dissociation (Fig. 7.3). There is typically a peritumoral stromal response, which may vary with different invasive patterns. Many tumors are accompanied by peritumoral *inflammatory* response (Figs. 7.2 and 7.3), which is more often seen in tumors with a fingerlike pattern of infiltration. Tumors with spray-like pattern of invasion on the other hand may show a strong peritumoral *desmoplastic* response (Figs. 7.2 and 7.3). The cell size is variable; earlier classification separated a large cell non-keratinizing SCC from a small cell variant [93]. The use of the term “small cell” non-keratinizing SCC in the pathology report is not recommended [108] to avoid confusion with small cell neuroendocrine carcinoma, a subtype associated with very poor prognosis [29]. In rare instances SCC may be mixed with a true small cell neuroendocrine carcinoma [29, 40]; the majority of mixed neuroendocrine carcinomas being associated with adenocarcinoma [29, 40].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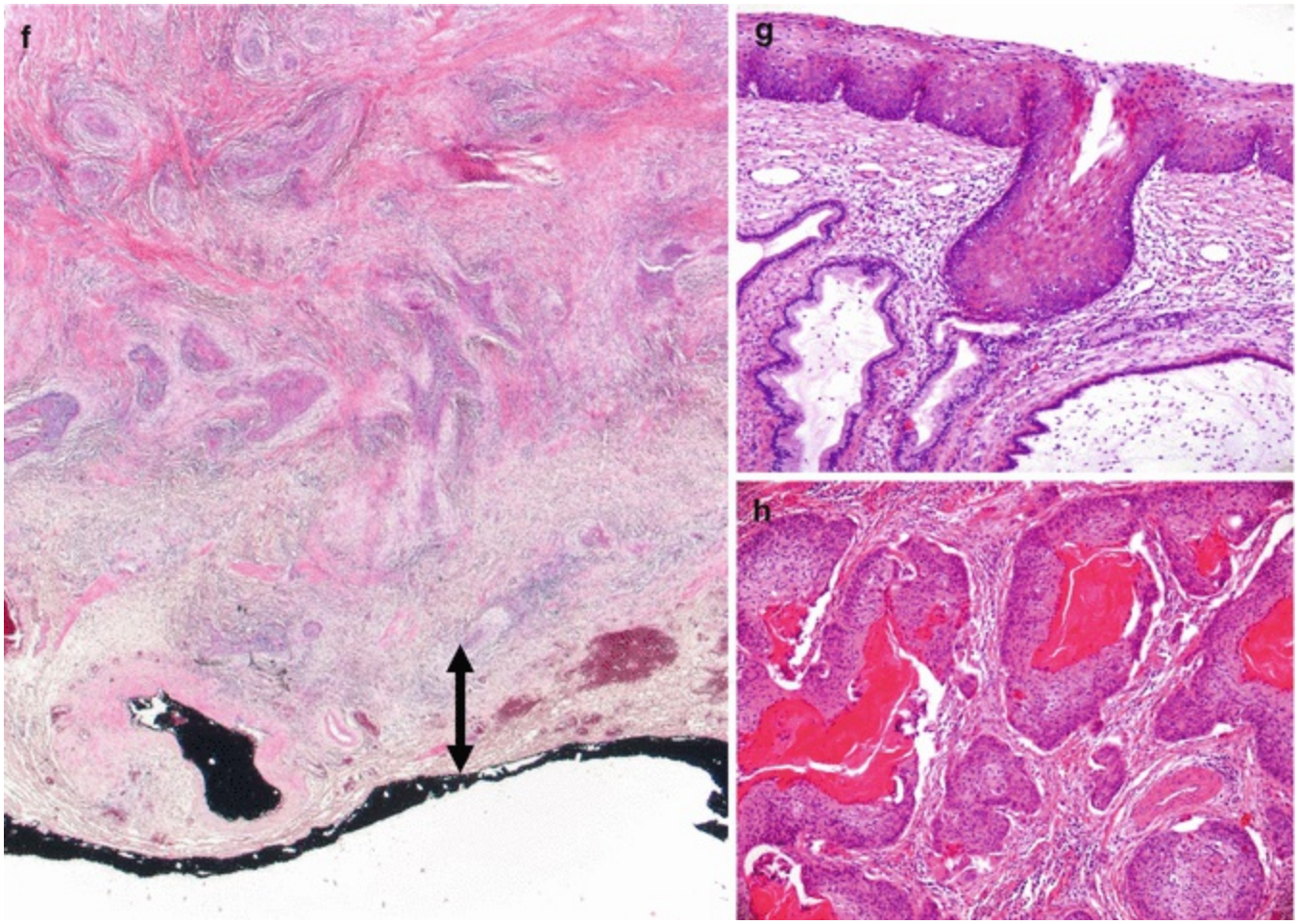


Fig. 7.2 Morphological spectrum of non-keratinizing and keratinizing squamous cell carcinoma (SCC). (**a**) Colposcopy-guided biopsy containing a non-keratinizing SCC, (**b**) low-power view of a non-keratinizing SCC with fingerlike pattern of invasion and strong peritumoral desmoplastic reaction and weak inflammatory response, (**c**) high-power view of a non-keratinizing SCC with fingerlike pattern of invasion without peritumoral desmoplastic reaction but moderate inflammatory response, (**d**) low-power view of a non-keratinizing SCC with mixed fingerlike and spray-like patterns of invasion and without peritumoral inflammatory response but strong desmoplastic reaction, (**e**) high-power view of a non-keratinizing SCC with peritumoral retraction artifacts, (**f**) inked resection margin of a hysterectomy specimen. The distance between the leading edge of the tumor and the margin is marked by a double-*headed arrow;* (**g**) example of keratinizing form of high-grade intraepithelial lesion involving an endocervical gland (**h**) keratinizing SCC

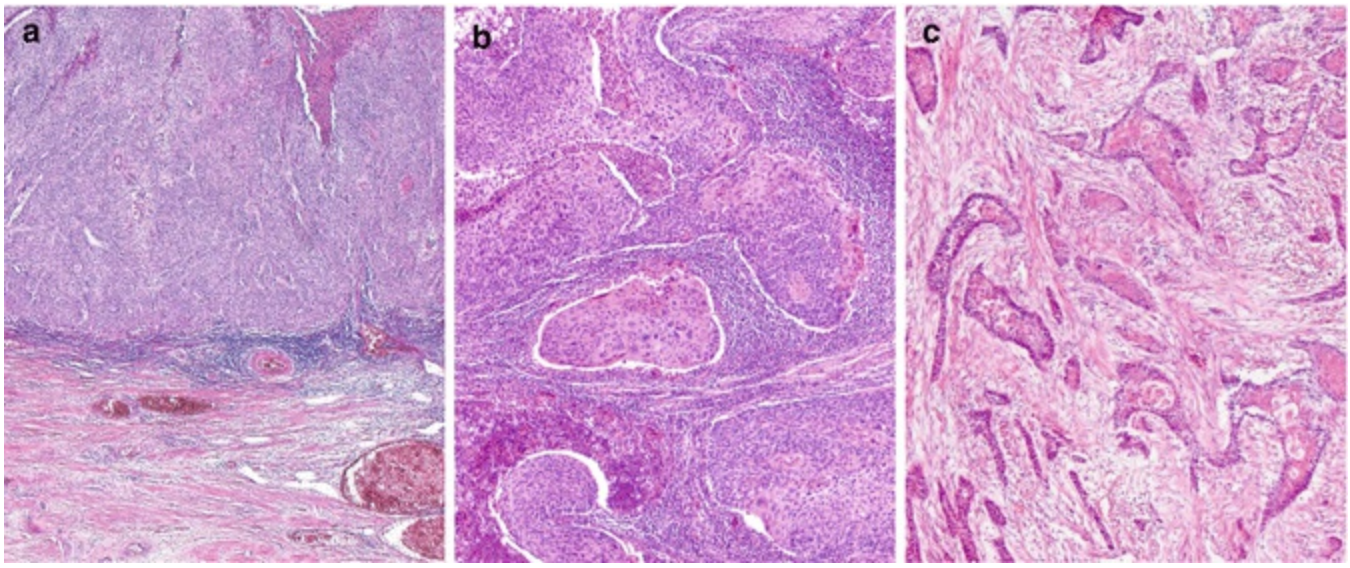


Fig. 7.3 Growth patterns and peritumoral stromal alterations (stromal remodeling) of squamous cell carcinoma of the uterine cervix: (**a**) closed or pushing pattern of invasion with cohesive tumor growth with well-delineated but infiltrating borders and "pushing" margins with a weak peritumoral inflammatory response, (**b**) fingerlike growth pattern with trabecular tumor growth in solid cords and cell groups with rounded edges, accompanied by a strong peritumoral inflammatory response with lymphocytes. Note that the inflammatory response is peritumoral and not intratumoral as seen in lymphoepithelioma-like carcinomas (see Fig. 7.7), (**c**) spray-like pattern with tumor growth in very small groups of infiltrating cells with sharpened tips with a strong peritumoral desmoplastic stromal reaction but no inflammatory response

On occasion, non-keratinizing SCC may show a deceptive "CIN 3-like" pattern, comprising islands of atypical squamous epithelial cells with well-circumscribed edges and a central luminal space filled with debris or occasionally keratin [3] (Fig. 7.4). These may be misdiagnosed as CIN 3 involving crypts. Features useful in distinction are the extent beyond the crypt field in the adjacent cervix, the presence of usual infiltration in other areas, and/or the presence of a mass lesion. The distinction is important as under-recognition can result in undertreatment, and there has been a report of a case with an unusual and late pattern of recurrence [121].

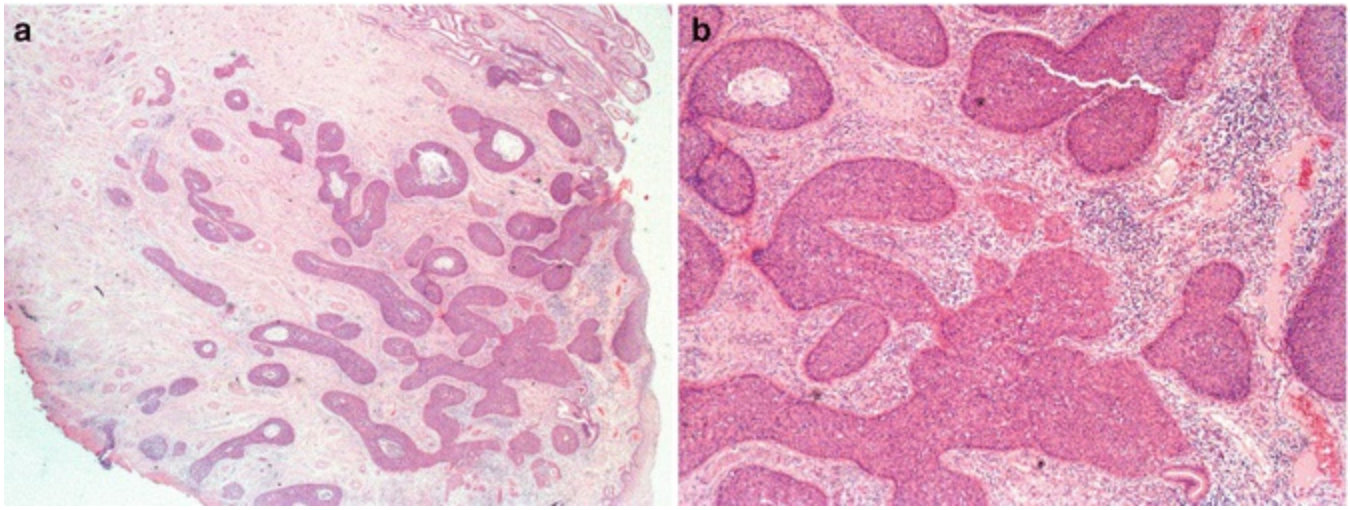


Fig. 7.4 (**a**) SCC invading in a pattern resembling CIN 3: the invasive islands have rounded edges with peripheral palisading but extend deeper than the crypt field, (**b**) conventional invasion comprising cells with eosinophilic cytoplasm forming islands with irregular edges is also present

Since non-keratinizing SCC represents the commonest histological subtype of cervical SCC and shows no special morphology, in the authors' opinions, this may be designated as SCC, not otherwise specified (NOS).

Keratinizing SCC

Keratinizing SCC (Fig. 7.2h) is less common than its non-keratinizing counterpart. This is characterized by morphological evidence of keratinization in the form of keratin pearls or intracytoplasmic keratinization. The cells and nuclei are usually larger and hyperchromatic with a coarse chromatin and lack easily seen nucleoli. There may be a correlation with ectocervical localization and the keratinizing form of CIN (Fig. 7.2g). It is possible, as in vulval carcinomas, that keratinization may indicate HPV-independent SCC [12].

Basaloid SCC

Basaloid SCC (Fig. 7.5) is HPV related [30] and composed of nests of immature small oval cells with scanty cytoplasm and dark nuclei, resembling cells seen in CIN 3 and showing brisk mitotic activity. Some cytoplasmic keratinization may occur but keratin pearls are not seen. The majority of tumors demonstrate geographical or comedo-like necrosis. Because of its aggressive behavior [30], the WHO classification considers basaloid SCC as a high-grade tumor (grade 3; [108]). Immunohistochemically, basaloid SCCs are positive for p16, cytokeratin 34βE12, and, sometimes, neuroendocrine markers, but negative for TTF-1 [30, 68]. The main differential diagnosis is with small cell neuroendocrine carcinoma (SCNEC). Immunohistochemically, SCNEC shows positivity for p16, neuroendocrine markers, and TTF-1 but is negative for cytokeratin

34βE12 [29, 30, 39, 40, 68].

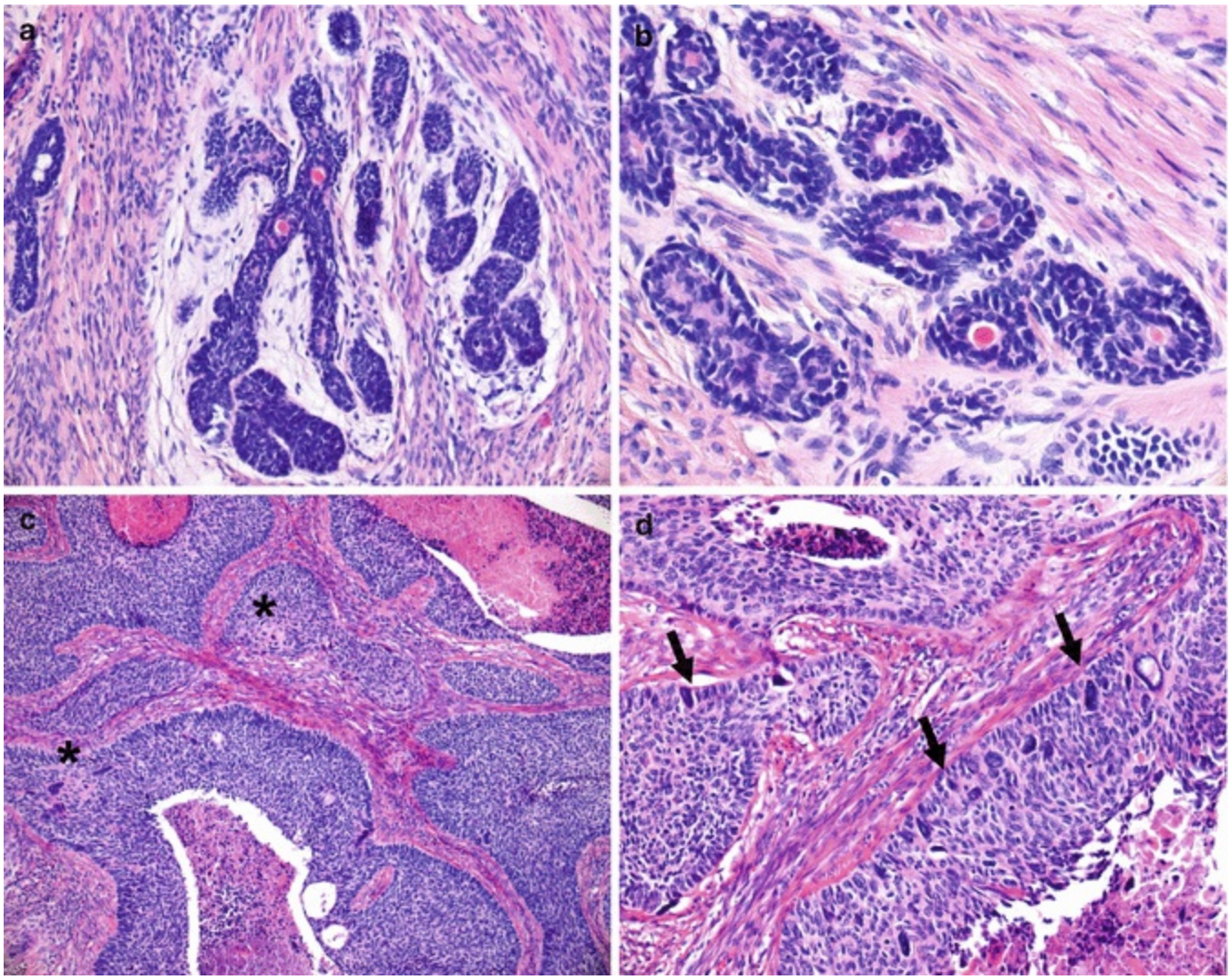


Fig. 7.5 Basaloid neoplasms of the uterine cervix (**a**, **b**) adenoid basal cell carcinoma: proliferation of cords and bands of bland-appearing basaloid cells with central gland formation. (**c**, **d**) Basaloid squamous cell carcinoma: nests of immature basal-type cells with peripheral palisading accompanied by comedo-like necrosis, some pleomorphic cells (*arrows*) and focal keratinization (*asterisks*)

Adenoid basal carcinoma (ABC; Fig. 7.5) of the cervix is composed of small, monomorphic, basaloid cells that are p16 positive, forming rounded nests or cords. Some adenoid formations may be seen containing necrotic debris. Contrary to basaloid SCC, there is no geographic/comedo necrosis and low mitotic activity. The tumors may be associated with another carcinoma subtype. Pure ABCs have a favorable prognosis [5], while mixed tumors share the prognostic outcome of the non-ABC component.

Verrucous SCC

Verrucous SCC is a distinct type of highly differentiated SCC which is similar

morphologically to its much more common vulval counterpart. This has a favorable prognosis [17, 31] and is distinguished from keratinizing SCC by the absence of nuclear atypia and destructive stromal invasion. The tumor characteristically shows a pushing border and a hyperkeratotic undulating surface, resembling condyloma and condylomatous SCC (see next).

Warty/Condylomatous SCC

Warty/condylomatous SCC typically shows a warty surface and a low-power architecture analogous to condyloma [108]. Morphologically it resembles its vulval counterpart [84]. The cells show keratinization and koilocytic atypia.

Squamotransitional/Transitional and Papillary SCC

Squamotransitional/transitional SCCs have a papillary architecture with fibrovascular cores covered by cells resembling CIN 3/HSIL [56, 57]. Rare cases of pure transitional cell carcinoma have been reported [1] that are indistinguishable from their urological counterparts. However, most of these tumors represent malignant squamous elements. The evidence relating transitional cell metaplasia of the uterine cervix [83, 119] and squamotransitional SCC is controversial.

Papillary SCCs consist of fibrovascular papillae with different thickness, covered by an epithelium representing CIN 3-like morphology. They differ from warty SCC by the lack of Bowenoid morphology and from squamotransitional SCC by their more overt squamous differentiation.

Squamotransitional and papillary SCC can be diagnostically challenging in small biopsies because invasion may not be seen within the stroma included in the slender fibrovascular cores present in biopsies, and sampling of deeper tissues is precluded by the papillary surface of the tumor (Fig. 7.6). The typical appearance with papillary architecture and small fibrovascular cores covered by CIN 3-like squamous epithelium favors the diagnosis of this cancer subtype, which should be raised even if stromal invasion is not seen. Correlation with the clinical appearance of a visible (papillary-exophytic) tumor of the uterine cervix is helpful. In doubtful cases re-biopsy or even conization may be necessary to establish the diagnosis of invasive carcinoma [56, 82]. Regardless of the sometimes superficial infiltration of squamotransitional and papillary SCC, the tumor should be staged according to the whole tumor size [2]. Because of its rarity, the prognostic impact of squamotransitional and papillary SCC is controversial [2, 22].

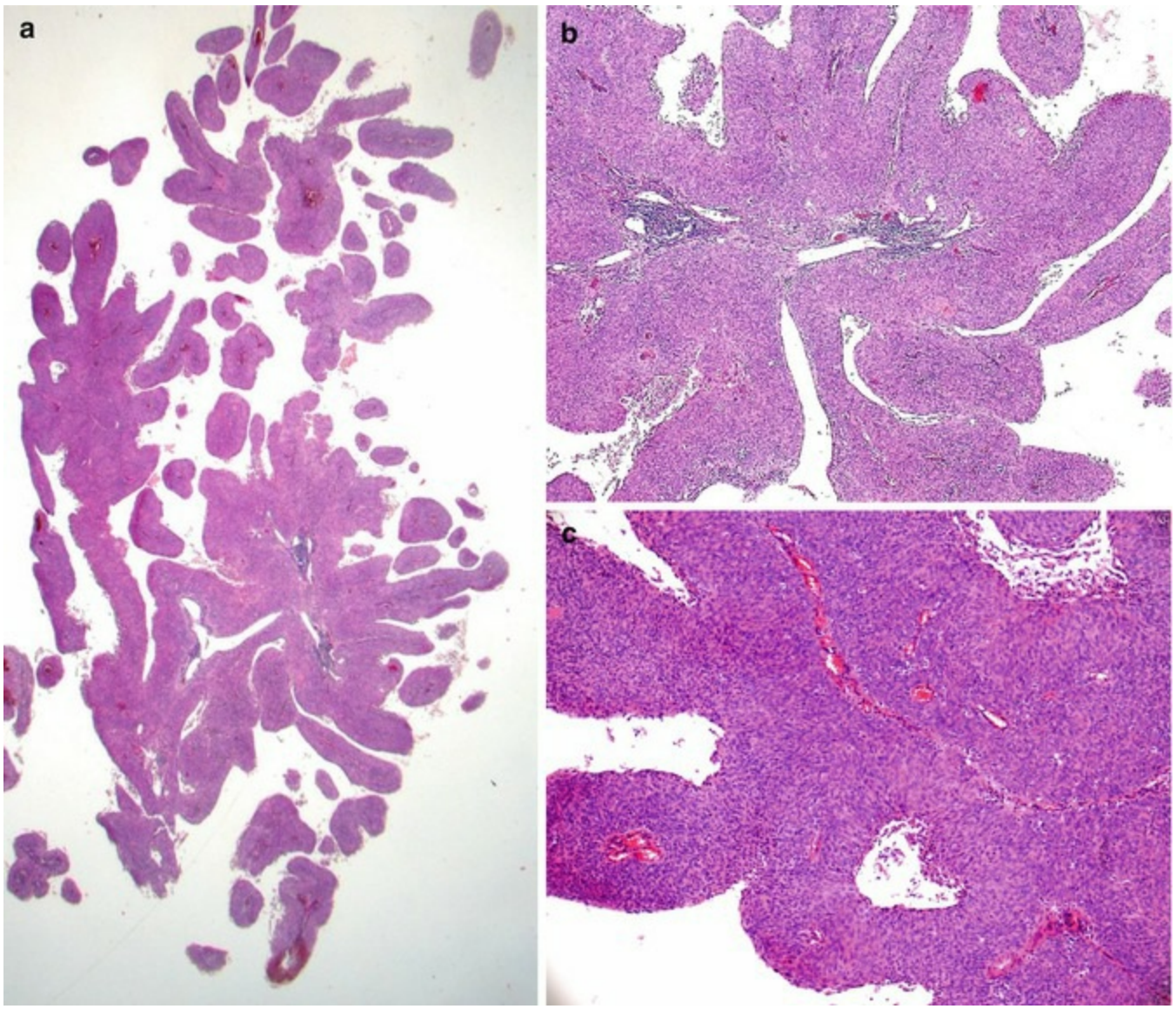


Fig. 7.6 Papillary squamous cell carcinoma of the uterine cervix. Cervical curettage specimen containing fragmented tissue 1.5 cm in diameter from a 69-year-old postmenopausal patient. The radical hysterectomy specimen contained a 2.4 × 1.9 × 2 cm well-differentiated papillary squamous cell carcinoma staged pT2b pN0 (0/36) MX L0 V0 Pn0, R0. (**a**) Fragmented tumor tissue with thin papillae resembling papillary noninvasive urothelial carcinoma. (**b**, **c**) Papillae covered by epithelium similar to high-grade squamous intraepithelial lesion. No invasion can be seen

In the authors' opinion, squamotransitional and papillary SCC should be regarded as a single category of (non-keratinizing) SCC with a papillary growth pattern, for several reasons. Firstly, both tumors morphologically differ only by the presence/degree of squamous differentiation, which is almost always CIN 3-like and challenging to grade; furthermore it may be more relevant to grade the deeper infiltrative component. Secondly, mixed forms have been reported, and it is likely that these terms are used interchangeably [56]. Thirdly, they share HPV 16-mediated etiology with other types of SCC [6, 22]. Fourthly, they share diagnostic difficulties in relation to sampling and recognition as above; a common term would encourage recognition and alert the

pathologist and clinician. Fifthly, this would remove the potential for confusion with origin from the urogenital tract. Finally and most importantly, their separation has no therapeutic implications.

Lymphoepithelioma-Like SCC

Lymphoepithelioma-like SCC (Fig. 7.7) bears a striking resemblance to its nasopharyngeal counterpart. This is a very rare tumor with high-grade morphology but a more favorable prognosis than keratinizing SCC, NOS [72, 110]. It is composed of ill-defined islands of undifferentiated squamous cells, associated with a marked lymphocytic, sometimes eosinophil-rich, infiltrate surrounding and infiltrating the tumor islands [80, 98]. The tumor cells have poorly defined cell borders, eosinophilic cytoplasm, and uniform vesicular nuclei with prominent nucleoli. The tumors are HPV related with no association to EBV infection [13, 98].

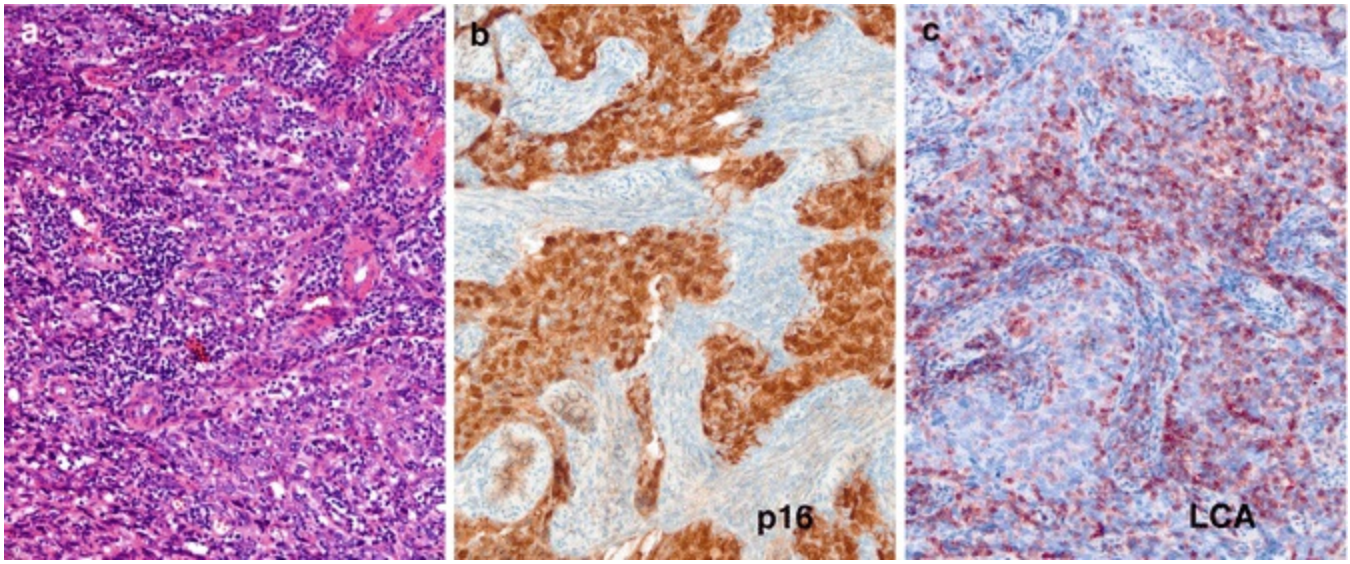


Fig. 7.7 Lymphoepithelioma-like carcinoma of the uterine cervix. (**a**) Poorly delineated islands of poorly differentiated squamous cells, accompanied by dense lymphocytic infiltration peri- and intratumoral (compare to Fig. 7.3b, non-keratinizing squamous cell carcinoma with strong peritumoral inflammatory response). (**b**) Strong p16 immunostaining of the tumor cells. (**c**) The lymphocytic infiltrate shows positive staining for leukocyte common antigen (*LCA*)

Main Differential Diagnoses of SCC

Some of the lesions that may cause diagnostic problems in colposcopy-guided biopsies are listed below. Useful immunohistochemical markers in this setting are summarized in Table 7.1.

Table 7.1 Immunohistochemical stains for the main differential diagnoses for squamous cell carcinoma of the uterine cervix [11, 27, 59, 63, 69, 74, 78, 86, 112, 118]

Cytokeratins		PAX-8	p16	GATA-3	p63	LCA	Desmin	Myogenin	HMB-45, melan-A
Tumors with a "squamous appearance"									
SCC non-keratinizing	+ve for: CK 7, CK 5/6, CK 17, pan-CK	+	+[a]	–/+[d]	+	–	–	–	–
TCC (bladder)	+ve for: pan-CKs CK 7, CK 20, CK 5/6	+	+/–	+	+	–	–	–	–
AC and ASQ G3	+ve	+	+	–	–	–	–	–	–
Glassy cell CX	+ve	+	+	–	–	–	–	–	–
Large cell NEC[b]	+/–	??	+	–	–/+	–	–	–	–
Malignant melanoma[c]	+/– (CK 7 –ve)	–	+/–	–	–	–	–	–	+
ETT	+ (esp. CK 18)	??	–	+	+	–	–	–	–
Ductal breast cancer[d]	+ve (CK 18)	–	–/+	+/–	–/+	–	–	–	–
Tumors with a small round blue cell appearance									
Lymphoma	–	–	–	–	–	–	–	–	–
Non-keratinizing SCC[e]	+ve, see above	+	+	–/+[d]	+	–	–	–	–
Small cell NEC[b]	–/+	+/–	+	–	–	–	–	–	–
Embryonal RMS	–	??	–	–	–	–	+ (cytoplasmic)	+ (nuclear)	–
Lobular breast cancer[d]	+	–	–/+	+/–	–	–	–	–	–

SCC squamous cell carcinoma, *TCC* transitional cell carcinoma, *CX* cervical carcinoma, *NEC* neuroendocrine carcinoma, *RMS* rhabdomyosarcoma, *AC* adenocarcinoma, *ASQ* adenosquamous carcinoma, *ETT* epithelioid trophoblastic tumor

[a]Rare cases of HPV-negative SCC may be –ve

[b]NEC may be positive for a variety of cytokeratins and some large cell NEC stain for p63. The best markers for neuroendocrine differentiation are CD56 and synaptophysin. Primary and secondary NEC may be positive for TTF-1. Primary cervical small cell NEC is associated with high-risk HPV infection and stains diffusely and strongly positive for p16. The knowledge of PAX-8 expression is very limited in small and large cell NEC of the female genital tract

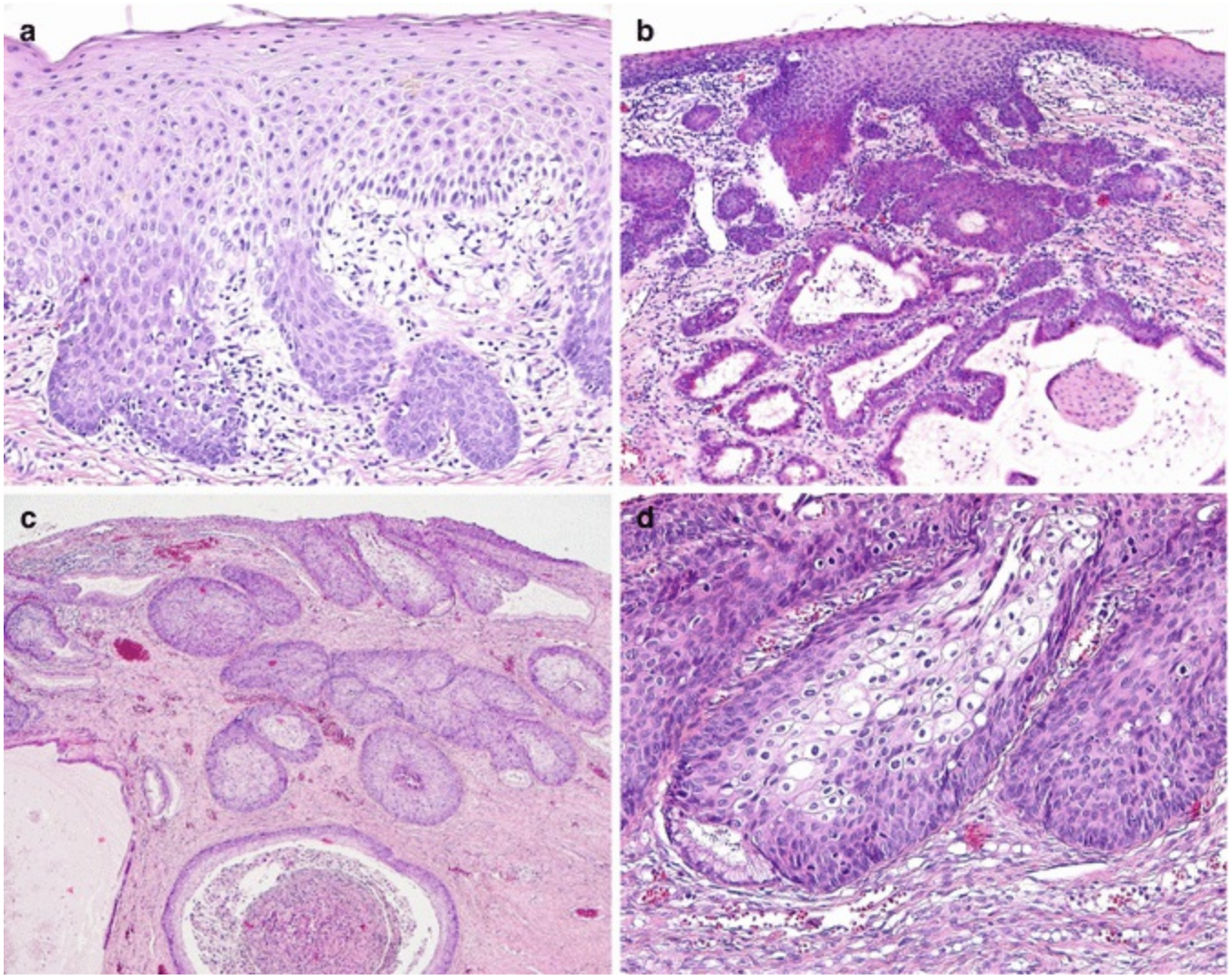
[c]Primary or secondary melanomas are positive for S-100, HMB-45, MART-1 (melan-A), and MiTF

[d]GATA-3 expression shows a strong correlation with estrogen receptor positivity. The majority of triple negative breast cancers and breast cancers with basal cell characteristics are negative. Some breast cancers are positive for p63. Invasive lobular cancer is almost always E-cadherin negative. Knowledge of GATA-3 in SCC is limited, but approximately 33% of the cases are positive. A subset of breast cancer with basal-like phenotype is positive for p63, vimentin, and CK 5/6

[e]Some non-keratinizing SCCs may consist of small cells mimicking small cell NEC, leading to a differential diagnostic challenge

Lesions Mimicking Invasive Disease (Fig. 7.8)

- Tangential cutting of (immature) metaplastic squamous epithelium and decidual change of cervical stroma. Both lesions have no nuclear atypia and low levels of mitotic activity. p16 is negative and decidual cells are positive for vimentin.
- Squamous epithelial hyperplasia with pseudoinvasion is a lesion that most commonly occurs within the ectocervix and represents a reactive change; this harbors no nuclear atypia, shows low mitotic activity, and is negative for p16.
- Placental site nodule (PSN) may mimic a squamous lesion because of its eosinophilic appearance and sometimes marked (regressive) nuclear pleomorphism. The intermediate trophoblastic cells of PSN are positive for CK 8/18 and GATA-3 as well as HPL, whereas CIN and SCC are negative. p16 may show focal patchy positivity in PSN as opposed to block staining.
- CIN 3 with extensive endocervical gland involvement can be mimicked by a rare but challenging pattern of invasion in SCC and is discussed in the paragraph of the morphologic appearance of non-keratinizing SCC (see above).


a
b
c
d

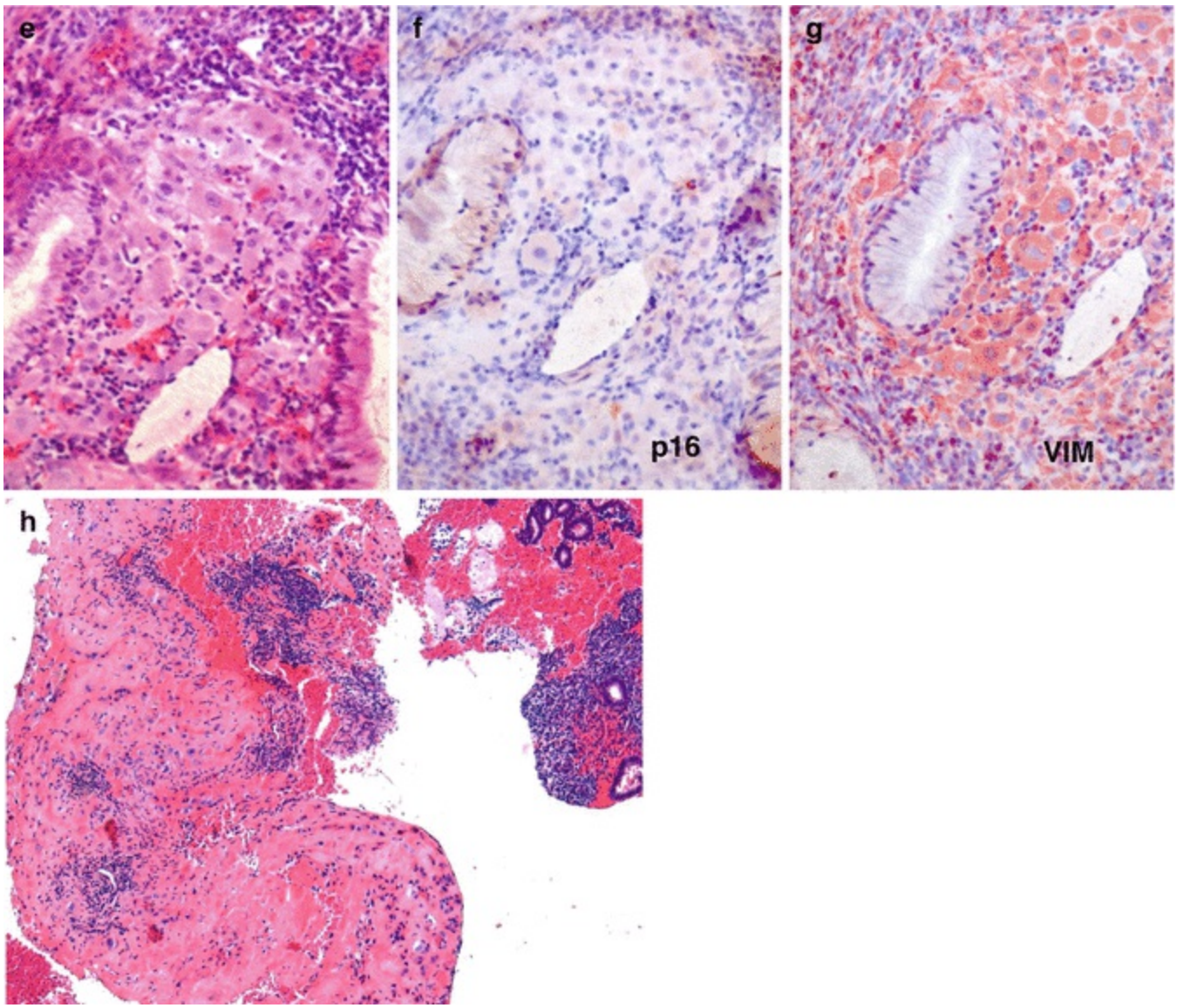


Fig. 7.8 Morphological mimics of early stromal invasion in SCC, (**a**) pseudoinvasion by elongated and thickened squamous epithelium protruding into the cervical stroma. Note the well-defined basal cell layer and the absence of cellular pleomorphism, (**b**) pseudoinvasion due to reserve cell hyperplasia and immature squamous cell metaplasia within an endocervical crypt showing squamous metaplasia, (**c**) extensive involvement of endocervical glands by CIN 3 mimicking invasive disease; note the round and smooth outline of the squamous islands without any stromal response, (**d**) higher magnification of **c** shows residual glandular epithelium at the tip of the endocervical gland, (**e**–**g**) stromal decidualization in pregnancy mimicking invasive SCC; decidualized cells are negative for p16 but positive for vimentin, (**h**) placental site nodule mimicking fragments of non-keratinizing SCC

Differential Diagnosis of Invasive Lesions

- Transitional cell carcinoma (TCC) of the urinary bladder may invade the uterine cervix and may mimic non-keratinizing SCC. Without knowledge of clinical history, the diagnosis may be challenging. TCCs are positive for p63 and GATA-3 and may show p16 expression (which is in general not diffuse and strong as in cervical HPV-related SCC). Low molecular weight cytokeratins (e.g., CK 34β-

E12) may not be helpful because these are positive in SCC.

- Poorly differentiated adeno- or adenosquamous carcinoma of the uterine cervix can be distinguished by its negativity for p63. Sometimes adenocarcinomas demonstrate PAS-positive secretions; very focal PAS positivity may occur in SCC.
- Glassy cell carcinoma (Fig. 7.9) is a poorly differentiated variant of adenosquamous carcinoma characterized by cells with sharp cytoplasmic margins, eosinophilic cytoplasm with "ground-glass" appearance, and large nuclei containing prominent eosinophilic nucleoli. Many tumors show a dense peritumoral inflammatory response containing numerous eosinophils, which is uncommon in SCC. The tumors are positive for p16, cytokeratins, and MUC-2 but negative for p63.
- Large cell neuroendocrine carcinomas (Fig. 7.10) have a diffuse, organoid trabecular, or cord-like pattern, similar to the fingerlike pattern of invasion of SCC, and are composed of cells that have abundant eosinophilic cytoplasm, large nuclei, and prominent nucleoli. The majority of tumors show areas of dirty tumor necrosis within the infiltrating tumor cell nests, a pattern not generally seen in SCC. The majority, but not all, of tumors express neuroendocrine markers and may be positive for p63 [74]. TTF-1 is not uncommonly positive.
- Malignant melanoma (MM) should always be included in the differential diagnosis in any unusual looking tumor, especially those with prominent eosinophilic nucleoli. Primary MM within the cervix is exceedingly rare. The presence of melanin pigment and immunostains for CK 7, p16, and melanocytic markers may aid diagnosis.
- Gestational trophoblastic disease may involve the uterine cervix, and particularly epithelioid trophoblastic tumor (ETT) may mimic SCC (Fig. 7.11). ETT is characterized by islands of relatively monomorphic cells with generally abundant eosinophilic cytoplasm and areas of eosinophilic geographic necrosis [69]. Among the tumor cells, there are small-sized vessels with normal-appearing vessel walls. ETT is positive for cytokeratins, especially CK 18 and GATA-3, HPL, inhibin, and cyclin E, but is negative for p16 and neuroendocrine and melanocytic markers. It should be noted that p63 stains positive in SCC as well as ETT and is therefore not useful in distinction. The Ki-67 labeling index is about 10–15% and tends to be much higher in SCC.
- Metastatic ductal carcinoma of the breast may mimic SCC [89]. Clinical data and the use of GATA-3, mammaglobin, and GCDFP-15 may be helpful. Some breast cancers are positive for p16 [102] and for p63 [112].
- Stratified mucin-producing carcinoma ("invasive SMILE") represents a recently

described variant of adenocarcinoma [62, 87] that may provoke some differential diagnostic problems with glassy cell and poorly differentiated SCC. The tumor cells infiltrate the cervical stroma in the form of nests of stratified, columnar cells with bland, round to ovoid nuclei without prominent nucleoli. A distinct pattern of peripheral nuclear palisading is present in the majority of cases [62]. Some neutrophilic and eosinophilic infiltration may surround the infiltrative tumor nests. Mucicarmine and/or PAS staining may highlight mucin production. Morphologic evidence of squamous differentiation in the form of keratinization or intercellular bridges is not present. Tumor cells stain diffusely positive with p16.

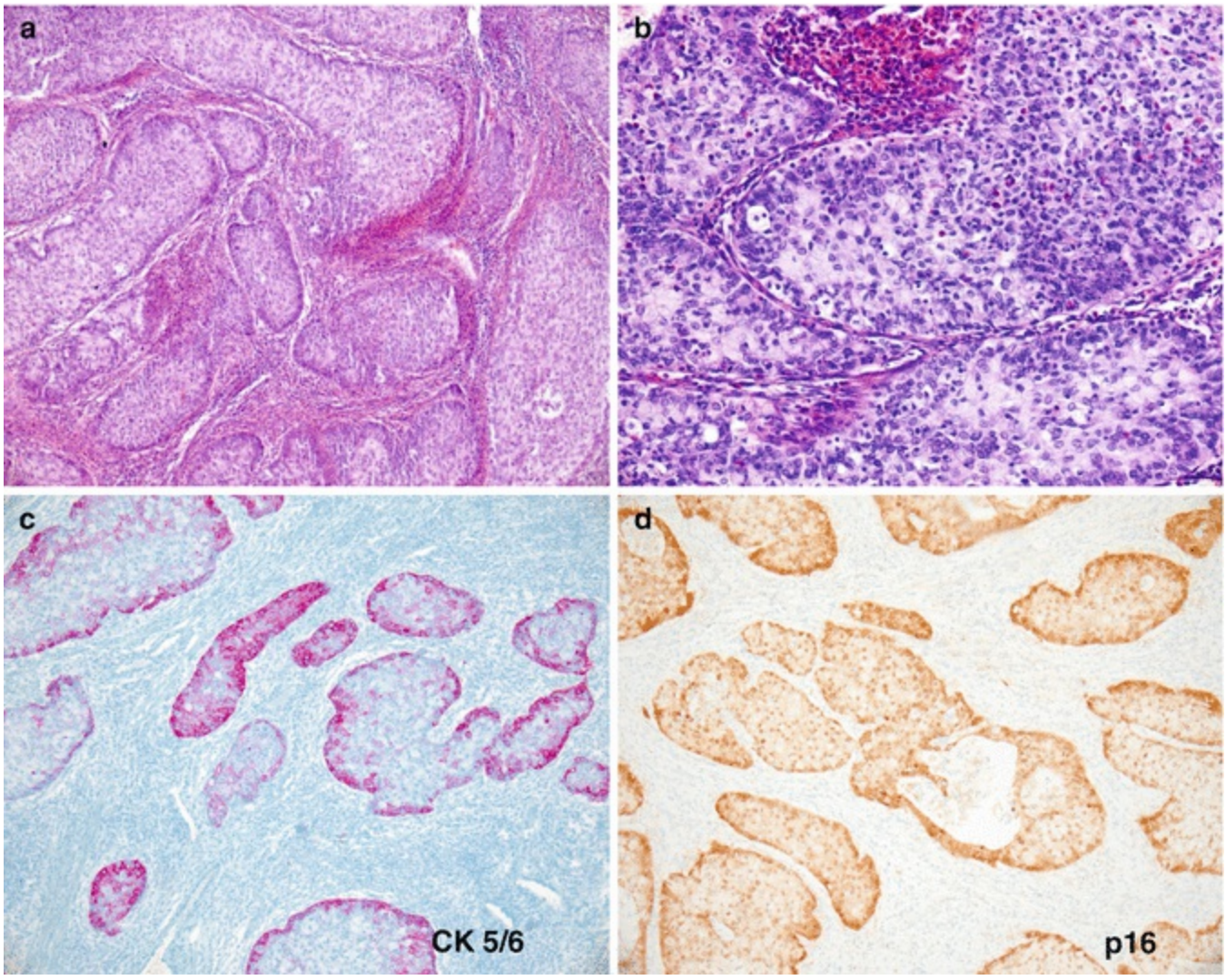


Fig. 7.9 Glassy cell carcinoma. (**a**, **b**) Infiltrating cords and bands of large cells with prominent nucleoli and basophilic pale cytoplasm. Peri- and intratumoral infiltrate rich in eosinophils. (**c**, **d**) Tumor cells are positive for CK 5/6 with peripheral staining and show positivity for p16

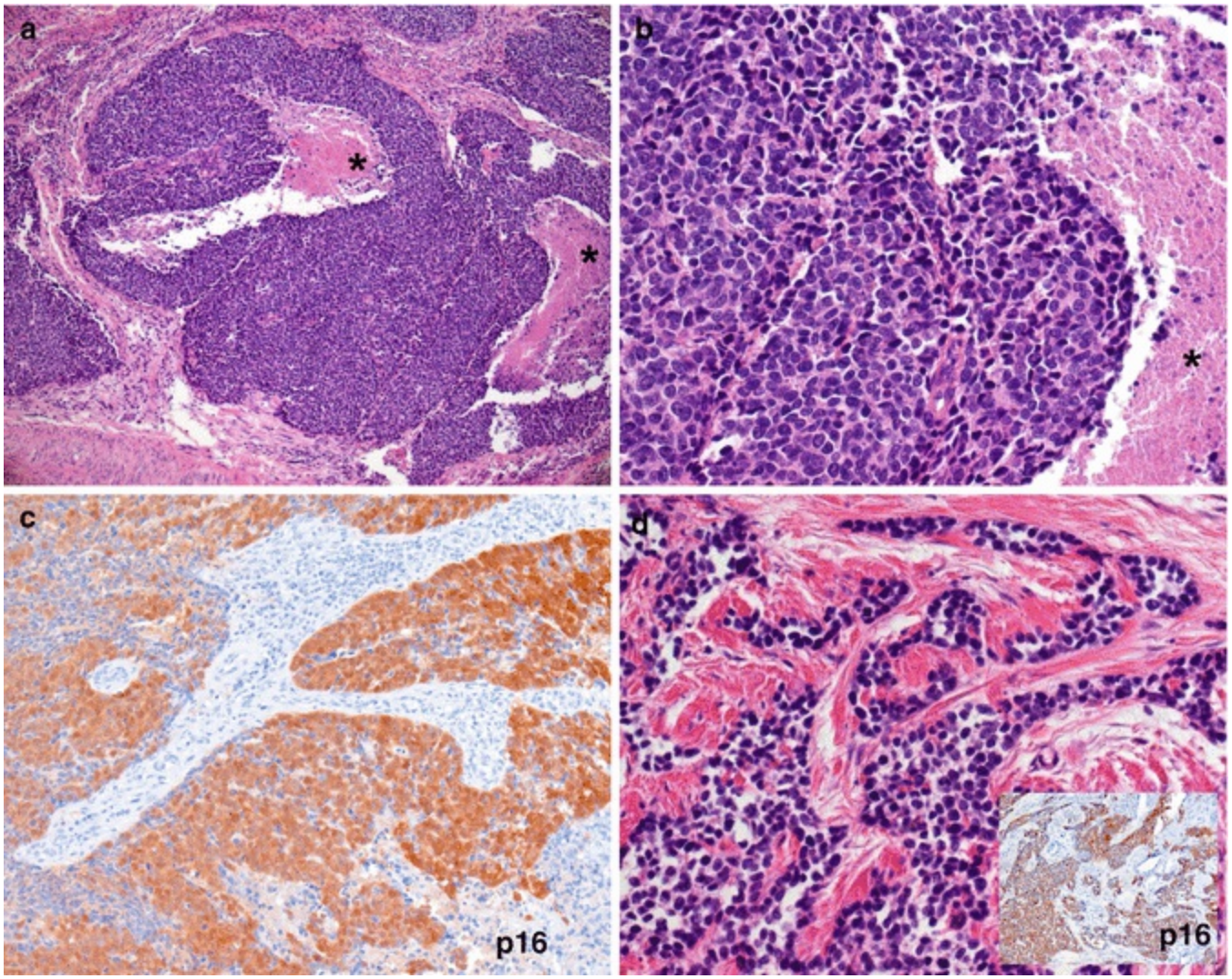


Fig. 7.10 Neuroendocrine carcinomas of the uterine cervix, (**a**, **b**) large cell neuroendocrine carcinoma with large islands of cells with scanty cytoplasm but pleomorphic nuclei with some nuclear molding. Within the tumor cell islands, there is comedo-like necrosis, (**c**) positive staining for p16 in a large cell neuroendocrine carcinoma, (**d**) small cell neuroendocrine carcinoma composed of small cells with scanty cytoplasm infiltrating the endocervical stroma. The inset shows positive staining for p16

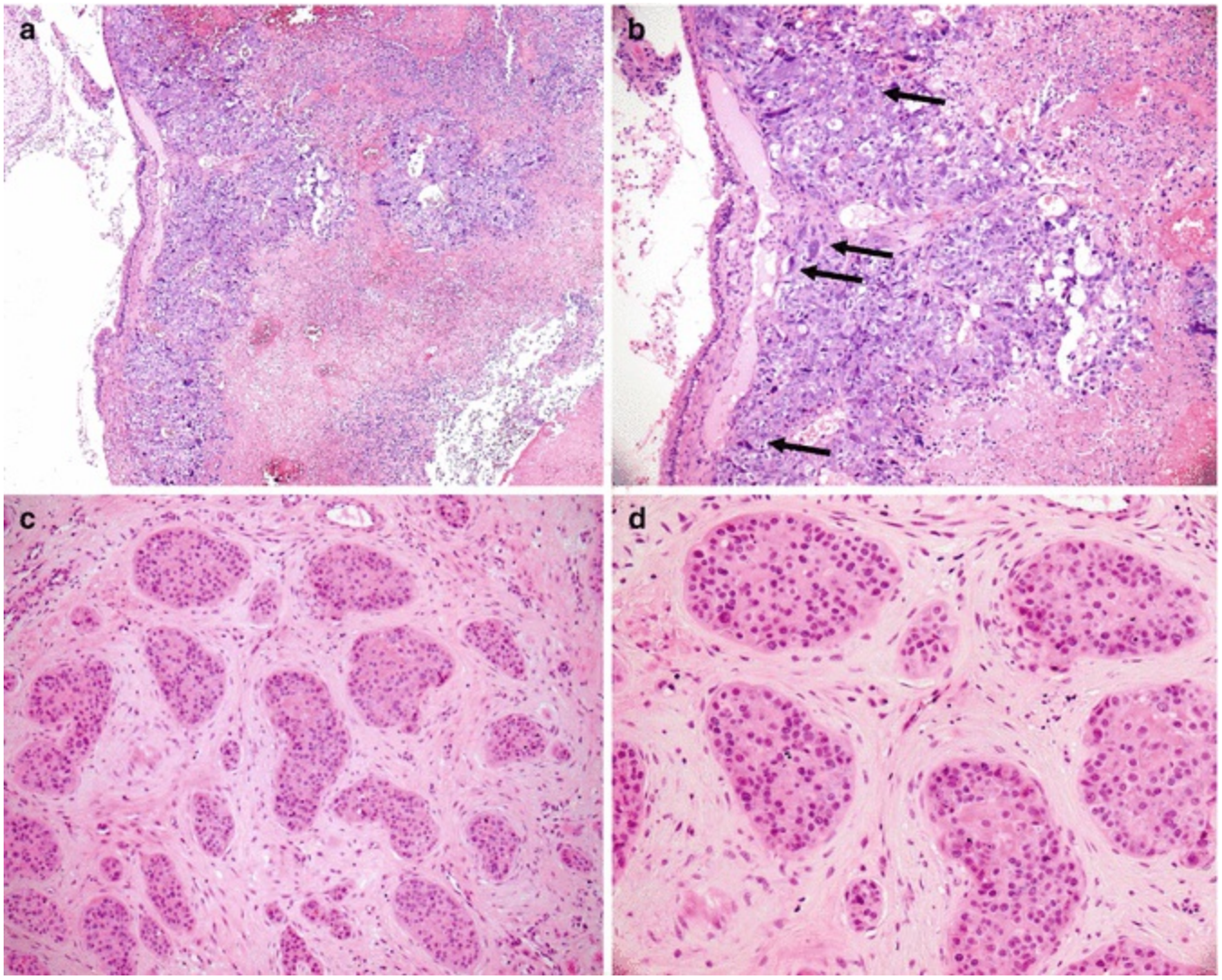


Fig. 7.11 Gestational trophoblastic disease involving the cervix, (**a**, **b**) gestational choriocarcinoma within the endocervical stroma surrounded by hemorrhagic necrosis. Note the syncytiotrophoblastic giant cells (**c**, **d**) epithelioid trophoblastic tumor mimicking non-keratinizing SCC with infiltrative islands of monomorphic cells, surrounded by pale eosinophilic necrosis. Tumor cells are monomorphic without mitotic figures. There is no peritumoral stromal remodeling

Tumors with a Small Round Blue Cell Appearance

- As mentioned above, non-keratinizing SCC may be composed of small cells and should not be misinterpreted as the following lesions. For immunohistochemical differential diagnoses, see Table 7.1.
- Small cell neuroendocrine carcinomas (Fig. 7.12) may show small areas of dirty tumor necrosis within the infiltrating tumor cell nests. The majority, but not all, of tumors express neuroendocrine markers, with CD56 and synaptophysin being the most sensitive. p63 is almost always negative. TTF-1 is not uncommonly positive and does not help to exclude a pulmonary primary.
- Lymphomas may primarily or secondarily occur within the cervix [59] and should

be included within the differential diagnosis. They are negative for epithelial markers and p16, but positive for lymphoid markers.

- Embryonal rhabdomyosarcoma affects women in their second and third decades. These present as polypoid lesions with small round or spindle cells with hyperchromatic dense nuclei. The cells typically show subepithelial condensation (cambium layer). Tumor cells are positive for vimentin and myogenic markers but negative for cytokeratins and p16.
- Rarely metastatic lobular breast cancer may involve the uterine cervix with a small round blue cell appearance [89]. As mentioned above, clinical data and the use of GATA-3, mammaglobin, E-cadherin and GCDFP-15 may be helpful in the differential diagnosis.

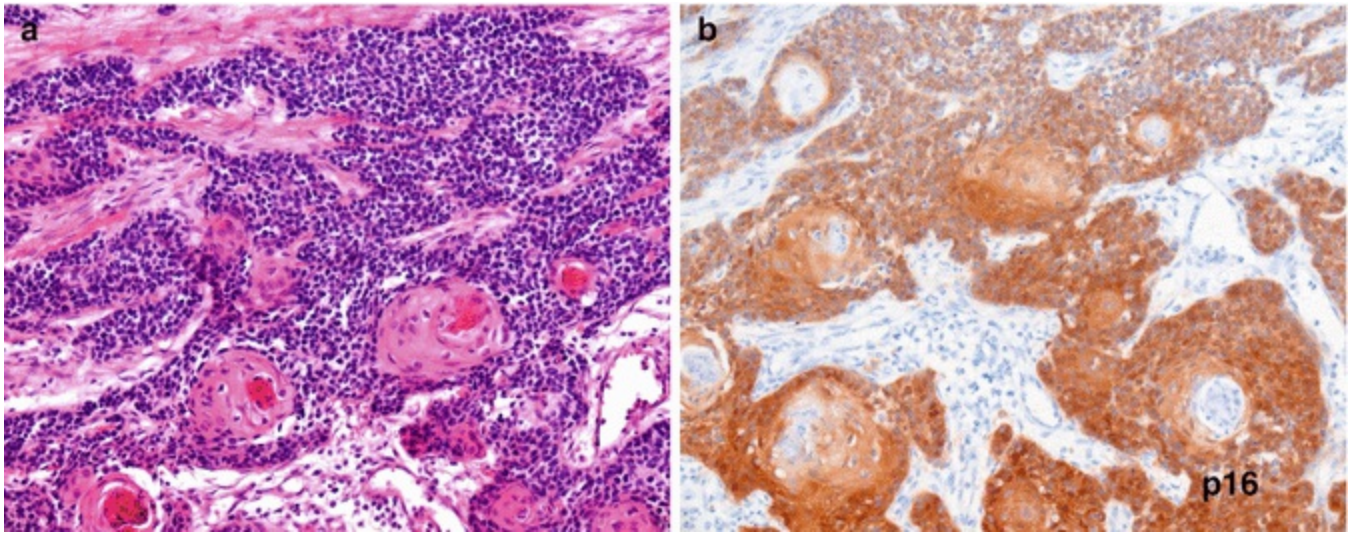


Fig. 7.12 Mixed squamous and small cell neuroendocrine carcinoma of the uterine cervix. (**a**) Foci of squamous epithelium within infiltrating small cells with dark blue nuclei and scanty cytoplasm, (**b**) both components stain positive for p16

Diagnostic Immunohistochemistry in SCC

There is a wide range of low and high molecular weight cytokeratin (CK) expression in SCC [105]. Broad-spectrum cytokeratins, such as AE 1/3, MNF 116, and Pan Plus as well as CK 5/6, CK 7, and CK 17, are positive in SCC [11]. Nearly all SCCs are positive for p40 and p63 [118] and for p16 [86]. The majority, but not all, of SCCs are positive for the Müllerian marker PAX-8 [63]. The main differential diagnoses and helpful immunohistochemical stains are summarized in Table 7.1.

Diagnosis and Measurement of Superficially Invasive

Squamous Cell Carcinoma

Invasive squamous cell carcinoma (SCC) is a lethal disease with a high death toll worldwide, justifying its treatment by radical measures. Cervical screening, designed to prevent cervical cancer through detection of precancerous changes, leads to detection of minimally invasive cases, before they are symptomatic or clinically apparent, which may not require radical treatment. Various terms have been used in the past to describe such cases including "early stromal invasion" and "microinvasive carcinoma," and there have been many different staging proposals. Despite these attempts various terms have been used imprecisely and inconsistently, and the measurement and staging of low-volume disease continues to be problematic, with potential for overtreatment and undertreatment of individual cases. The use of imprecise terms is strongly discouraged in favor of universally recommended terms and staging systems [34].

Recently there has been a concerted effort to unify the terminology and reporting parameters of all HPV-related lower anogenital tract squamous lesions, in recognition that these have similar biology and management implications [19]. This project, termed the Lower Anogenital Squamous Terminology (LAST) project, included a working group that focused on defining criteria for superficially invasive squamous cell carcinoma (SISCCA), aiming to clearly delineate cases that can be managed conservatively from those requiring radical treatment. The group identified 1863 publications and drew data from 194, most of which related to cervical disease. They concluded that the definition of SISCCA differed for different anogenital sites and that for the cervix SISCCA corresponds to FIGO stage IA1. The presence of vascular invasion and the pattern of invasion do not influence the FIGO stage of cervical carcinoma. Conservative options are not recommended for FIGO stage IA2 and beyond.

Diagnosis of Stromal Invasion

In low-volume disease, stromal invasion may be obvious, but is sometimes subtle. Features that may be helpful in identifying stromal invasion are listed below:

- Small, angulated buds of atypical squamous epithelial cells with a more differentiated or "hypermature" appearance (Fig. 7.13); this phenomenon, termed "paradoxical" differentiation, is believed to result through an epithelial-mesenchymal transition(EMT)-like mechanism whereby invading cells acquire mesenchymal phenotypic and functional alterations that facilitate invasiveness. Immunohistochemistry for EMT-related markers such as cyclin D1 has been put forward to facilitate diagnosis of stromal invasion but must be interpreted with caution.
- Invasive buds may be seen in continuity with surface epithelium or gland crypts involved by high-grade cervical intraepithelial neoplasia/squamous intraepithelial

lesion (CIN/SIL), and these may be single or multiple.

- Alternatively they may be detached from any epithelium, at least in the plane of sectioning, and seen as discrete islands.
- Irrespective of whether they are attached or separate, their "hypermature" appearance is characterized by more abundant eosinophilic cytoplasm than that in the cells within the CIN/SIL from which they arise.
- Nuclei are paler than those of the neighboring high-grade CIN/SIL, tend to be more vesicular and pleomorphic, and may contain nucleoli; in some cases nuclei may appear to be blander than those in the adjacent CIN/SIL.
- Unlike the orderly basal palisade of high-grade CIN/SIL, these islands exhibit altered and haphazard nuclear polarity.
- Absence or loss of a sharply defined surrounding basement membrane may facilitate recognition; this may be aided by immunohistochemistry for laminin or collagen IV.
- Dyskeratosis may be present.
- Characteristically there is a stromal reaction, which may be inflammatory, edematous, or desmoplastic; this may be difficult to discern if the adjacent CIN/SIL is also surrounded by an intense inflammatory reaction.
- There are different patterns of stromal invasion, such as a "spray bud" pattern (which comprises tiny nests of hypermature squamous cells), a confluent pattern, and a pattern with invasive "tongues"; these patterns do not independently influence prognosis over and above invasive depth.

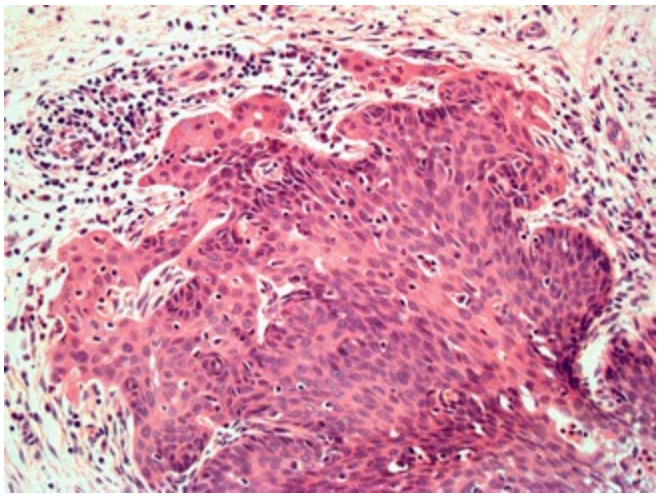


Fig. 7.13 Stromal invasion characterized by "paradoxical maturation"; cells with abundant eosinophilic cytoplasm form nests with irregular edges and a surrounding inflammatory reaction; adjacent noninvasive epithelium shows regular borders and consists of cells with scanty cytoplasm, a basaloid appearance, and darker, monotonous nuclei

Measurement of Stromal Invasion

Two tumor dimensions are required for FIGO staging: depth and horizontal spread [88]; a FIGO stage IA1 carcinoma is defined as one which:

- Is *not* a grossly visible lesion
- Has an invasive depth of ≤3 mm from the basement membrane of the point of origin
- Has a horizontal spread of ≤7 mm in maximal extent
- Has been completely excised

The definition of SISCCA requires, in addition, three further elements [19]:

- A comment for cases with positive margins; this means cases showing the invasive component at endocervical, ectocervical, or deep margins; a margin positive for only CIN/SIL of any grade does not negate the diagnosis of SISCCA but should be mentioned:
  - The examined invasive tumor exceeds the dimensions for a SISCCA.
  - The examined invasive tumor component is less than or equal to the dimensions for a SISCCA and concludes that the tumor is "at least a superficially invasive squamous carcinoma."
- The presence or absence of lymphovascular space involvement (LVSI).
- The presence, number, and size of independent multifocal carcinomas (after excluding the possibility of a single carcinoma) (see below).

FIGO provides some guidance on measuring the depth of invasion, but does not specify how to measure width, i.e., horizontal spread/lateral extent, or how to deal with cases showing multiple discrete foci of stromal invasion. It should be noted that tumor volume is reported to be one of the prognostic factors for early-stage tumors [8, 114] but is cumbersome to apply in routine diagnostic practice. In everyday practice, measurement of tumors in two dimensions (depth and maximum width) is an adequate surrogate for tumor volume. These dimensions should be measured as follows (Figs. 7.14 and 7.15):

- Depth of invasion (Fig. 7.14) is taken from the base of the epithelium (surface or glandular) from which the carcinoma arises, as specified in the FIGO classification:
  - When the invasion is in continuity with CIN/SIL, the measurement is from the farthest edge of invasion to the base of the CIN/SIL epithelium.
  - When the invasive focus is not attached to CIN/SIL in the plane of sectioning

but there is nearby crypt or surface epithelium showing CIN/SIL, the measurement is from the farthest edge of invasion to the base of the CIN/SIL epithelium.

  - When invasion is seen without any CIN/SIL in the vicinity in the plane of sectioning, the depth must be measured from the deepest focus of tumor invasion to the base of the nearest non-neoplastic surface epithelium.

  - Inevitably there is potential for over- and underestimation of invasive depth in cases where the invasive foci are detached from the epithelium of origin.

- Horizontal extent should be assessed not only in an individual section but also in adjacent blocks (Fig. 7.15), and it is the largest horizontal dimension that should be taken into account for staging purposes.

  - For tumors showing a single confluent focus of invasion, a "spray bud" pattern, multiple seemingly discrete foci arising from a single continuous complex area of high-grade CIN/SIL involving surface and crypt epithelium, or a combination of these, the measurement is from one lateral edge of the invasive component to the other and not limited to individual confluent areas. This measurement is straightforward if the maximum extent of disease is included in a single section.

  - When there is invasion present in three consecutive sections and ≤7 mm in any one of these, the lateral extent is taken as likely to represent >7 mm, and the case should be reported as exceeding the lateral dimensions for FIGO stage IA1.

  - When there is invasion in four or more consecutive sections, the horizontal extent should be measured on individual sections as well as estimated by the number of slices involved and the block thickness, and the greater of the two measurements should be provided in the report.

- Multifocal invasion is reported to occur in 12% of invasive carcinomas [7, 20]. When these present as a coalescent confluent lesion, measurement is relatively straightforward as above; however measurement of lateral extent in cases showing two, and occasionally more, separate discrete foci of stromal invasion forms a special category. The foci must be far apart in the same or different blocks, separated by tissue devoid of any sign of stromal invasion. Levels should be examined to exclude invasion in intervening foci. An arbitrary minimum distance of 2 mm between foci has been put forward in two recent studies [20, 75]. Two methods have been proposed to deal with this situation:

  - The first is to add the individual widths of the discrete foci [94]; in practice

this is unlikely to over stage an individual case and provide a good estimate of tumor volume. It has the potential, however, of overestimating the lesion in biological terms.

– The second [75] is to state the number and dimensions of individual foci and stage the case according to the dimensions of the largest. This appears to be scientifically valid as prognosis in such cases is likely to be determined by the largest lesion; 22 cases reported in this way with 2–4 discrete foci and an arbitrary distance of 2 mm between foci showed good clinical outcomes; had these cases been staged as above, by measuring the distance between the farthest foci, 50% (11 of 22) would have been categorized as FIGO stage IB1 and potentially overtreated.

– In the absence of robust randomized and controlled prospective studies, the second approach is favored [50], as this provides more complete information. All such cases should be discussed at multidisciplinary meetings to offer optimized treatment options to each individual case. This approach also concurs with the recent LAST guidance, which states that the "presence, number, and size of independent multifocal carcinomas (after excluding the possibility of a single carcinoma)" should be included in the pathology report.

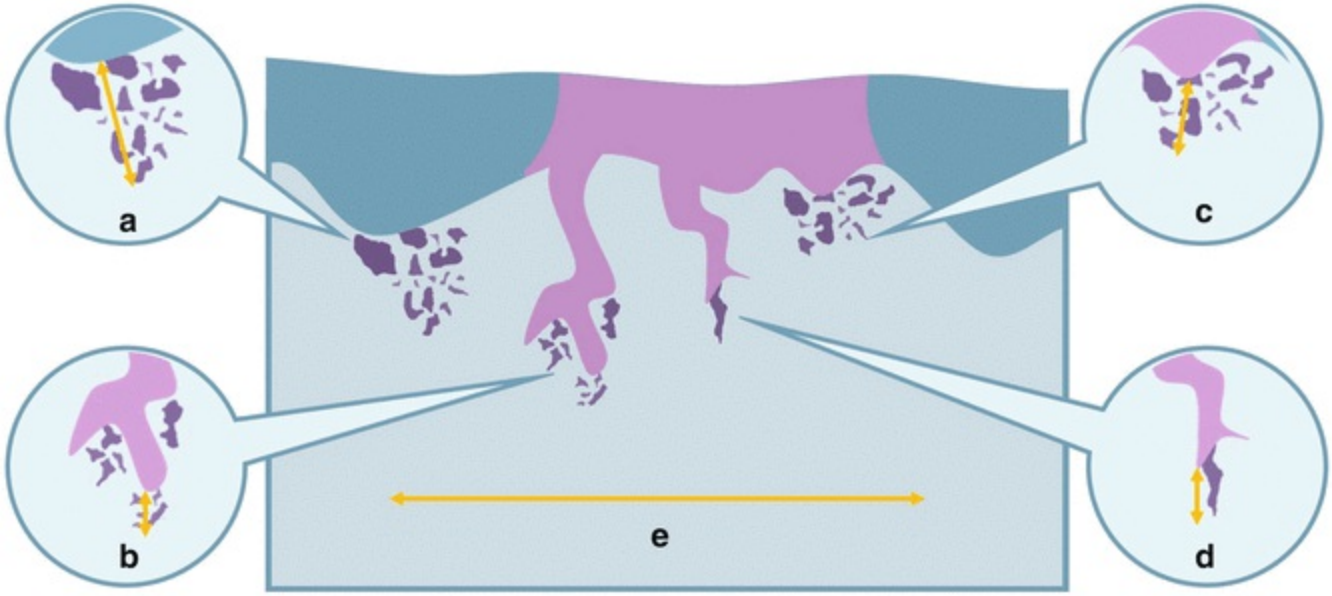


Fig. 7.14 Measuring stromal invasion. Depth of invasion is measured from the deepest point of invasion till the base of the closest intraepithelial neoplastic epithelium, cryptal or surface, from which it arises (**b**–**d**), whether it occurs in continuity with the epithelium (**d**) or detached from it (**b**, **c**). When there is no obvious intraepithelial lesion of origin, the measurement is to the base of the nearest surface epithelium (**a**). Horizontal width in non-confluent disease is measured from the outermost edge of the first focus till the opposite edge of the farthest focus (**e**); please also see Fig. 7.15 for further details on measuring horizontal extent (Courtesy of Lucas Catalan Galan and Laura Casey)

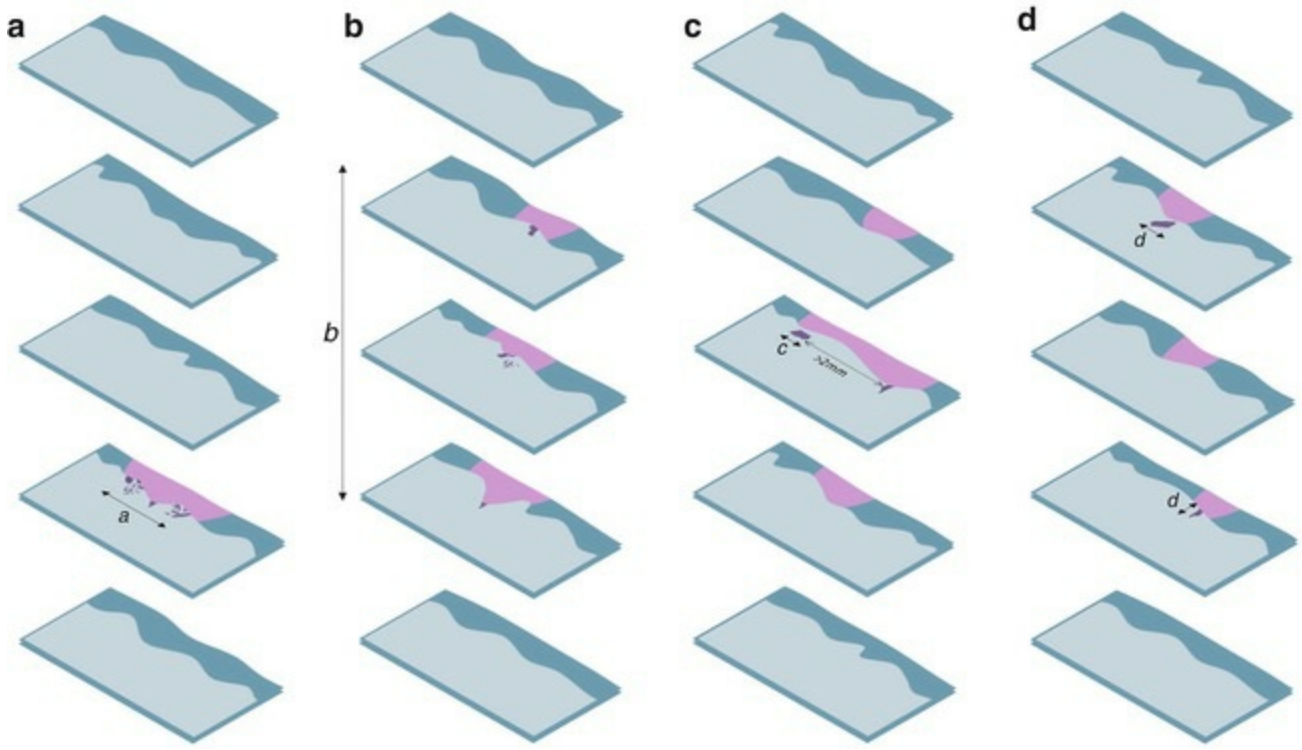


Fig. 7.15 Measuring tumor width: (**a**) If the maximum extent of disease is included in a single section, this is measured as indicated (*a*). (**b**) When there is invasion present in three consecutive sections and ≤7 mm in any one of these, the lateral extent is taken as likely to represent >7 mm, and the case should be reported as exceeding the lateral dimensions for FIGO stage Ia1. If seen in four slices or more, the maximum width should be measured on a single slice as well as estimated according to dimensions of the specimen and number of slices involved; the larger of the two measurements should be used for staging. (**c**, **d**) When two, or occasionally more, separate discrete foci of stromal invasion are seen in the same (**c**) or different (**d**) blocks, separated by 2 mm or more of tissue devoid of stromal invasion, these should be measured as separate foci (*c*, *d*), and the larger of the two should be used for staging (Courtesy of Lucas Catalan Galan and Laura Casey)

It is not possible to draw up guidance for measurement that covers each individual case, and ultimately the decision on how to measure an individual case rests with the reporting pathologist. The value of obtaining and examining multiple levels and seeking additional opinions cannot be overemphasized.

Factors Affecting Prognosis and Staging

A range of morphological factors is reported to have prognostic significance in cervical SCC [103, 104, 113]; these are briefly summarized below.

Tumor Stage

Stage is the strongest prognostic factor in cervical cancer. It is recommended that both FIGO and TNM staging systems be applied, as the former does not include lymph node involvement; these are detailed in Appendix 3. Staging of FIGO stage IA or subclinical

carcinomas is carried out through histological examination. The tumor is measured on histological sections on an excisional biopsy or hysterectomy specimen according to the guidance above. Clinically evident tumors, regardless of the manner of presentation, are automatically FIGO stage IB or higher. Traditionally FIGO staging is carried out clinically through examination under anesthetic, though in recent years and where resources are available, this is supplemented by imaging studies, principally magnetic resonance imaging (MRI).

Tumor Grade

Grading is clearly the most controversial of all the histopathological factors dealing with prognostic outcome. The major reason for this is the lack of standard criteria. There is no recommendation for a uniform grading system in the WHO classification of cervical cancer [108], although it is stated that SCC can be grouped into poorly, moderately, and well-differentiated tumors (conventional grading) based on the degree of keratinization (regardless of the nuclear morphology). It is also mentioned that the prognosis of keratinizing tumors (G1 or G2) is not necessarily better. In clinical practice, the majority of cervical SCCs do not show keratinization. As a result, the data on the prognostic relevance of grading cervical cancers are contradictory [60, 103, 104, 113].

It is of historical interest that cervical SCC grading is based on the method described by Broders [7] who established a grading system of squamous cell carcinomas of the lip that is oriented on the degree of keratinization – which seems reasonable for carcinomas in this location. Wentz and Reagan [120] modified this system, defining small and large cell squamous cell carcinomas while preserving the parameter of keratinization. This system was incorporated into the WHO classification of cervical cancer in 1975 [90] and has continued ever since.

Due to the insufficient prognostic evidence of the (conventional) grading of cervical cancer, the current German S3 guidelines determine that there is significance to cervical cancer grading in terms of therapeutic decisions only in combination with other parameters, not by itself (AWMF 2014, [41]). Current results suggest that a binary grading model based on the conventional grading system differentiating low- from high-grade tumors shows better prognostic significance [43]. A grading system that does not integrate the degree of keratinization of cervical carcinomas but morphological parameters on the invasive front has also been put forward [24, 60]. In addition the pattern of invasion (see Fig. 7.3) seems to play a role in surgically treated and in advanced (FIGO III/IV) cervical SCC, though not as an independent parameter [39, 40, 47]. Clearly, further studies are required to establish the utility of a grading system that is prognostically valuable and that can be universally applied.

Tumor Diameter

The tumor size, given as its maximum dimension, represents a strong prognostic factor [44, 55] and is important for substaging of pT1b and pT2a tumors. During recent years there have been several attempts to reduce the radicality in the surgical approach for treatment ([54, 92] and see Chap. 4). Several recent studies have reported that tumors of ≤2 cm in largest dimension are associated with a lower incidence of lymphovascular space involvement (LVSI) and pelvic lymph node involvement and showed an improved prognostic outcome [42, 54, 55, 117]. A maximum size of 2 cm has been a criterion for patient selection for trachelectomy [100]. Two ongoing studies are designed to prospectively examine the oncologic safety of a reduced surgical approach in patients with small tumors of ≤2 cm [38, 99].

Tumor Volume

Tumor volume based on the three measured tumor dimensions has been shown to predict prognosis more reliably than measurements in only one or two dimensions in early-stage cervical cancers: a volume of less than 420mm^3 has been suggested to be associated with no lymph node metastasis [8, 114]. This is the basis for recommending the recording of three tumor dimensions in pathology reports, two of horizontal extent, and one of depth of invasion or tumor thickness. However, this is generally considered cumbersome, and volume is not taken into account in planning management in most centers.

Surgical Margin Status

There is no doubt that the presence of tumor at surgical margins affects outcome [104]. A recent study reported 5-year overall survival (OS) rates of 85.4% for negative and 60.9% for positive surgical margins (p <0.005; [111]).

The impact of the tumor distance from surgical margins (Fig. 7.2) is more controversial. An earlier study stated that a margin clearance at the site of colpectomy of ≤5 mm is an important risk factor for local recurrence with and without simultaneous distant metastases [26]. One analysis stated that a surgical margin of ≤2 mm was significantly associated with an increased risk of overall disease recurrence (36% vs. 9%, p = 0.009) and locoregional recurrence (22% vs. 4%, p = 0.0034, [73]). Viswanathan and colleagues estimated an overall recurrence rate of 20% and a local recurrence rate of 11% in cases with close paracervical margins (>0/<1 cm) as compared to 11% and 10%, respectively, for negative (≥1 cm) margins [116]. A study using the surgical approach of total mesometrial resection (TMMR) showed that there is no increased risk for recurrent disease and shorter OS, even when the distance of the tumor to the margin is less than 1 mm [37].

Lymphovascular Space Involvement (LVSI)

The diagnosis of lymphovascular space involvement (LVSI) requires the demonstration of tumor cells (single cells or groups) within channels that are unequivocally lined with endothelium (Fig. 7.16a; [122]). It has been stated within the TNM supplement of the UICC that spaces around tumor cell nests caused by shrinkage during tissue processing (Fig. 7.20) and spaces which cannot be clearly defined as lymphatic vessels should be classified as negative forLVSI (i.e., L0; [122]). To avoid the misinterpretation of shrinking artifacts as LVSI, this should be evaluated at the invasive front. Areas of desmoplastic change should be excluded from the evaluation for LVSI.D2-40 may help to identify lymphatic invasion immunohistochemically (Fig. 7.16b; [66, 115]), whereas CD 31 stains endothelial cells both in blood and lymphatic vessels. The ICCR states that caution should be exercised in the interpretation of D2-40 because some cells of SCC, representing basal cells, may show positive staining, leading to misinterpretation as lymphatic channels [50] completely filled (and expanded) by tumor. There have been some attempts to quantify ("grade") the LVSI, which may have prognostic impact [33, 109, 115]. At present there is no widely accepted method for quantification of LVSI, but its absence or presence should be given within pathology report, with a descriptive comment on whether it is focal or widespread.

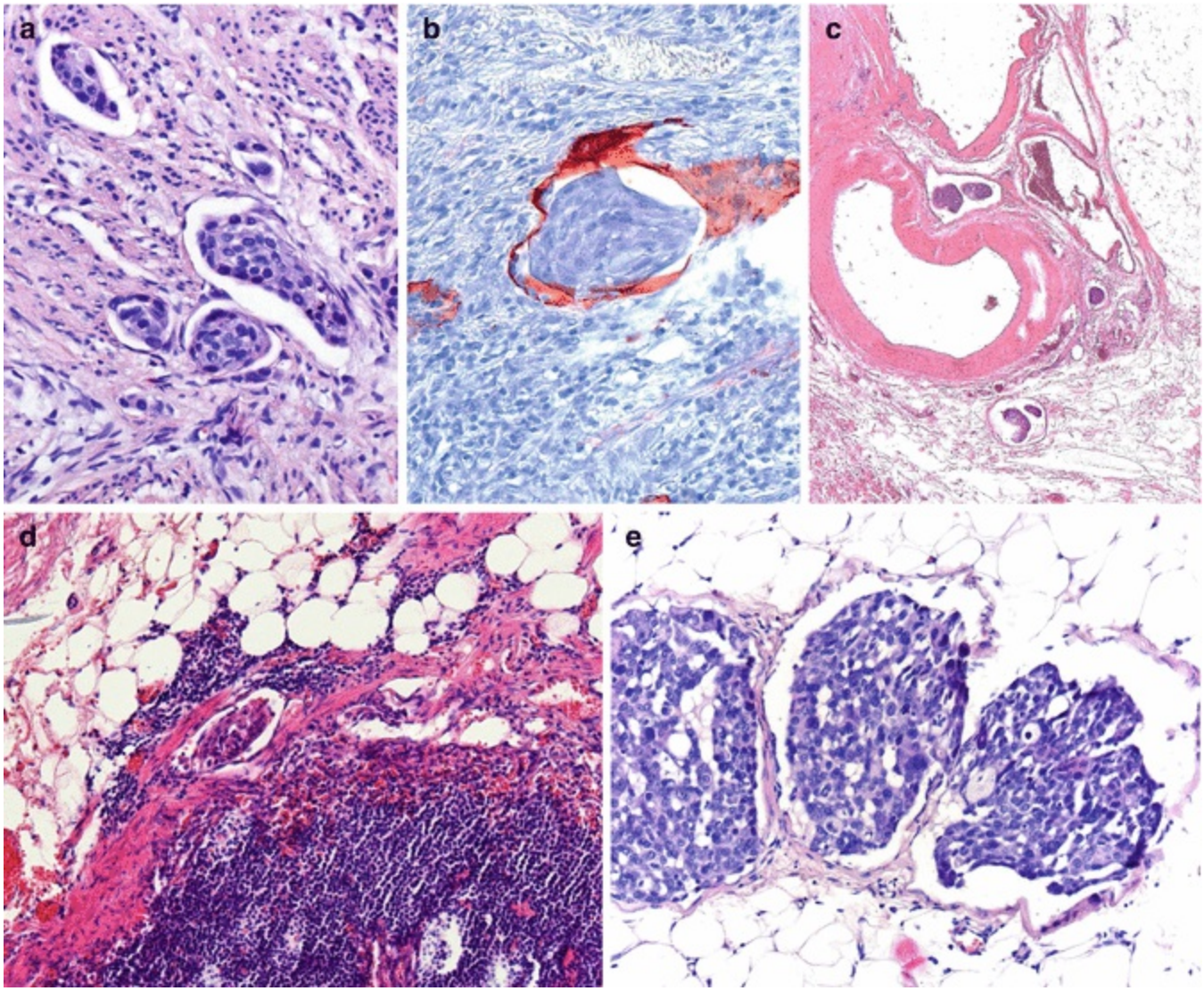


Fig. 7.16 Different features of lymphovascular space involvement (LVSI). (**a**) LVSI within the cervical stroma. Note that the spaces surrounding the tumor cells are lined by flat endothelial cells, (**b**) LVSI highlighted by immunohistochemistry using D2-40 (podoplanin), (**c**) LVSI within lymphatic vessels surrounding large vessels within parametrial tissue. This feature should be categorized as LVSI (L1) and not as parametrial involvement (stage pT2b). (**d**) LVSI within the capsule of a pelvic lymph node. This feature should be categorized as LVSI (L1) and not as lymph node involvement (pN1). Please compare with Fig. 7.17d picturing isolated tumor cells within a lymph node. (**e**) LVSI within the fatty tissue surrounding a pelvic lymph node. This feature should be categorized as LVSI (L1) and not as involvement of the pelvic soft tissue (stage pT2b)

The prognostic relevance of LVSI is controversial. In a review of 25 studies evaluating over 6500 patients, only three reports found LVSI to be an independent risk factor [16]. It may be that LVSI is of more prognostic impact in small-sized tumors (<2 cm; [81]).

The most recent TNM supplement has defined (venous) *vessel invasion* if there is a tumor invasion within the vessel wall. This does not necessarily require demonstration of tumor cells in the lumen of the vessels (Fig. 7.17a, b; [122]).

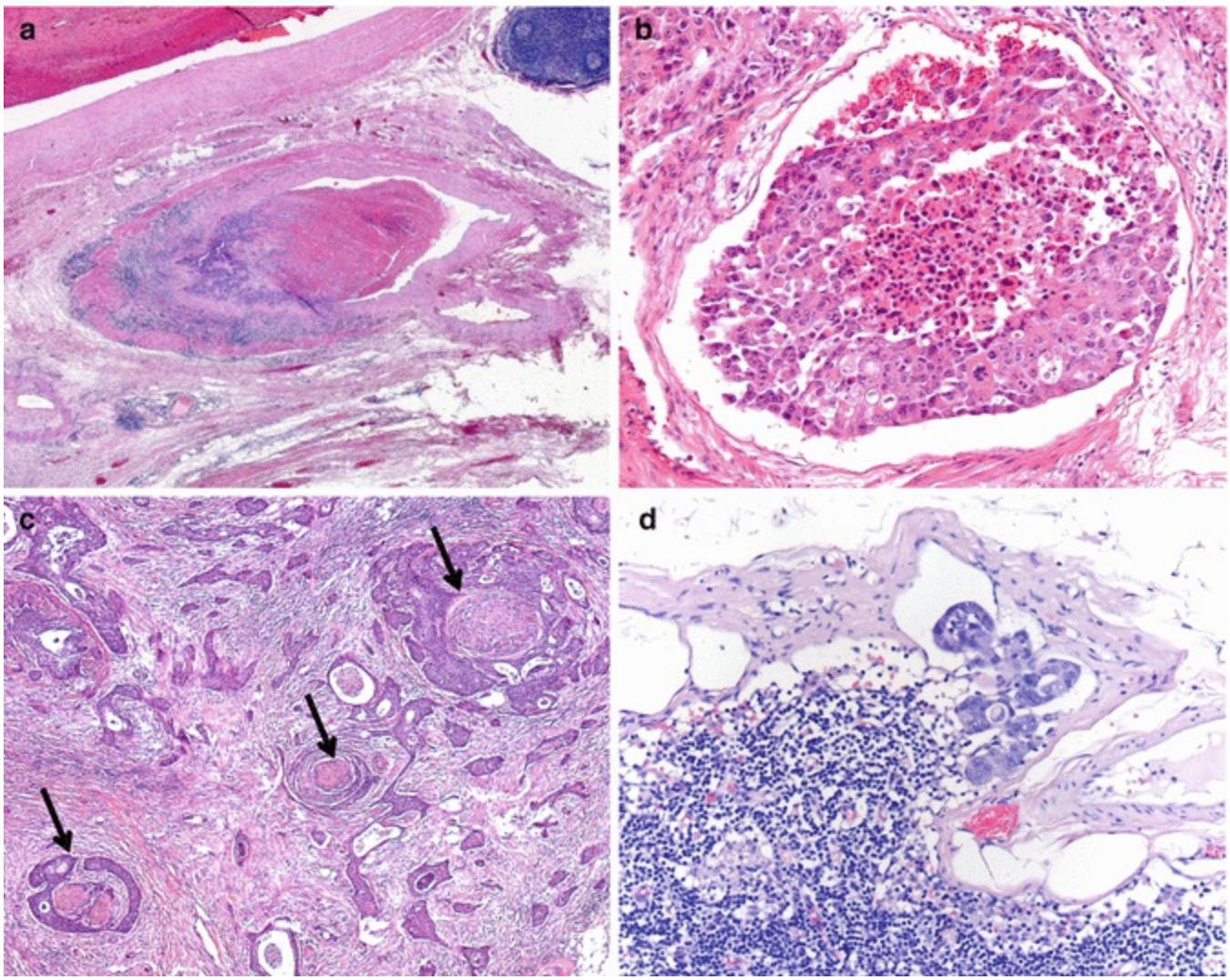


Fig. 7.17 Definition of parameters relevant to staging. (**a**) Vascular involvement with fibrinoid thrombus containing tumor cells adherent to the vessel wall (V1). (**b**) Vascular involvement comprising tumor cells within the lumen of a small caliber vessel surrounded by erythrocytes (V1). (**c**) Perineural involvement (*arrows*) by a non-keratinizing SCC (Pn1). (**d**) Isolated tumor cells within a pelvic lymph node (categorized as pN0 i + according to the TNM classification [106]). Note that some tumor cells are within the sinusoidal spaces of the lymph node, but others are in close contact to the lymphoid cells of the lymph node. This is a different feature from that illustrated in Fig. 7.16d

Perineural Involvement

Perineural involvement (PNI) has been defined as the detection of malignant cells in the perineural space of nerves, regardless of whether the nerve itself is infiltrated by the tumor and regardless of the extent of involvement of the perineural tissue (Fig. 7.17c; [23, 65]). The prognostic impact of PNI is not well studied [18]. Some authors reported no impact on recurrence and OS [25]. In a recent study, the 5-year survival was relatively decreased in cases with PNI, without reaching significance (92% versus 95%; $p = 0.346$ [14]). Others have reported a significantly decreased 5-year overall survival in cases with PNI (51.1% [95% CI 38.0–64.2] vs. 75.6% [95% CI 67.8–83.4];

$p = 0.001$), but the difference in 5-year disease-free survival was not significant [48]. The presence of PNI is associated with tumor size, depth of invasion, and LVSI [14, 25, 76]. The presence or absence of PNI and its localization (cervical stroma versus parametrial tissue) should be given within the pathology report.

Pattern of Invasion

Different patterns of invasion have been recently defined as prognostically significant for cervical adenocarcinomas [96]. Different patterns of invasion also exist in SCC (Fig. 7.3), these being closed or pushing, fingerlike, and spray-like patterns [53, 60]. In surgically treated SCC, a spray-like pattern of invasion was accompanied by a reduced 5-year overall survival when compared to the fingerlike and closed patterns (68.7% vs. 80.9% vs. 88.5%; $p = 0.0004$ [7, 39]). Examining cases of advanced SCC FIGO stage III and IV where only diagnostic biopsies were available to diagnose the cervical carcinoma, the spray-like pattern was associated with a reduced two-year overall survival when compared to the fingerlike pattern (14.0% vs. 29.1%, respectively; $p = 0.012$ [47]).

There are several morphological features associated with peritumoral stromal remodeling (Figs. 7.2 and 7.3) in SCC. Strong peritumoral stromal reaction, low peritumoral inflammatory response, and strong neovascularization may be associated with poor prognostic outcome in SCC, but currently the reported data are inconclusive [47, 49, 53].

Depth of Cervical Stromal Invasion

Deep cervical stromal invasion represents a prognostic factor and is relevant for adjuvant treatment selection. Unfortunately, there are no well-accepted cut-off points for the definition of deep cervical stromal invasion. Different studies have used different cut-off points, ranging from >25% to >75% [41, 55, 61, 124]. Regardless of the lack of generally accepted cut-off values, the *relative* depth of invasion should be given in the pathology report. The size of the uterine cervix is very variable, and therefore the relative depth of invasion (as in assessing myometrial invasion in endometrial cancer) gives additional information to the absolute value of tumor depth/thickness.

Depth of invasion is measured from the level of the cervical mucosa up to the deepest point of invasion. The relative depth of invasion is calculated by the relationship between the deepest point of invasion and the full thickness of the cervical wall (see Fig. 7.18). The presence of lymphovascular space involvement outside the deepest point of tumor stromal infiltration does not alter the depth of invasion.

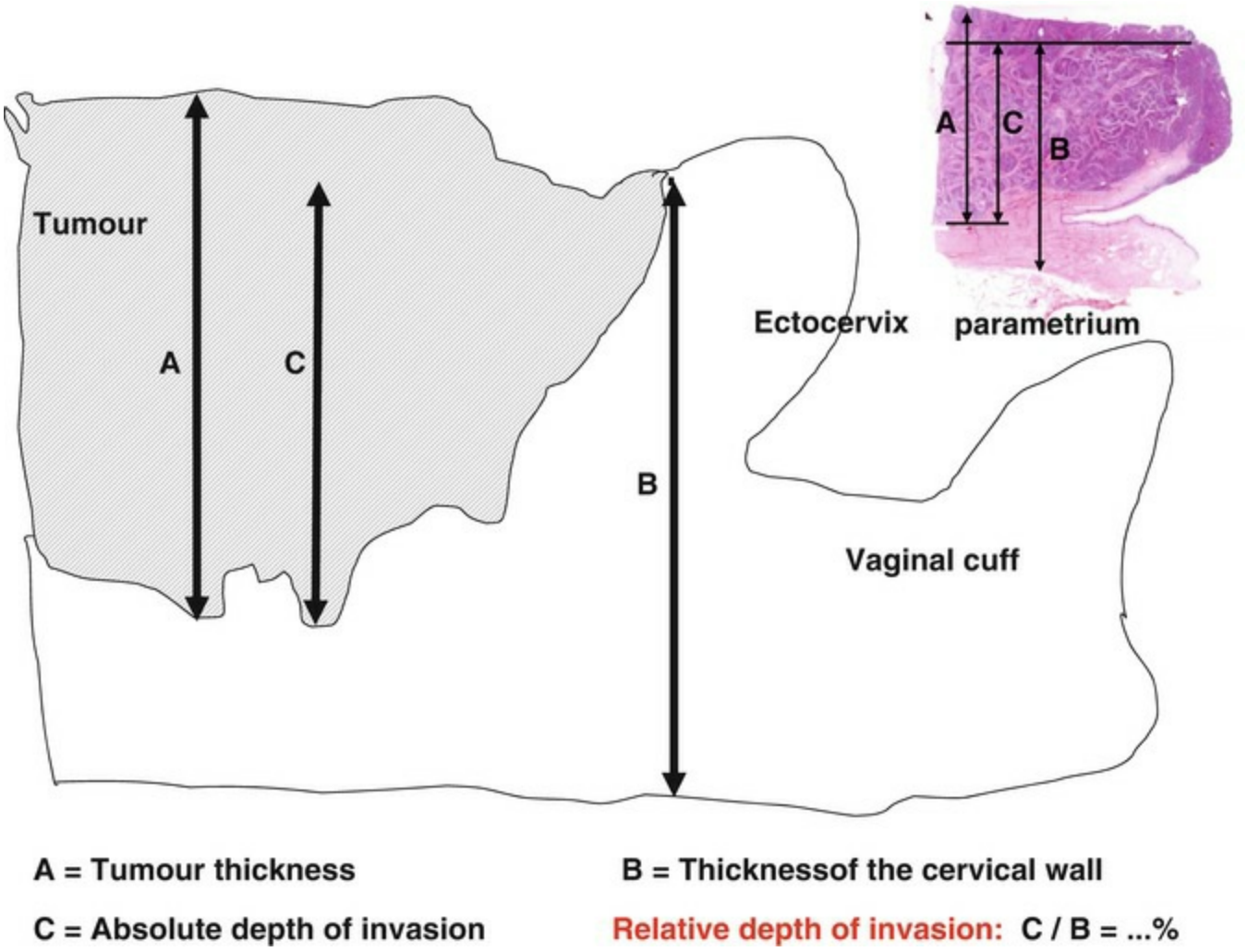


Fig. 7.18 Measurement of the relative depth of invasion of the tumor within the cervical wall (please see text)

Parametrial Involvement

Parametrial involvement is an important issue in cervical cancer for staging and determining adjuvant treatment approaches. Microscopically, there is no clear transition between the endocervical stroma and the parametrial/mesometrial tissue; however, if tumor is seen outside the endocervical stroma with infiltrative growth within the fibrous paracervical tissue, parametrial/mesometrial involvement should be diagnosed (Fig. 7.19). There is no doubt that the presence of tumor between large vessels and fatty tissue represents stage pT2b. Metastatic involvement of parametrial/mesometrial lymph nodes represents pelvic lymph node involvement and should be staged as pN1 and not as stage pT2b.

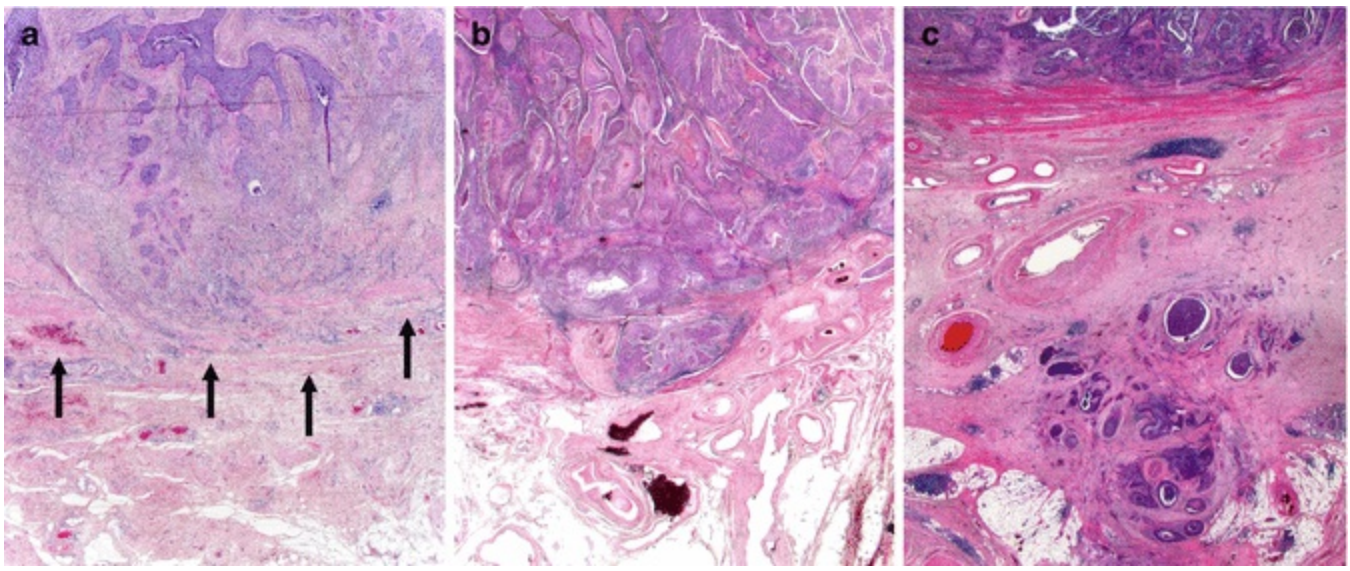


Fig. 7.19 Features of parametrial/mesometrial involvement, (**a**) complete infiltration of the cervical wall, but no tumor growth is seen beyond the cervical stroma, (**b**) very early parametrial/mesometrial infiltration with involvement of the fibrous paracervical tissue (stage pT2b), (**c**) parametrial/mesometrial infiltration with involvement of the fibroadipose tissue (stage pT2b); tumor cells are surrounded by a heavy peritumoral desmoplastic response

Especially in larger tumors and/or those showing deep cervical stromal invasion, careful embedding of the transition zone between the cervical stroma and the adjacent mesometrial/parametrial tissue is mandatory using perpendicular sections (see Fig. 7.19). Ideally all parametrial tissue should be histologically examined in every case; however this may not be permissible due to resource limitations. In small tumors where there is macroscopically no evidence for mesometrial/parametrial invasion, one block from the right and left side may be appropriate for embedding. In larger tumors or gross suspicion of mesometrial/parametrial involvement, embedding of two blocks from each side may be recommended to demonstrate microscopic parametrial/mesometrial involvement.

Uterine Corpus and Adnexal Involvement

Although involvement of the lower uterine segment (LUS) and the uterine corpus has no impact on staging in current systems, this feature should be noted within the pathology report due to an increased risk of (para-aortic) lymph node spread [79] and higher frequency of ovarian metastases [52]. In each case of macroscopically suspected corpus infiltration (especially in larger tumors), one or two blocks from the cranial end of the tumor including the corpus uteri should be embedded.

There is one major unclear topic within the staging of uterine cervical cancer [35]. The involvement of the fallopian tube, ovary, or adjacent mesoadnexal tissue ("adnexal involvement") is not recognized within the TNM/FIGO system [106]. There are only limited studies dealing with the prognostic impact of this finding [51, 52, 101, 104].

Overall, the impact of "adnexal involvement" is unclear at present. In the authors' opinion, adnexal involvement by SCC represents a feature of local advanced disease and poor prognostic outcome [52], deserving categorization as distant metastatic disease, or pM1, and should be emphasized in the pathology report. It is emphasized that these comments apply to SCC; the situation is different for adenocarcinoma, where indolent metastases are reported to occur in a subset of cases without adverse impact on outcomes [97].

Lymph Node Metastasis

Cervical cancer mortality results largely from its local spread, in particular ureteric involvement and renal failure. Nodal involvement is not a part of FIGO staging for this reason. Nodal involvement increases with clinical stage, and its detection determines the need and extent of adjuvant treatment. Pelvic and para-aortic node dissection may be carried out prior to local resection, and detection of nodal metastasis may preclude surgical resection in favor of chemoradiation treatment. The role of sentinel node procedures is currently under evaluation.

The detection and reporting of isolated tumor cells (ITC) may be an issue in cervical cancer patients treated by the use of the sentinel node technique. To the best of our knowledge, there are no studies regarding prognostic impact of isolated tumor cells within pelvic lymph nodes. Two reports deal with "low-volume lymph node involvement" in patients with cervical carcinoma [15, 107]; cases with ITC and micrometastases were merged within these studies. According to the UICC and AJCC definitions, ITCs are single tumor cells or small clusters of cells not more than 0.2 mm in greatest extent that can be detected by routine H&E staining (Fig. 7.17d; or immunohistochemically [122]). ITCs do not show evidence of metastatic activity (e.g., proliferation or stromal reaction) or penetration of lymphatic sinus walls [122]. The presence of ITCs within lymph nodes should not be categorized as pN1 but as pN0(i+) if the ITCs are morphologically identified (H&E or by immunohistochemistry) and as pN0(mol+) if they are diagnosed by non-morphological techniques (e.g., DNA cytometry or molecular techniques [122]).

Micrometastases within lymph nodes (Fig. 7.20a) are defined as tumor cell deposits ≥0.2 mm but <0.2 cm [122]. The TNM/UICC suggests the use of pN1(mi) within the tumor classification [106, 122]. For easier communication, the authors prefer the use of pN1mic for the designation of micrometastases within lymph nodes according to the earlier TNM classification in breast cancer. The vast majority of studies have reported a prognostic impact of micrometastases [28, 45, 64, 70], while a few did not show significance [107]. Within one study, patients with pN1mic showed a poor prognostic outcome compared to node-negative patients but improved outcome when compared to those with macrometastatic disease [45].

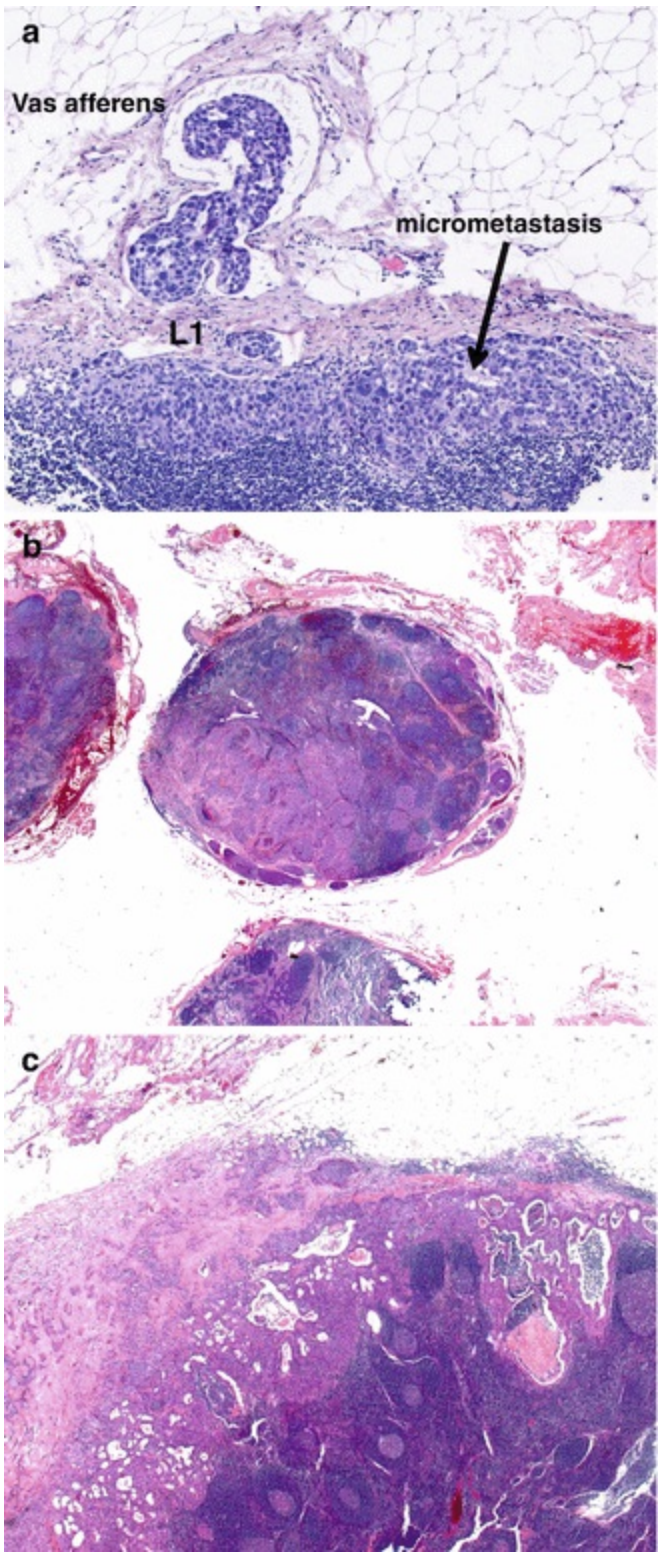


Fig. 7.20 Patterns of lymph node involvement in SCC of the uterine cervix. (**a**) Formal pathogenetic pathway of pelvic lymph node involvement: tumor cells within vasa efferentia reach the lymph node → infiltration of lymphatic vessels within the lymph node capsule → infiltration of the marginal sinusoids of the lymph node. (**b**) Lymph node metastasis within a pelvic lymph node (macrometastasis) without extracapsular extension (ECS 0). (**c**) Lymph node metastasis within a pelvic lymph node (macrometastasis) with extracapsular extension (ECS 1)

There is no information regarding the prognostic impact of lymphovascular involvement within the fatty tissue surrounding the lymph nodes or within the lymph node capsule (Fig. 7.16e). Both features are categorized within the L-category of the TNM system but not as lymph node involvement (N-category).

Extracapsular extension (Fig. 7.20b, c) of the metastatic deposits into the perinodal fatty tissue (i.e., extracapsular spread; ECS) is an important issue for staging and prognostication in vulvar cancer. For carcinoma of the uterine cervix, it has been reported that ECS may also be of prognostic impact [46, 77].

(Chemo)Radiation-Induced Changes

Neoadjuvant chemotherapy (NACT) followed by surgical treatment is reported to show good outcomes in patients with advanced disease. In addition, some patients with previous (chemo) radiation undergo additional sampling to control treatment effects [71] or secondary hysterectomy to control the disease [4]. Radiation-induced changes in SCC (Fig. 7.21) include nuclear enlargement and hyperchromasia associated with abundant eosinophilic cytoplasm, evidence of cytoplasmic degeneration with small vacuoles, smudged nuclear chromatin, and low mitotic index. There is no universally accepted response score for SCC previously treated by NACT or chemoradiation, and only limited data are available for reporting these changes [10, 58, 125]. The following response score has been proposed and correlated with outcome, though independent validation has not been carried out [10]:

- Complete response: No residual viable tumor cells in the surgical specimen (primary tumor and lymph nodes)
- Near-complete or microscopic response: The presence of one or more foci of malignant viable cells measuring less than 1 millimeter
- Partial response: The presence of residual tumor deposits measuring larger than 1 mm
- No response: No evidence of treatment change

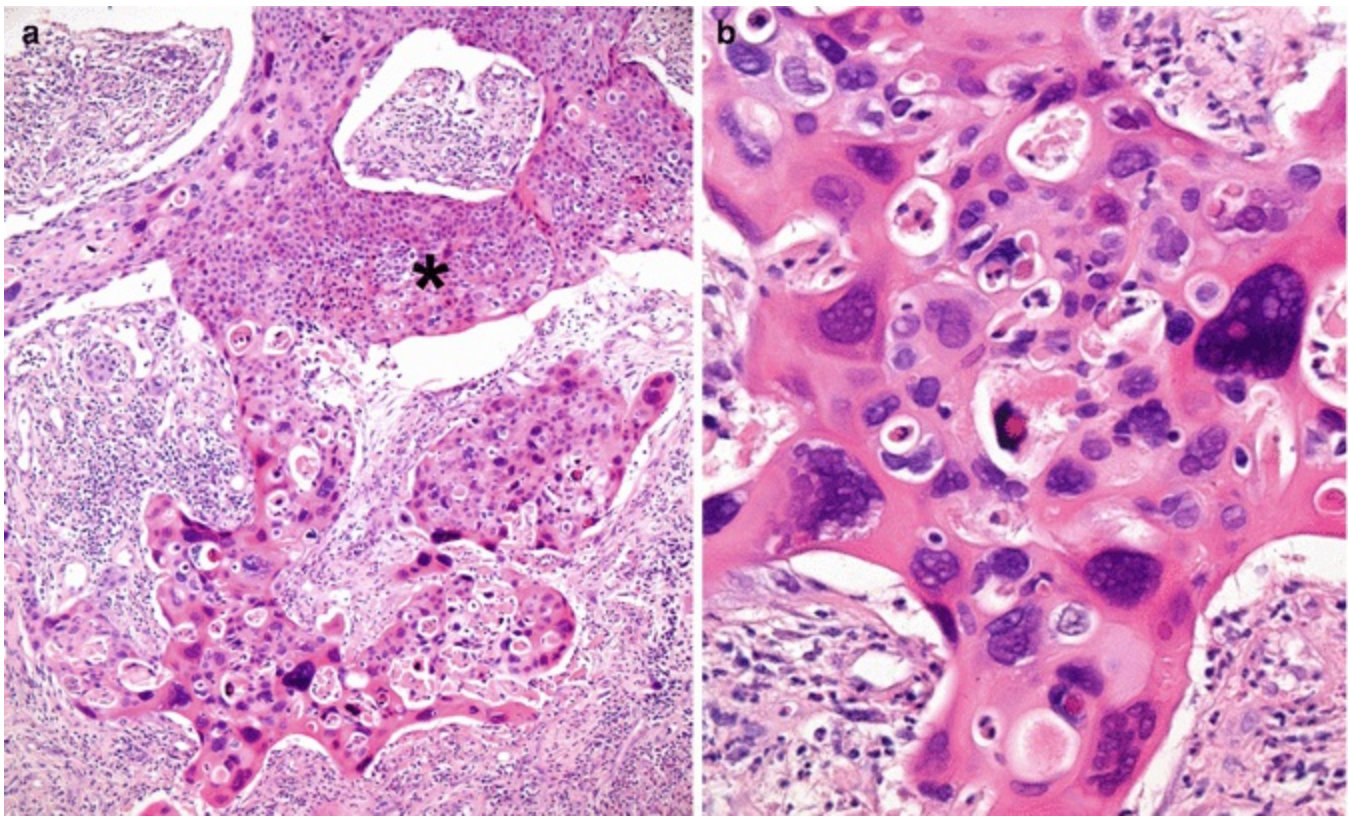


Fig. 7.21 Radiation-induced alterations, (**a**) more or less retained morphology of a moderately differentiated non-keratinizing squamous cell carcinoma (*asterisk*) and (**b**) marked nuclear abnormalities with apoptotic bodies, mononuclear giant cells, and cytoplasmic eosinophilia

Molecular Biomarkers

At present there are no molecular biomarkers or profiling data that influence treatment in cervical SCC [85, 123].

References

1. Albores-Saavedra J, Young RH. Transitional cell neoplasms (carcinomas and inverted papillomas) of the uterine cervix. A report of five cases. Am J Surg Pathol. 1995;19(10):1138–45. [PubMed]

2. Al-Nafussi AI, Al-Yusis R. Papillary squamotransitional cell carcinoma of the uterine cervix: an advance stage disease despite superficial location: report of two cases and review of the literature. Eur J Gynaecol Oncol. 1998;5:455–7.

3. Al-Nafussi AI, Monaghan H. Squamous carcinoma of the uterine cervix with CIN 3-like growth pattern: an under-diagnosed lesion. Int J Gynecol Cancer. 2000;10(2):95–9. [PubMed]

4. Boers A, Arts HJ, Klip H, Nijhuis ER, Pras E, Hollema H, Wisman GB, Nijman HW, Mourits MJ, Reyners AK, de Bock GH, Thomas G, van der Zee AG. Radical surgery in patients with residual disease after

(chemo)radiation for cervical cancer. Int J Gynecol Cancer. 2014;24(7):1276–85.
[PubMed]

5. Brainard JA, Hart WR. Adenoid basal epitheliomas of the uterine cervix: a reevaluation of distinctive cervical basaloid lesions currently classified as adenoid basal carcinoma and adenoid basal hyperplasia. Am J Surg Pathol. 1998;22(8):965–75.
[PubMed]

6. Brinck U, Jakob C, Bau O, Füzesi L. Papillary squamous cell carcinoma of the uterine cervix: report of three cases and a review of its classification. Int J Gynecol Pathol. 2000;19(3):231–5.
[PubMed]

7. Broders AC. Carcinoma grading and practical application. Arch Pathol. 1926;2:376–81.

8. Burghardt E, Holzer E. Diagnosis and treatment of microinvasive carcinoma of the cervix uteri. Obstet Gynecol. 1977;49:641–53.
[PubMed]

9. Cancer Research UK. 2016. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/cervical-cancer#heading-Zero. Accessed 28 February 2017.

10. Candelaria M, Chanona-Vilchis J, Cetina L, Flores-Estrada D, López-Graniel C, González-Enciso A, Cantú D, Poitevin A, Rivera L, Hinojosa J, de la Garza J, Dueñas-Gonzalez A. Prognostic significance of pathological response after neoadjuvant chemotherapy or chemoradiation for locally advanced cervical carcinoma. Int Semin Surg Oncol. 2006;3:3.
[PubMed][PubMedCentral]

11. Carrilho C, Alberto M, Buane L, David L. Keratins 8, 10, 13, and 17 are useful markers in the diagnosis of human cervix carcinomas. Hum Pathol. 2004;35(5):546–51.
[PubMed]

12. Casey S, Harley I, Jamison J, Molijn A, van den Munckhof H, McCluggage WG. A rare case of HPV-negative cervical squamous cell carcinoma. Int J Gynecol Pathol. 2015;34(2):208–12.
[PubMed]

13. Chao A, Tsai CN, Hsueh S, Lee LY, Chen TC, Huang SL, Chao FY, Lai CH. Does Epstein-Barr virus play a role in lymphoepithelioma-like carcinoma of the uterine cervix? Int J Gynecol Pathol. 2009;28(3):279–85.
[PubMed]

14. Cho HC, Kim H, Cho HY, Kim K, No JH, Kim YB. Prognostic significance of perineural invasion in cervical cancer. Int J Gynecol Pathol. 2013;32(2):228–33.
[PubMed]

15. Cibula D, Abu-Rustum NR, Dusek L, Zikán M, Zaal A, Sevcik L, Kenter GG, Querleu D, Jach R, Bats AS, Dyduch G, Graf P, Klat J, Lacheta J, Meijer CJ, Mery E, Verheijen R, Zweemer RP. Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. Gynecol Oncol. 2012;124(3):496–501.
[PubMed]

16. Creasman WT, Kohler MF. Is lymph vascular space involvement an independent prognostic factor in early cervical cancer? Gynecol Oncol. 2004;92(2):525–9.
[PubMed]

17. Crowther ME, Lowe DG, Shepherd JH. Verrucous carcinoma of the female genital tract: a review. Obstet

Gynecol Surv. 1988;43(5):263–80.
[PubMed]

18. Cui L, Shi Y, Zhang GN. Perineural invasion as a prognostic factor for cervical cancer: a systematic review and meta-analysis. Arch Gynecol Obstet. 2015;292(1):13–9.
[PubMed]

19. Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC, Members of the LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Int J Gynecol Pathol. 2013;32(1):76–115.
[PubMed]

20. Day E, Duffy S, Bryson G, Syed S, Shanbhag S, Burton K, Lindsay R, Siddiqui N, Millan D. Multifocal FIGO stage IA1 squamous carcinoma of the cervix: criteria for identification, staging, and its good clinical outcome. Int J Gynecol Pathol. 2016;35(5):467–74.
[PubMed]

21. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menéndez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T, Garcia-Barriola V, Clavel C, Ordi J, Andújar M, Castellsagué X, Sánchez GI, Nowakowski AM, Bornstein J, Muñoz N, Bosch FX, Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048–56.
[PubMed]

22. Drew PA, Hong B, Massoll NA, Ripley DL. Characterization of papillary squamotransitional cell carcinoma of the cervix. J Low Genit Tract Dis. 2005;9(3):149–53.
[PubMed]

23. Dunn M, Morgan MB, Beer TW. Perineural invasion: identification, significance, and a standardized definition. Dermatol Surg. 2009;35:214–21.
[PubMed]

24. Eggen T, Arnes M, Moe B, Straume B, Orbo A. Prognosis of early cervical cancer (FIGO Stages IA2, IB, and IIA) in northern Norway predicted by malignancy grading score and objective morphometric image analysis. Int J Gynecol Pathol. 2007;26(4):447–56.
[PubMed]

25. Elsahwi KS, Barber E, Illuzzi J, Buza N, Ratner E, Silasi DA, Santin AD, Azodi M, Schwartz PE, Rutherford TJ. The significance of perineural invasion in early-stage cervical cancer. Gynecol Oncol. 2011;123(3):561–4.
[PubMed]

26. Estape RE, Angioli R, Madrigal M, Janicek M, Gomez C, Penalver M, Averette H. Close vaginal margins as a prognostic factor after radical hysterectomy. Gynecol Oncol. 1998;68(3):229–32.
[PubMed]

27. Euscher E, Malpica A. Use of immunohistochemistry in the diagnosis of miscellaneous and metastatic tumors of the uterine corpus and cervix. Semin Diagn Pathol. 2014;31(3):233–57.

[PubMed]

28. Fregnani JH, Latorre MR, Novik PR, Lopes A, Soares FA. Assessment of pelvic lymph node micrometastatic disease in stages IB and IIA of carcinoma of the uterine cervix. Int J Gynecol Cancer. 2006;16:1188–94. [PubMed]

29. Ganesan R, Hirschowitz L, Dawson P, Askew S, Pearmain P, Jones PW, Singh K, Chan KK, Moss EL. Neuroendocrine carcinoma of the cervix: review of a series of cases and correlation with outcome. Int J Surg Pathol. 2016;24:490–6.

30. Grayson W, Cooper K. A reappraisal of "basaloid carcinoma" of the cervix, and the differential diagnosis of basaloid cervical neoplasms. Adv Anat Pathol. 2002;9(5):290–300. [PubMed]

31. Gualco M, Bonin S, Foglia G, Fulcheri E, Odicino F, Prefumo F, Stanta G, Ragni N. Morphologic and biologic studies on ten cases of verrucous carcinoma of the vulva supporting the theory of a discrete clinico-pathologic entity. Int J Gynecol Cancer. 2003;13(3):317–24. [PubMed]

32. Hellman K, Hellström AC, Pettersson BF. Uterine cervix cancer treatment at Radiumhemmet: 90 years' experience. Time trends of age, stage, and histopathology distribution. Cancer Med. 2014;3(2):284–92. [PubMed][PubMedCentral]

33. Herr D, König J, Heilmann V, Koretz K, Kreienberg R, Kurzeder C. Prognostic impact of satellite-lymphovascular space involvement in early-stage cervical cancer. Ann Surg Oncol. 2009;16(1):128–32. [PubMed]

34. Hirschowitz L, editor. Histopathology reporting in cervical screening – an integrated approach. 2nd ed. NHSCSP Publication number 10. Sheffiled: NHS Cancer Screening Programmes; 2012.

35. Hirschowitz L, Nucci M, Zaino RJ. Problematic issues in the staging of endometrial, cervical and vulval carcinomas. Histopathology. 2013;62(1): 176–202. [PubMed]

36. Höckel M, Hentschel B, Horn LC. Association between developmental steps in the organogenesis of the uterine cervix and locoregional progression of cervical cancer: a prospective clinicopathological analysis. Lancet Oncol. 2014;15(4):445–56. [PubMed]

37. Höckel M, Horn LC, Manthey N, Braumann UD, Wolf U, Teichmann G, Frauenschläger K, Dornhöfer N, Einenkel J. Resection of the embryologically defined uterovaginal (Müllerian) compartment and pelvic control in patients with cervical cancer: a prospective analysis. Lancet Oncol. 2009;10(7):683–92. [PubMed]

38. Höckel M, Horn LC, Tetsch E, Einenkel J. Pattern analysis of regional spread and therapeutic lymph node dissection in cervical cancer based on ontogenetic anatomy. Gynecol Oncol. 2012;125(1):168–74. [PubMed]

39. Horn LC, Fischer U, Raptis G, Bilek K, Hentschel B, Richter CE, Braumann UD, Einenkel J. Pattern of invasion is of prognostic value in surgically treated cervical cancer patients. Gynecol Oncol. 2006;103(3):906–11. [PubMed]

40. Horn LC, Hentschel B, Bilek K, Richter CE, Einenkel J, Leo C. Mixed small cell carcinomas of the uterine

cervix: prognostic impact of focal neuroendocrine differentiation but not of Ki-67 labeling index. Ann Diagn Pathol. 2006;10(3):140–3.
[PubMed]

41. Horn LC, Beckmann MW, Follmann M, Koch MC, Mallmann P, Marnitz S, Schmidt D. S3 guidelines on diagnostics and treatment of cervical cancer: demands on pathology. Pathologe. 2015;36(6):585–93.
[PubMed]

42. Horn LC, Bilek K, Fischer U, Einenkel J, Hentschel B. A cut-off value of 2 cm in tumor size is of prognostic value in surgically treated FIGO stage IB cervical cancer. Gynecol Oncol. 2014;134(1):42–6.
[PubMed]

43. Horn LC, Bilek K, Fischer U, Hentschel B. Prognostic impact of conventional tumor grade in surgically treated FIGI stage IB to IIB squamous cell cancer. Int J Gynecol Cancer. 2016;25(Suppl 2):823–4.

44. Horn LC, Fischer U, Raptis G, Bilek K, Hentschel B. Tumor size is of prognostic value in surgically treated FIGO stage II cervical cancer. Gynecol Oncol. 2007;107(2):310–5.
[PubMed]

45. Horn LC, Hentschel B, Fischer U, Peter D, Bilek K. Detection of micrometastases in pelvic lymph nodes in patients with carcinoma of the cervix uteri using step sectioning: frequency, topographic distribution and prognostic impact. Gynecol Oncol. 2008;111(2):276–81.
[PubMed]

46. Horn LC, Hentschel B, Galle D, Bilek K. Extracapsular extension of pelvic lymph node metastases is of prognostic value in carcinoma of the cervix uteri. Gynecol Oncol. 2008;108(1):63–7.
[PubMed]

47. Horn LC, Hommel N, Roschlau U, Bilek K, Hentschel B, Einenkel J. Peritumoral stromal remodeling, pattern of invasion and expression of c-met/HGF in advanced squamous cell carcinoma of the cervix uteri, FIGO stages III and IV. Eur J Obstet Gynecol Reprod Biol. 2012;163(1):76–80.
[PubMed]

48. Horn LC, Meinel A, Fischer U, Bilek K, Hentschel B. Perineural invasion in carcinoma of the cervix uteri – Prognostic impact. J Cancer Res Clin Oncol. 2010;136(10):1557–62.
[PubMed]

49. Horn LC, Schreiter C, Canzler A, Leonhardt K, Einenkel J, Hentschel B. CD34(low) and SMA(high) represent stromal signature in uterine cervical cancer and are markers for peritumoral stromal remodeling. Ann Diagn Pathol. 2013;17(6):531–5.
[PubMed]

50. International Collaboration on Cancer Reporting (ICCR). 2016. www.iccr-cancer.org.

51. Jaiman S, Surampudi K, Gundabattula SR, Garg D. Bilateral ovarian metastatic squamous cell carcinoma arising from the uterine cervix and eluding the Mullerian mucosa. Diagn Pathol. 2014;9:109.
[PubMed][PubMedCentral]

52. Kato T, Watari H, Takeda M, Hosaka M, Mitamura T, Kobayashi N, Sudo S, Kaneuchi M, Kudo M, Sakuragi N. Multivariate prognostic analysis of adenocarcinoma of the uterine cervix treated with radical hysterectomy and systematic lymphadenectomy. J Gynecol Oncol. 2013;24(3):222–8.
[PubMed][PubMedCentral]

53.

Khunamornpong S, Settakorn J, Sukpan K, Suprasert P, Lekawanvijit S, Siriaunkgul S. Prognostic value of pathological characteristics of invasive margins in early-stage squamous cell carcinomas of the uterine cervix. Asian Pac J Cancer Prev. 2013;14(9):5165–9.
[PubMed]

54. Kim M, Ishioka S, Endo T, Baba T, Mizuuchi M, Takada S, Saito T. Possibility of less radical treatment for patients with early invasive uterine cervical cancer. J Obstet Gynaecol Res. 2016;42:876–82.

55. Kodama J, Fukushima C, Kusumoto T, Nakamura K, Seki N, Hongo A, Hiramatsu Y. Stage IB1 cervical cancer patients with an MRI-measured tumor size < or =2 cm might be candidates for less-radical surgery. Eur J Gynaecol Oncol. 2013;34(1):39–41.
[PubMed]

56. Koenig C, Turnicky RP, Kankam CF, Tavassoli FA. Papillary squamotransitional cell carcinoma of the cervix: a report of 32 cases. Am J Surg Pathol. 1997;21(8):915–21.
[PubMed]

57. Kokka F, Verma M, Singh N, Faruqi A, Yoon J, Reynolds K. Papillary squamotransitional cell carcinoma of the uterine cervix: report of three cases and review of the literature. Pathology. 2006;38(6):584–6.
[PubMed]

58. Kornovski Y, Gorchev G. Histopathological findings in postoperative specimens in cervical cancer patients with stages IB2-IVA after neoadjuvant chemotherapy and preoperative plus postoperative radiotherapy. J BUON. 2007;12(1):57–63.
[PubMed]

59. Kosari F, Daneshbod Y, Parwaresch R, Krams M, Wacker HH. Lymphomas of the female genital tract: a study of 186 cases and review of the literature. Am J Surg Pathol. 2005;29(11):1512–20.
[PubMed]

60. Kristensen GB, Abeler VM, Risberg B, Trop C, Bryne M. Tumor size, depth of invasion, and grading of the invasive tumor front are the main prognostic factors in early squamous cell cervical carcinoma. Gynecol Oncol. 1999;74(2):245–51.
[PubMed]

61. Landoni F, Maneo A, Colombo A, Placa F, Milani R, Perego P, Favini G, Ferri L, Mangioni C. Randomised study of radical surgery versus radiotherapy for stage IB-IIA cervical cancer. Lancet. 1997;350:535–40.
[PubMed]

62. Lastra RR, Park KJ, Schoolmeester JK. Invasive Stratified Mucin-producing Carcinoma and Stratified Mucin-producing Intraepithelial Lesion (SMILE): 15 cases presenting a spectrum of cervical neoplasia with description of a distinctive variant of invasive adenocarcinoma. Am J Surg Pathol. 2016;40(2):262–9.
[PubMed]

63. Laury AR, Perets R, Piao H, Krane JF, Barletta JA, French C, Chirieac LR, Lis R, Loda M, Hornick JL, Drapkin R, Hirsch MS. A comprehensive analysis of PAX8 expression in human epithelial tumors. Am J Surg Pathol. 2011;35(6):816–26.
[PubMed]

64. Lentz SE, Muderspach LI, Felix JC, Ye W, Groshen S, Amezuca CA. Identification of mircometastases in histologically negative nodes of early-stage cervical cancer patients. Obstet Gynecol. 2004;103:1204–10.
[PubMed]

65. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a Review of the Literature. Cancer. 2009;115:3379–91.
[PubMed]

66. Lim CS, Alexander-Sefre F, Allam M, Singh N, Aleong JC, Al-Rawi H, Jacobs IJ. Clinical value of immunohistochemically detected lymphovascular space invasion in early stage cervical carcinoma. Ann Surg Oncol. 2008;15(9):2581–8.
[PubMed]

67. Lynge E, Rygaard C, Baillet MV, Dugué PA, Sander BB, Bonde J, Rebolj M. Cervical cancer screening at crossroads. APMIS. 2014;122(8):667–73.
[PubMed]

68. Majhi U, Murhekar K, Sundersingh S, Srinivasan V. Basaloid squamous cell carcinoma of cervix showing neuroendocrine differentiation. J Cancer Res Ther. 2015;11(2):492–3.
[PubMed]

69. Mao TL, Seidman JD, Kurman RJ, Shih IM. Cyclin E and p16 immunoreactivity in epithelioid trophoblastic tumor – An aid in differential diagnosis. Am J Surg Pathol. 2006;30(9):1105–10.
[PubMed]

70. Marchiole P, Buenerd A, Benchaib M, Nezhat K, Dargent D, Mathevet P. Clinical significance of lympho vascular space involvement and lymph node micrometastases in early-stage cervical cancer: a retrospective case-control surgico-pathological study. Gynecol Oncol. 2005;97:727–32.
[PubMed]

71. Marnitz S, Abt EC, Martus P, Tsunoda A, Köhler C. Is routine curettage a useful tool to evaluate persistent tumor in patients who underwent primary chemoradiation for locally advanced and/or lymph node positive cervical cancer? Int J Gynecol Cancer. May 2015. [Epub ahead of print].

72. Martorell MA, Julian JM, Calabuig C, García-García JA, Pérez-Vallés A. Lymphoepithelioma-like carcinoma of the uterine cervix. Arch Pathol Lab Med. 2002;126(12):1501–5.
[PubMed]

73. McCann GA, Taege SK, Boutsicaris CE, Phillips GS, Eisenhauer EL, Fowler JM, O'Malley DM, Copeland LJ, Cohn DE, Salani R. The impact of close surgical margins after radical hysterectomy for early-stage cervical cancer. Gynecol Oncol. 2013;128(1):44–8.
[PubMed]

74. McCluggage WG, Kennedy K, Busam KJ. An immunohistochemical study of cervical neuroendocrine carcinomas: neoplasms that are commonly TTF1 positive and which may express CK20 and P63. Am J Surg Pathol. 2010;34(4):525–32.
[PubMed]

75. McIlwaine P, Nagar H, McCluggage WG. Multifocal FIGO stage 1A1 cervical squamous carcinomas have an extremely good prognosis equivalent to unifocal lesions. Int J Gynecol Pathol. 2014;33(3):213–7.
[PubMed]

76. Meinel A, Fischer U, Bilek K, Hentschel B, Horn LC. Morphological parameters associated with perineural invasion (PNI) in carcinoma of the cervix uteri. Int J Surg Pathol. 2011;19(2):159–63.
[PubMed]

77. Metindir J, Bilir Dilek G. Evaluation of prognostic significance in extracapsular spread of pelvic lymph node

metastasis in patients with cervical cancer. Eur J Gynaecol Oncol. 2008;29(5):476–8.
[PubMed]

78. Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, Langfort R, Waloszczyk P, Biernat W, Lasota J, Wang Z. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol. 2014;38(1):13–22.
[PubMed][PubMedCentral]

79. Mileshkin L, Paramanathan A, Kondalsamy-Chennakesavan S, Bernshaw D, Khaw P, Narayan K. Smokers with cervix cancer have more uterine corpus invasive disease and an increased risk of recurrence after treatment with chemoradiation. Int J Gynecol Cancer. 2014;24(7):1286–91.
[PubMed]

80. Mills SE, Austin MB, Randall ME. Lymphoepithelioma-like carcinoma of the uterine cervix. A distinctive, undifferentiated carcinoma with inflammatory stroma. Am J Surg Pathol. 1985;9(12):883–9.
[PubMed]

81. Morice P, Piovesan P, Rey A, Atallah D, Haie-Meder C, Pautier P, Sideris L, Pomel C, Duvillard P, Castaigne D. Prognostic value of lymphovascular space invasion determined with hematoxylin-eosin staining in early stage cervical carcinoma: results of a multivariate analysis. Ann Oncol. 2003;14(10):1511–7.
[PubMed]

82. Nakamura E, Shimizu M, Fujiwara K, Yamauchi H, Monobe Y, Hirokawa M, Kohno I, Manabe T. Papillary squamous cell carcinoma of the uterine cervix: diagnostic pitfalls. APMIS. 1998;106(10):975–8.
[PubMed]

83. Ng WK, Cheung LK, Li AS, Cheung FM, Chow JC. Transitional cell metaplasia of the uterine cervix is related to human papillomavirus: molecular analysis in seven patients with cytohistologic correlation. Cancer. 2002;96(4):250–8.
[PubMed]

84. Ng WK, Cheung LK, Li AS. Warty (condylomatous) carcinoma of the cervix. A review of 3 cases with emphasis on thin-layer cytology and molecular analysis for HPV. Acta Cytol. 2003;47(2):159–66.
[PubMed]

85. Noordhuis MG, Eijsink JJ, Roossink F, de Graeff P, Pras E, Schuuring E, Wisman GB, de Bock GH, van der Zee AG. Prognostic cell biological markers in cervical cancer patients primarily treated with (chemo)radiation: a systematic review. Int J Radiat Oncol Biol Phys. 2011;79(2):325–34.
[PubMed]

86. O'Neill CJ, McCluggage WG. p16 expression in the female genital tract and its value in diagnosis. Adv Anat Pathol. 2006;13(1):8–15.
[PubMed]

87. Onishi J, Sato Y, Sawaguchi A, Yamashita A, Maekawa K, Sameshima H, Asada Y. Stratified mucin-producing intraepithelial lesion with invasive carcinoma: 12 cases with immunohistochemical and ultrastructural findings. Hum Pathol. 2016;55:174–81.
[PubMed]

88. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103–4.
[PubMed]

89. Pérez-Montiel D, Serrano-Olvera A, Salazar LC, Cetina-Pérez L, Candelaria M, Coronel J, Montalvo LA, de León DC. Adenocarcinoma metastatic to the uterine cervix: a case series. J Obstet Gynaecol Res. 2012;38(3):541–9.
[PubMed]

90. Poulsen HE, Taylor CW, Sobin LH. Histological typing of female genital tract tumors. International Classification of Tumours. No 13. Geneva: WHO; 1975.

91. Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, Heintz AP, Ngan HY, Pecorelli S. Carcinoma of the cervix uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet. 2006;95(Suppl 1):S43–103.

92. Ramirez PT, Pareja R, Rendón GJ, Millan C, Frumovitz M, Schmeler KM. Management of low-risk early-stage cervical cancer: should conization, simple trachelectomy, or simple hysterectomy replace radical surgery as the new standard of care? Gynecol Oncol. 2014;132(1):254–9.
[PubMed]

93. Reagan JW, Hamonic MJ, Wentz WB. Analytical study of the cells in cervical squamous-cell cancer. Lab Invest. 1957;6(3):241–50.
[PubMed]

94. Reich O, Pickel H. Multifocal stromal invasion in microinvasive squamous cell carcinoma of the cervix: how to measure and stage these lesions. Int J Gynecol Pathol. 2002;21(4):416–7.
[PubMed]

95. Rodríguez-Carunchio L, Soveral I, Steenbergen RD, Torné A, Martinez S, Fusté P, Pahisa J, Marimon L, Ordi J, del Pino M. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG. 2015;122(1):119–27.
[PubMed]

96. Roma AA. Patterns of invasion of cervical adenocarcinoma as predicators of outcome. Adv Anat Pathol. 2015;22(6):345–54.
[PubMed]

97. Ronnett BM, Yemelyanova AV, Vang R, Gilks CB, Miller D, Gravitt PE, Kurman RJ. Endocervical adenocarcinomas with ovarian metastases: analysis of 29 cases with emphasis on minimally invasive cervical tumors and the ability of the metastases to simulate primary ovarian neoplasms. Am J Surg Pathol. 2008;32(12):1835–53.
[PubMed]

98. Saylam K, Anaf V, Fayt I, Noel JC. Lymphoepithelioma-like carcinoma of the cervix with prominent eosinophilic infiltrate: an HPV-18 associated case. Acta Obstet Gynecol Scand. 2002;81(6):564–6.
[PubMed]

99. Schmeler KM, Frumovitz M, Ramirez PT. Conservative management of early stage cervical cancer: is there a role for less radical surgery? Gynecol Oncol. 2011;120(3):321–5.
[PubMed][PubMedCentral]

100. Schneider A, Erdemoglu E, Chiantera V, Reed N, Morice P, Rodolakis A, Denschlag D, Kesic V. Clinical recommendation radical trachelectomy for fertility preservation in patients with early-stage cervical cancer. Int J Gynecol Cancer. 2012;22(4):659–66.
[PubMed]

101.

Shimada M, Kigawa J, Nishimura R, Yamaguchi S, Kuzuya K, Nakanishi T, Suzuki M, Kita T, Iwasaka T, Terakawa N. Ovarian metastasis in carcinoma of the uterine cervix. Gynecol Oncol. 2006;101(2):234–7. [PubMed]

102. Shin E, Jung WH, Koo JS. Expression of p16 and pRB in invasive breast cancer. Int J Clin Exp Pathol. 2015;8(7):8209–17. [PubMed][PubMedCentral]

103. Silverberg SG, Ioffe OB. Pathology of cervical cancer. Cancer J. 2003;9(5):335–47. [PubMed]

104. Singh N, Arif S. Histopathologic parameters of prognosis in cervical cancer – A review. Int J Gynecol Cancer. 2004;14(5):741–50. [PubMed]

105. Smedts F, Ramaekers F, Troyanovsky S, Pruszczynski M, Link M, Lane B, Leigh I, Schijf C, Vooijs P. Keratin expression in cervical cancer. Am J Pathol. 1992;141(2):497–511. [PubMed][PubMedCentral]

106. Sobin LH, Gospodarowicz MK, Wittekind C. TNM-classification of malignant tumors. 7th ed. London: Wiley-Blackwell; 2009.

107. Stany MP, Stone PJ, Felix JC, Amezcua CA, Groshen S, Ye W, Kyser KL, Howard RS, Zahn CM, Muderspach LI, Lentz SE, Chernofsky MR. Lymph node micrometastases in early-stage cervical cancer are not predictive of survival. Int J Gynecol Pathol. 2015;34(4):379–84. [PubMed]

108. Stoler M, Bergeron C, Colgan TJ, Ferency AS, Herrington CS, Kim KR, Loening T, Schneider A, Sherman ME, Wilbur DC, Wright T. Squamous cell tumors of the uterine cervix and its precursors. In: Kurman RJ, Carcangiou ML, Herrington S, Young RH, editors. WHO classification of tumours of female reproductive organs. Lyon: IARC Press; 2014. p. 172–82.

109. Sykes P, Allen D, Cohen C, Scurry J, Yeo D. Does the density of lymphatic vascular space invasion affect the prognosis of stage Ib and IIA node negative carcinoma of the cervix? Int J Gynecol Cancer. 2003;13(3):313–6. [PubMed]

110. Takai N, Nakamura S, Goto K, Hayashita C, Kira N, Urabe S, Narahara H, Matsumoto H. Lymphoepithelioma-like carcinoma of the uterine cervix. Arch Gynecol Obstet. 2009;280(5):725–7. [PubMed]

111. Teke F, Yöney A, Teke M, Adanaş G, Urakçı Z, Türkcü G, Eren B, İnal A, Ünsal M. Evaluation of outcome and prognostic factors in 739 patients with uterine cervix carcinoma: a single institution experience. Contemp Oncol (Pozn). 2015;19(2):130–6.

112. Thike AA, Cheok PY, Jara-Lazaro AR, Tan B, Tan P, Tan PH. Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol. 2010;23(1):123–33. [PubMed]

113. Tiltman AJ. The pathology of cervical tumours. Best Pract Res Clin Obstet Gynaecol. 2005;19(4):485–500. [PubMed]

114. Trattner M, Graf AH, Lax S, et al. Prognostic factors in surgically treated stage Ib–IIb cervical carcinomas with special emphasis on the importance of tumour volume. Gynecol Oncol. 2001;82:11–6.

[PubMed]

115. Urabe A, Matsumoto T, Kimura M, Sonoue H, Kinoshita K. Grading system of lymphatic invasion according to D2-40 immunostaining is useful for the prediction of nodal metastasis in squamous cell carcinoma of the uterine cervix. Histopathology. 2006;49(5):493–7.
[PubMed]

116. Viswanathan AN, Lee H, Hanson E, Berkowitz RS, Crum CP. Influence of margin status and radiation on recurrence after radical hysterectomy in Stage IB cervical cancer. Int J Radiat Oncol Biol Phys. 2006;65(5):1501–7.
[PubMed]

117. Wagner AE, Pappas L, Ghia AJ, Gaffney DK. Impact of tumor size on survival in cancer of the cervix and validation of stage IIA1 and IIA2 subdivisions. Gynecol Oncol. 2013;129:517–21.
[PubMed]

118. Wang TY, Chen BF, Yang YC, Chen H, Wang Y, Cviko A, Quade BJ, Sun D, Yang A, McKeon FD, Crum CP. Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: a study of 250 cases. Hum Pathol. 2001;32(5):479–86.
[PubMed]

119. Weir MM, Bell DA, Young RH. Transitional cell metaplasia of the uterine cervix and vagina: an underrecognized lesion that may be confused with high-grade dysplasia. A report of 59 cases. Am J Surg Pathol. 1997;21(5):510–7.
[PubMed]

120. Wentz WB, Reagan JW. Survival in cervical cancer with respect to cell type. Cancer. 1959;12:384–8.
[PubMed]

121. Wilkens J, Beattie G, Al-Nafussi A. Metastatic CIN3-like squamous carcinoma. Diagn Histopathol. 2011;17(3):140–3.

122. Wittekind C, Compton CC, Brierley J, Sobin LH. TNM-supplement. A commentary on uniform use. Oxford: Wiley-Blackwell; 2012. p. 9–11; 21–22.

123. Zagouri F, Sergentanis TN, Chrysikos D, Filipits M, Bartsch R. Molecularly targeted therapies in cervical cancer. A systematic review. Gynecol Oncol. 2012;126(2):291–303.
[PubMed]

124. Zaino RJ, Ward S, Delgado G, Bungy B, Gore H, Fetter G, Ganjei P, Frauenhoffer E. Histopathological predictors of the behavior of surgically treated stage Ib squamous cell carcinoma of the cervix. Cancer. 1992;69:1750–8.
[PubMed]

125. Zannoni GF, Vellone VG, Carbone A. Morphological effects of radiochemotherapy on cervical carcinoma: a morphological study of 50 cases of hysterectomy specimens after neoadjuvant treatment. Int J Gynecol Pathol. 2008;27(2):274–81.
[PubMed]

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https://doi.org/10.1007/978-3-319-51257-0_8

8. Endocervical Adenocarcinoma In Situ/Cervical Glandular Intraepithelial Neoplasia and Adenocarcinoma of the Usual Type

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Abstract
Glandular carcinomas of the cervix and their precursor lesions comprise a minority of all cervical cancers; however, their relative prevalence and possibly absolute prevalence are increasing. Better sampling methods and recognition of the cytological features of early neoplasia make early detection feasible. Of all the variant types of glandular cervical neoplasia, the usual type of adenocarcinoma and its in situ precursor comprise the vast majority of cases. This chapter details the demographics and pathobiology of cancers and precursor lesions of the endocervix, the histopathological and cytopathological features, and presents a discussion of the morphological mimics of the usual type of endocervical adenocarcinoma, both in situ and invasive, including the differential diagnosis of atypical glandular cells in cytological preparations.

Keywords Cervix – Adenocarcinoma – Usual type – Adenocarcinoma in situ – Atypical glandular cells – Pap test – Cervical cytology

Epidemiology

Adenocarcinoma comprises approximately 20–25% of all primary cancers of the cervix in Western countries. This represents an increased percentage of nearly fourfold over the past 50 years [1, 2]. This increased proportion is due primarily to the dramatic decrease in squamous carcinoma during that period, mainly due to the success of cytological cervical cancer screening programs. However, US SEER program data show that there has also been an absolute increase in cervical adenocarcinoma of 0.6 cancers/100,000 women when comparing the periods 1973–1989 to 1990–2008 [3–5]. Studies from European countries have shown similar effects [6]. However, glandular cancer rates have more recently been stable or declining, the likely result of improved detection of glandular precursor lesions. The introduction in the late 1980s of new cytological sampling devices increased the amount of cellular material obtained from above the squamocolumnar junction and the upper endocervical canal. With greater experience, enhanced recognition of the cytological features of adenocarcinoma in situ subsequently impacted early detection in a manner completely analogous to the decline in squamous lesions following detailed cytological descriptions of their precursor lesions in the early part of the twentieth century. In fact, recent data show that the absolute incidence of cervical adenocarcinoma in situ has increased as much as sevenfold in recent years, almost certainly due to better detection [4].

Pathobiology

Just as in squamous neoplasia of the cervix, usual type adenocarcinoma of the cervix and its precursors are virtually always associated with high-risk human papillomavirus (hrHPV) infection (see Chap. 2) [7]. Many of the same risk factors are also present, such as young age for first sexual intercourse and increased numbers of sexual partners; however, there are additional differing risk factors for glandular cancers which include the use of oral contraceptives and hormone replacement therapy [8–10]. Glandular cancers show less association with smoking and high parity when compared to squamous cancers [11–13]. As in squamous cancers, HPV types 16 and 18 are most common in adenocarcinoma; however, the relative proportion of types is different, with adenocarcinomas showing an increased proportion of type 18 and its closely associated type 45 (Table 8.1) [14–18].

Table 8.1 Prevalence of HPV types in endocervical adenocarcinoma (EACA)

Study	No. EACA tested	% cases positive				
		Any HPV (%)	HPV 16 (%)	HPV 18 (%)	HPV 45 (%)	HPV other (%)
Li (2011)	3525	82	36.3	36.8	5.2	21.7
De Sanjose (2010)	760	62	50	32	12	6
Tornesello (2011)	39	72	57	18	7	18

An (2005)	135	90	42	36	1	21
Clifford (2008)	2521	80.3	35.3	37.9	5.6	21.2

Precursor Lesions (Adenocarcinoma In Situ, Usual Type or High-Grade Cervical Glandular Intraepithelial Lesion (HG-CGIN))

The prototypical cervical glandular neoplastic lesion is adenocarcinoma in situ (AIS) of the usual type. In the British literature, AIS has also been named "high-grade cervical glandular intraepithelial neoplasia" (HG-CGIN). These terms are now considered synonyms in the newest WHO classification system, which also includes stratified mucin-producing intraepithelial lesion (SMILE) as a variant of AIS/HG-CGIN [19]. AIS was originally described in 1953 by Friedell and McKay, and its histological features were recognized at that time [20]. The cytological morphology was described much later in the 1970s and 1980s [21–23]. There is significant evidence that AIS is a true precursor lesion to invasive carcinoma. AIS has a mean age which is reported to be from 12 to 18 years earlier than that of invasive endocervical adenocarcinoma [22, 24–26]. AIS is also more prevalent than invasive carcinoma indicating a larger pool of AIS from which only a portion goes on to develop invasive disease. AIS shows diffuse p16 immunostaining completely analogous to invasive carcinoma indicative of a neoplastic transformation of the epithelium and is positive for hrHPV of similar types to those seen in invasive endocervical cancer [27, 28]. In addition, areas of AIS are very commonly found adjacent to invasive endocervical adenocarcinomas. The usual type of AIS, in parallel with its invasive counterpart, is by far the most common in situ adenocarcinoma of the endocervix. In comparison to the other recognized in situ adenocarcinoma variants, namely, endometrioid and mucinous, the usual type constitutes more than 90% of all cases. AIS is generally not visible on colposcopic examination and may not be associated with symptoms or have only minor symptoms such as abnormal vaginal discharge [29, 30]. It is therefore most commonly identified via Pap or hrHPV testing [31–33]. Pap testing (as described below) can show diagnostic features of AIS but just as commonly shows atypical glandular cells which may be insufficient for a definitive interpretation of AIS. This equivocal finding is recognized to be very important for patient management and has been well incorporated into current management guidelines [34]. All cases of atypical glandular cells on a Pap test should prompt a colposcopic examination and histological sampling of the endocervical canal. Recent improvements in Pap test sampling device technology, which have allowed greater sampling of the endocervical canal and better recognition of the diagnostic cytological features, are thought to be the reason for the increased prevalence of AIS

discovered today in screening programs. hrHPV testing is also sensitive for AIS; however, the specificity is less than with Pap testing because of the high prevalence of benign hrHPV infections in the background population. AIS most often grows in a contiguous fashion, and therefore during excisional procedures, clear margins do generally indicate complete removal [35]. However, rare examples of discontinuous AIS have been reported, and clinical follow-up following excision is necessary. Because virtually all AIS of the usual type is associated with hrHPV types, testing for hrHPV can be helpful in assessing residual disease following excision. Fortunately, complete excision of AIS is curative.

Histopathology [36, 37]

The normal endocervical epithelium adjacent to the cervical transformation zone consists of a simple columnar epithelium with basal nuclei and a mucous cap of frothy cytoplasm (Fig. 8.1). AIS manifests as a replacement of the normal endocervical epithelium by neoplastic glandular cells without evidence of invasion through the basement membrane. When AIS replaces the normal endocervical epithelium, the simple epithelium is transformed to a pseudostratified epithelium in which the mucous cap is diminished with a much increased nucleus to cytoplasm ratio. This appearance is often referred to as “mucin-poor.” At low magnification examination, the first histopathological clue may be the density of the nuclei in the epithelium which lends a hyperchromatic (dark) appearance to the surface epithelium (Fig. 8.2). Usually areas where the hyperchromatic epithelium directly abuts normal endocervical epithelium are noted which accentuates the stark contrast between the normal and neoplastic epithelia (Fig. 8.3). At high magnification, the cells of AIS show enlarged nuclei, on average about two times the size of normal endocervical cell nuclei. The nuclei are elongate and show significant overlapping in the pseudostratified areas. Nucleoli are present but may not always be prominent. Nuclear chromatin is typically coarsely granular and evenly distributed, and nuclear envelops are irregular. Mitotic figures are common, and apoptotic debris (nuclear breakdown fragments indicative of cell turnover) is also common (Fig. 8.4). Architecturally, AIS does not generally show areas of solid or cribriform growth. If such areas are identified, a careful examination of the specimen for early invasion is indicated.

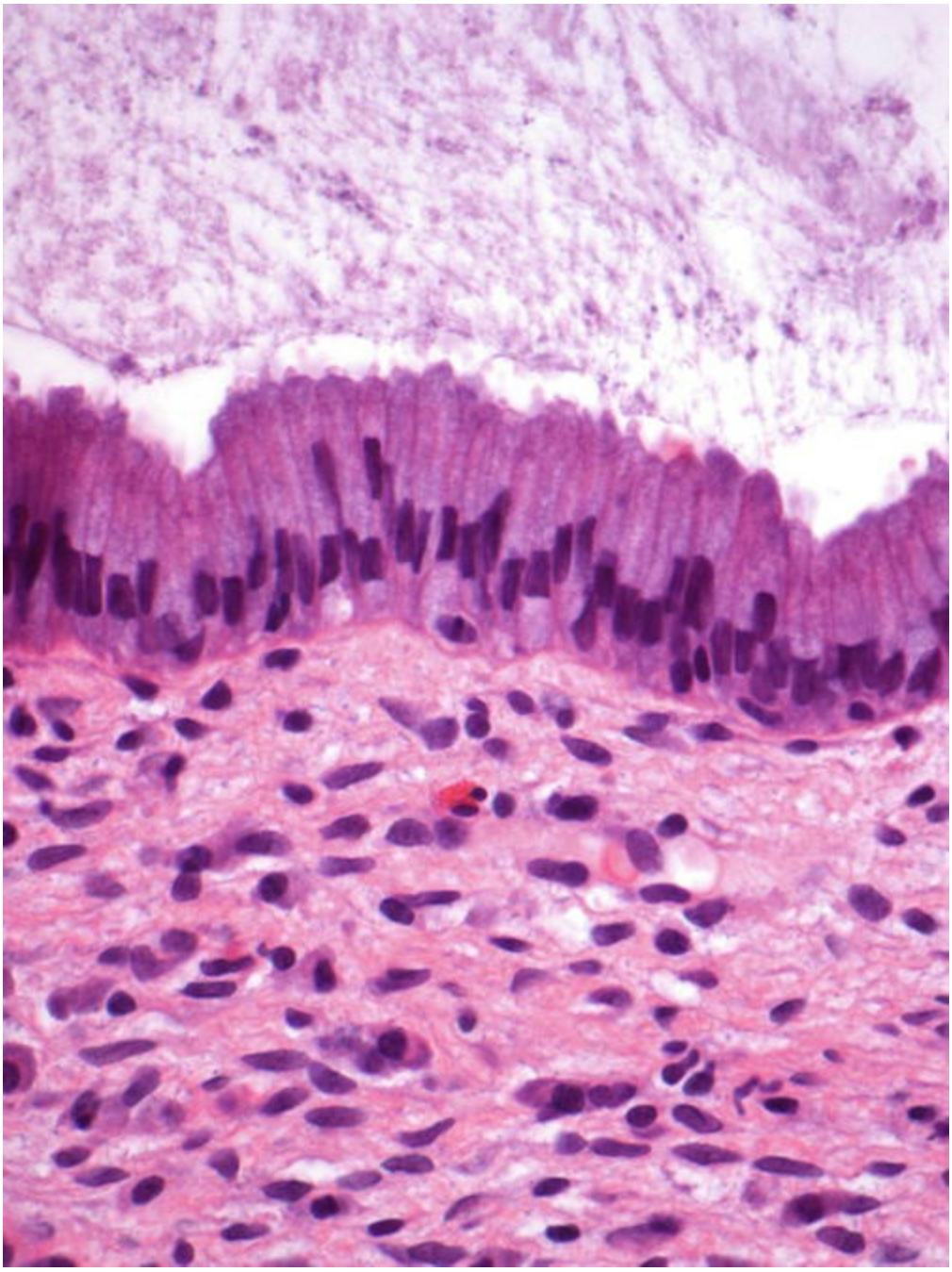

Fig. 8.1 Normal endocervical epithelium in the area of the transformation zone is simple, columnar, and non-stratified. Uniform nuclei are present in the basal portion of the cell, and the luminal columnar cytoplasm contains frothy mucus (hematoxylin and eosin stain, high magnification)

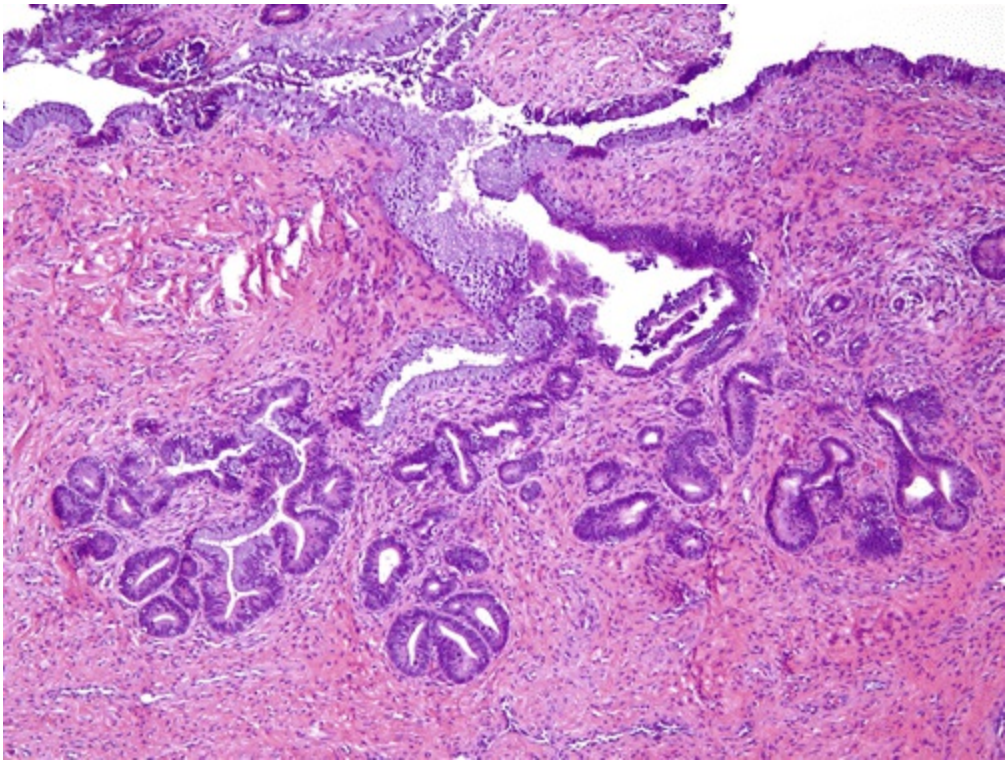

Fig. 8.2 Endocervical adenocarcinoma in situ is most often initially noted during low magnification scanning due to the hyperchromasia of the stratified nuclei in comparison to the pallor of the normal mucus-rich endocervical epithelium (hematoxylin and eosin stain, low magnification)

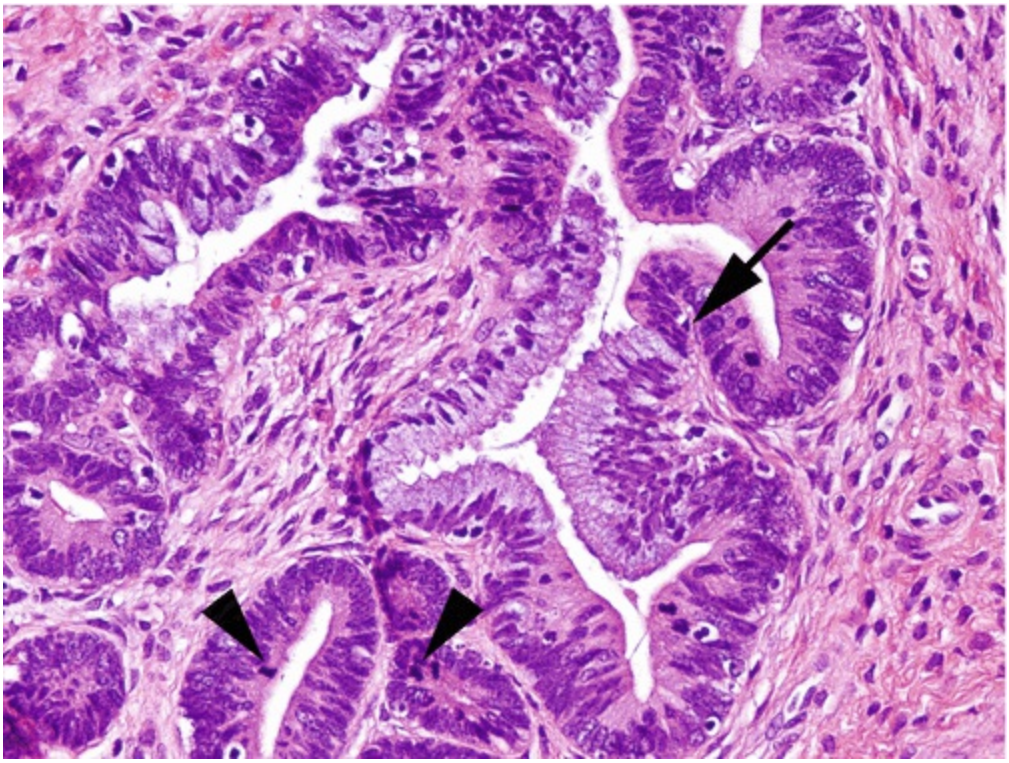

Fig. 8.3 Endocervical adenocarcinoma in situ commonly shows a sharp margin with adjacent normal endocervical epithelium. Note the very clear demarcation between lesional and normal columnar epithelium (*arrow*). AIS shows

prominent pseudostratification with loss of the mucus cap compared to the normal simple architecture with prominent frothy mucus. Note the prominent mitotic activity (*arrow head*) (hematoxylin and eosin stain, high magnification)

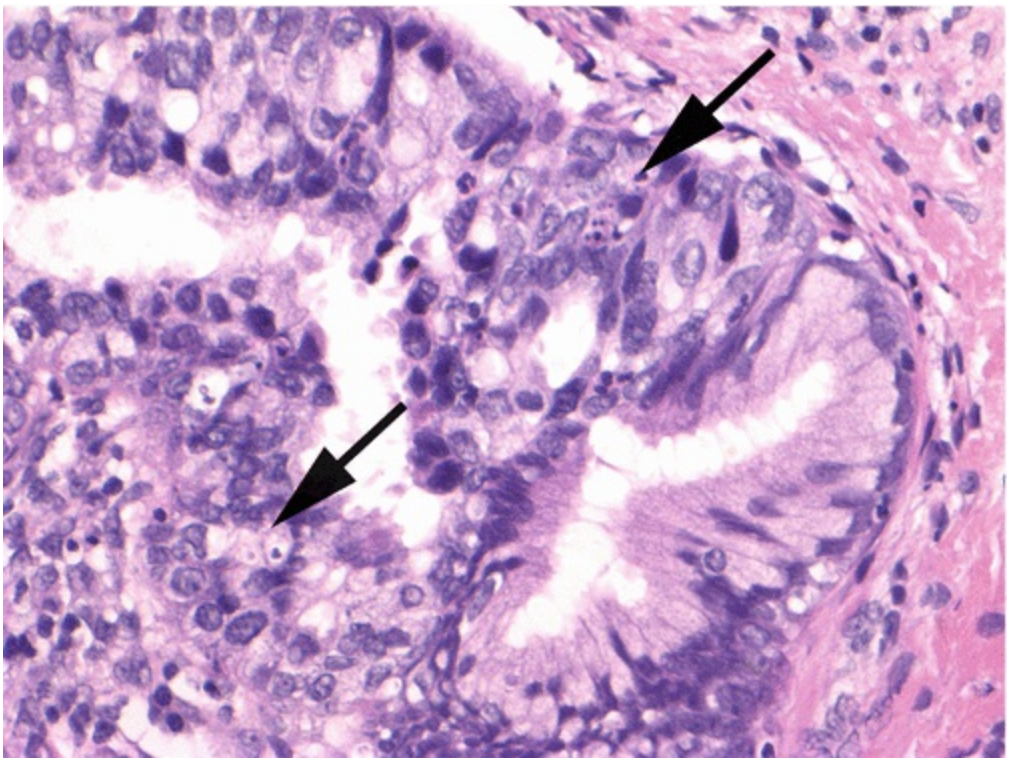

Fig. 8.4 The nuclei of endocervical adenocarcinoma in situ are typically about two times the size of normal endocervical cell nuclei. Apoptotic debris is commonly present in neoplastic endocervical epithelium and is indicative of increased cell turnover (*arrow*) (hematoxylin and eosin stain, high magnification)

Cytopathology [37–39]

The cytopathological appearance of AIS recapitulates the histopathology closely. Cytological specimens from patients with AIS may show abundant individual endocervical cells and hyperchromatic crowded groups (HCGs) of endocervical cells. Any specimen containing abundant endocervical material should be examined very carefully for the presence of abnormality within these cells. However, overall cellularity is dependent on the sampling of the lesion and may show few abnormal cells if the lesion is small, high in the canal, or not directly brushed. On initial low magnification examination, the hyperchromatic crowded groups typically show nuclear and cytoplasmic protrusion at the group margins. This phenomenon is referred to as “feathering” and can be seen in any endocervical proliferation but is particularly accentuated in AIS. In conventionally prepared specimens, feathering is most prominent due to flattening of the groups during the smearing process (Fig. 8.5). In liquid-based specimens, where groups are more three-dimensional, feathering can be more subtle, but is still present in most cases. The sentinel finding of AIS is the presence of strips of pseudostratified columnar epithelium with depletion of the mucous cap on the luminal

portion of the cell (Fig. 8.6). Polarity of the cells with identification of a basal and luminal aspect of the strip is important in order to discriminate AIS from high-grade squamous intraepithelial lesion (HSIL) (see Chap. 7) growing into a gland. In the latter, an appearance of a pseudostratified epithelium without a luminal-basal orientation is a key to that interpretation (Fig. 8.7). Other dense groups of glandular cells with high nucleus to cytoplasm ratio and hyperchromatic nuclei (HCGs) are also usually present. Often partial or complete gland formations (epithelial "rosettes") are noted in association with the HCGs (Fig. 8.8). Individual abnormal cells showing columnar configuration and atypical nuclei can be found distributed across the slide (Fig. 8.9). The individual cells show nuclei that are about two times the size of a normal endocervical cell. Nuclei are hyperchromatic with granular chromatin which is evenly distributed. Uneven chromatin distribution (so-called chromatin clearing or chromatin heterogeneity) should prompt a consideration of invasive endocervical adenocarcinoma. Nucleoli are present, but not generally prominent. Mitotic figures and apoptotic debris are frequently identified. The background of the slide does not show a tumor diathesis, which is caused by tissue destruction and tumor necrosis, both of which are not present in AIS and if present should also suggest the possibility of an invasive carcinoma. The background may show an increased number of acute inflammatory cells.

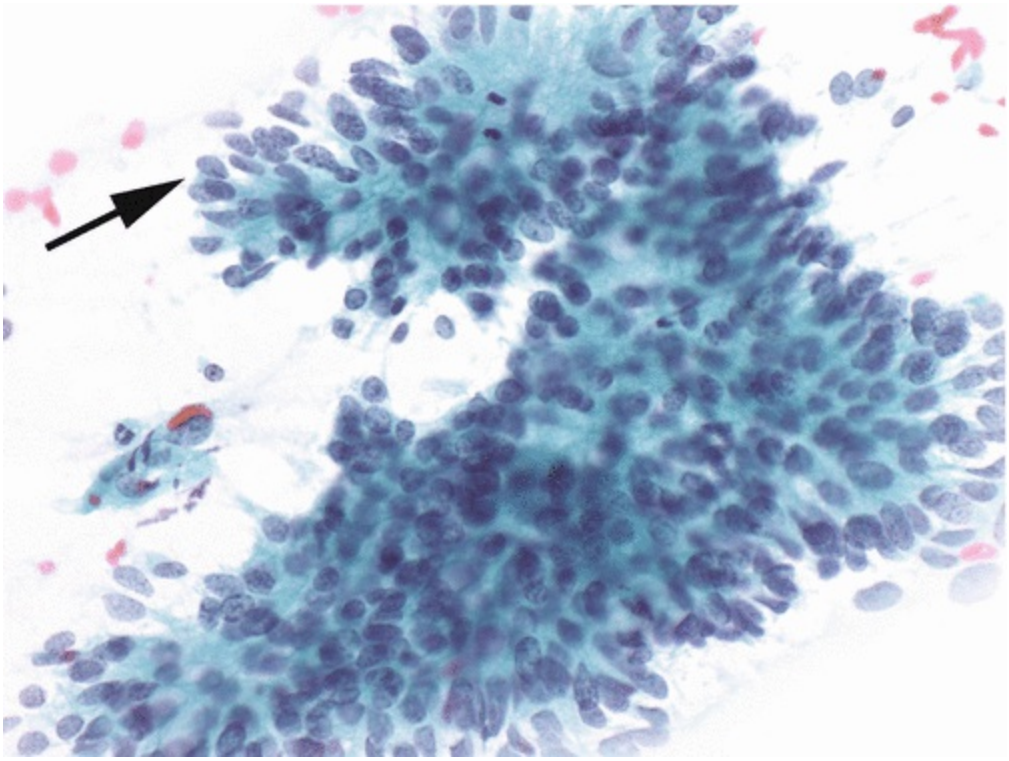

Fig. 8.5 Even at low magnification, groups of cells showing marginal protrusion of nuclei and cytoplasm ("feathering") is characteristic of endocervical adenocarcinoma in situ and may be the first indication of a neoplastic process (*arrows*). Feathering tends to be more prominent in conventionally prepared specimens due to flattening of the groups during the smearing and fixation process

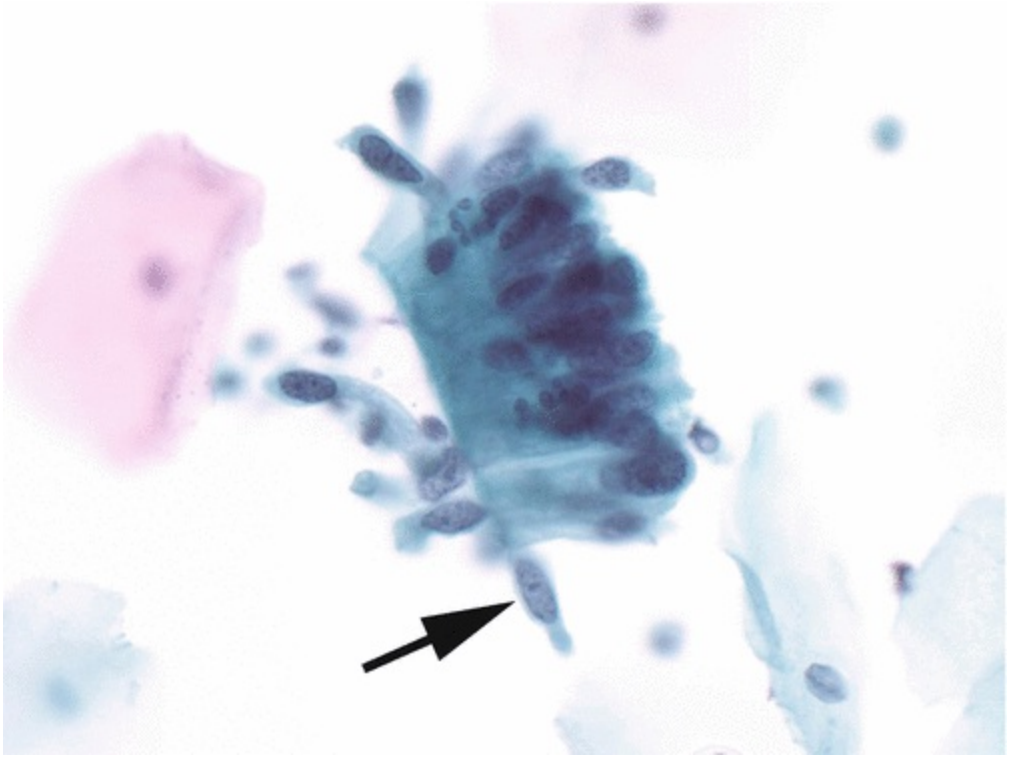

Fig. 8.6 In cytological specimens, pseudostratified strips of cells are a key feature of endocervical adenocarcinoma in situ. Note the coarse granularity with even distribution of the nuclear chromatin in this example (*arrow*) (Papanicolaou stain, medium magnification)

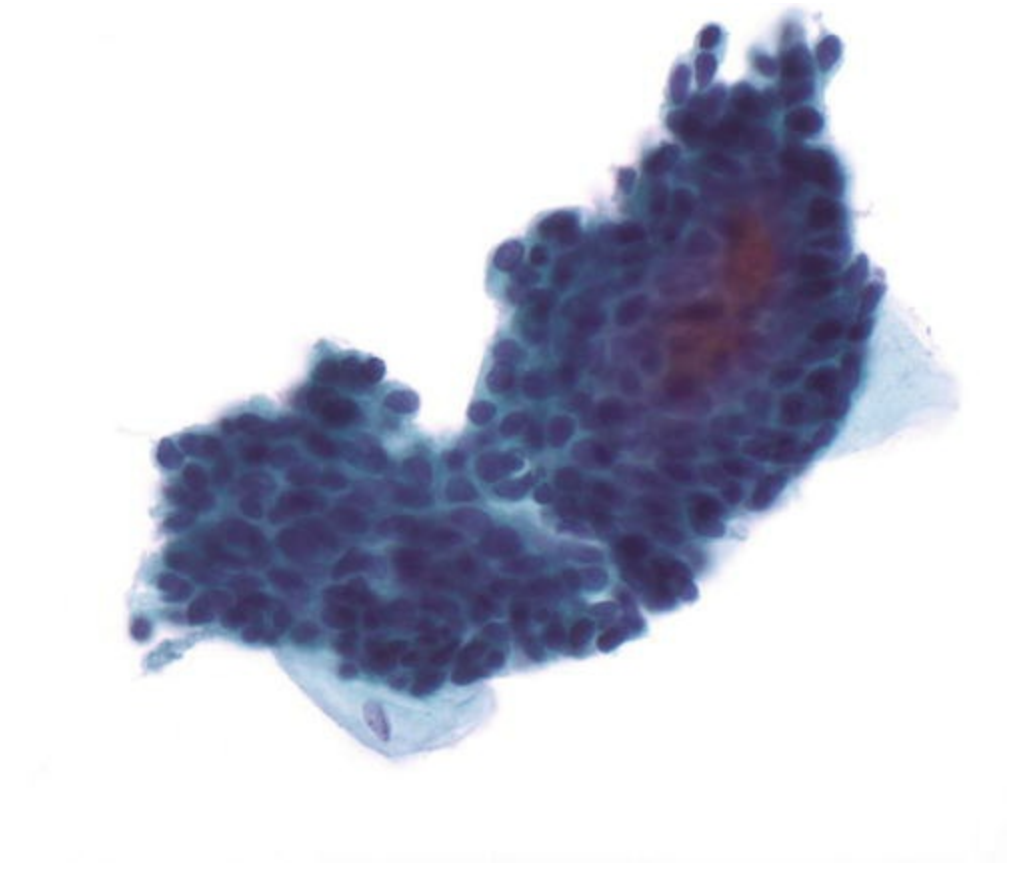

Fig. 8.7 A mimic of endocervical adenocarcinoma in situ on cytology can be seen in crowded groups of high-grade squamous intraepithelial lesion (HSIL) involving an endocervical gland (Papanicolaou stain, medium magnification)

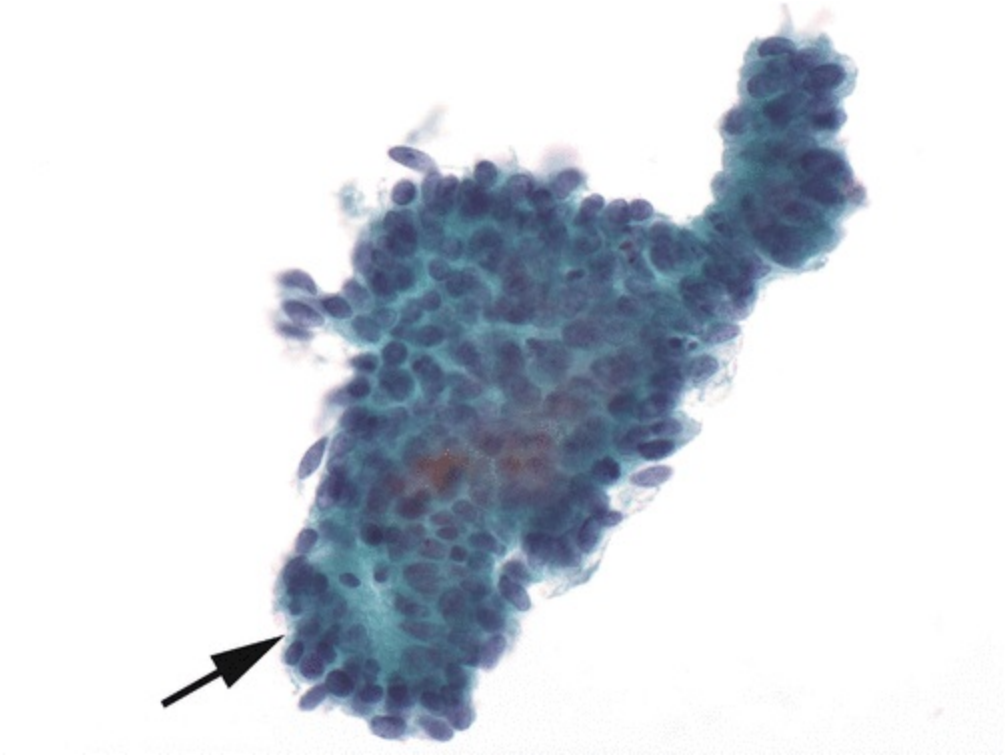

Fig. 8.8 In addition to pseudostratified strips of cells and feathering, endocervical adenocarcinoma often shows full or partial rosette arrangements of cells, indicative of gland-like formations (*arrow*) (Papanicolaou stain, medium magnification)

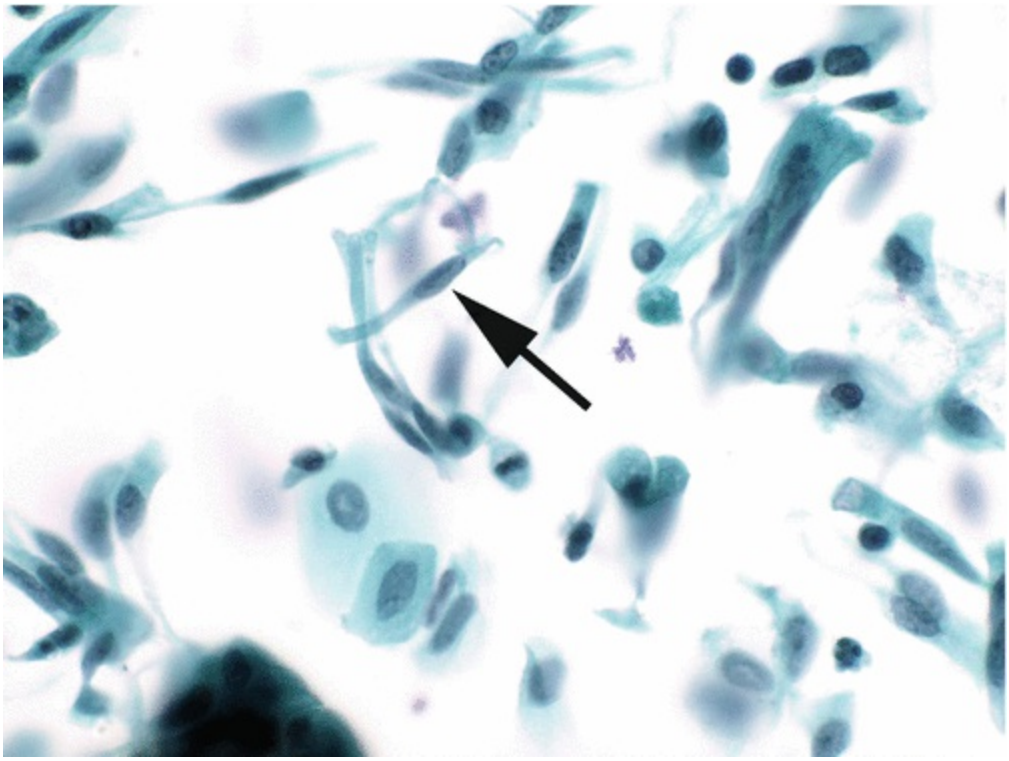

Fig. 8.9 In addition to crowded groups of cells, cases with endocervical adenocarcinoma in situ typically show atypical individual cells scattered in the background. The cells are tall and slender, with enlarged nuclei and coarse but evenly distributed nuclear chromatin (*arrow*) (Papanicolaou stain, high magnification)

Invasive Endocervical Adenocarcinoma, Usual Type

The usual type of invasive endocervical adenocarcinoma, just as in its correlate precursor lesion, is the most common type of cervical adenocarcinoma. It generally is recognized as comprising 80–90% of all adenocarcinomas, although several recent reports have shown that in Japanese populations, mucinous adenocarcinomas of gastric type may comprise as much as 30% of the total [40]. As in AIS, early superficially invasive adenocarcinomas may present with no symptoms, or occasionally with only minor symptoms, such as abnormal discharge or bleeding. In this early stage, Pap or HPV testing is most likely to show the only abnormal initial findings. Colposcopy may show areas of abnormality when the lesion occupies the lower portion of the endocervical canal. In larger tumors, symptoms of abnormal bleeding are almost always present and lesions are grossly visible on colposcopic examination. In larger tumors, the Pap test nearly always shows abnormality which may be present as either a diagnostic appearance of carcinoma or as atypical glandular cells.

The prognosis of invasive cervical adenocarcinoma is dependent on stage at presentation, with low stage disease generally having a good (curative) outcome (Tables 8.2 and 8.3). Most studies have shown that stage for stage cervical adenocarcinoma has a worse prognosis than squamous cell carcinoma [41–45]. Differences in dissemination and recurrence have been found for endocervical adenocarcinoma versus squamous cell carcinoma. Ovarian metastases are more common in endocervical adenocarcinoma than in squamous cell carcinoma [46]. Higher rates of distant metastasis have also been noted for endocervical adenocarcinoma [42, 47].

Table 8.2 Staging of cervical adenocarcinoma (TNM and FIGO)

TNM categories	FIGO stages	Definitions
Primary tumor (T)		
Tx		Primary tumor cannot be assessed
T0		No evidence of primary tumor
Tis		Carcinoma in situ (preinvasive carcinoma)
T1	I	Cervical carcinoma confined to the uterus (ignore corpus extension)
T1a	IA	Invasive carcinoma diagnosed on microscopy only
T1a1	IA1	Stromal invasion ≤3.0 mm in depth, ≤ 7.0 mm in width
T1a2	IA2	Stromal invasion 3.0–5.0 mm in depth, ≤ 7.0 mm in width

T1b	IB	Clinically visible tumor confined to cervix or microscopic size > T1a/IA2
T1b1	IB1	Clinically visible tumor ≤4.0 cm
T1b2	IB2	Clinically visible tumor >4.0 cm
T2	II	Cervical carcinoma invades beyond the uterus but not to the pelvic wall or lower one third of vagina
T2a	IIA	No parametrial invasion or involvement of the lower one third of the vagina
T2a1	IIA1	Clinically visible lesion ≤4.0 cm involving < the upper two third of the vagina
T2a2	IIA2	Clinically visible lesion >4.0 cm involving < the upper two third of the vagina
T2b	IIB	Tumor with parametrial invasion
T3a	IIIA	Tumor involves the lower one third of the vagina, no extension to the pelvic wall
T3b	IIIB	Tumor extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney
T4	IVA	Tumor invades the mucosa of the bladder or rectum and/or extends beyond the true pelvis
Regional lymph nodes (N)		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Regional lymph node metastasis
Distant metastasis (M)		
M0		No distant metastasis
M1	IVB	Distant metastasis

Table 8.3 Anatomical stage/prognostic groups of cervical adenocarcinoma

Anatomical stage/prognostic groups			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3	N0	M0
Stage IIIB	T1–3	N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

Histopathology [36, 37]

The usual type of endocervical adenocarcinoma is typically of moderate differentiation but can also present as well- or poorly differentiated lesions. In the best differentiated lesions, particularly in cases showing only superficial invasion, it may be difficult to distinguish between AIS and invasive cancer. Involvement of endocervical glandular structures that are below the level in the cervical wall of normal endocervical glands and changes in the stromal tissue surrounding the abnormal glands, such as inflammation, myxoid change, or “swirling” of stromal fibroblasts around the nests of

neoplastic cells, are clues to superficial invasion (Fig. 8.10). In addition, changes in the neoplastic cells compared to those directly adjacent to the areas of suspected invasion can also present a clue to invasion. Increased amounts of cytoplasm and the presence of more prominent macronucleoli can be seen in association with increase in metabolic activity necessary to penetrate the basement membrane and spread into the stromal tissues (Fig. 8.11). In these superficially invasive lesions, the neoplastic cells replace the normal endocervical cell lining of the glands. In distinction to the pseudostratified architecture of AIS, early invasive carcinoma may also show more complex architecture including solid patterns and areas of cribriform growth. Identification of either of these two architectural features should prompt consideration of an invasive tumor.

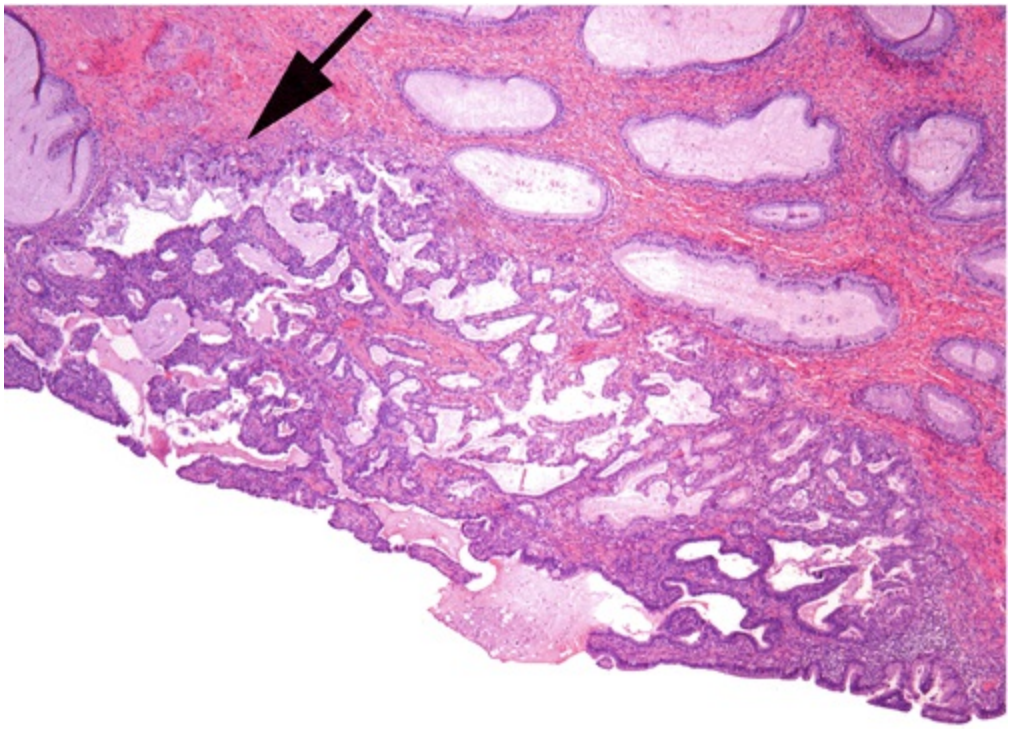

Fig. 8.10 Superficially invasive endocervical adenocarcinoma shows markedly irregular glandular spaces which impinge on the adjacent normal endocervical glands. Stromal reaction and inflammation often surrounds the early invasive nests (*arrow*) which is a key diagnostic feature differentiating an invasive from an in situ lesion (hematoxylin and eosin stain, low magnification)

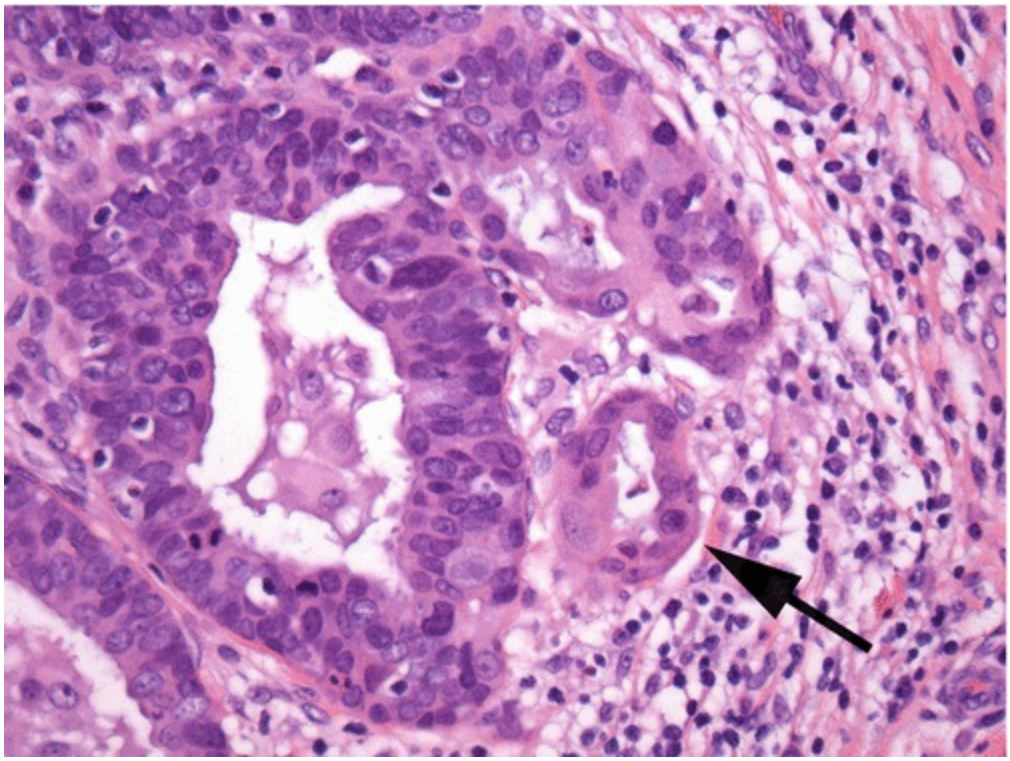

Fig. 8.11 Two early clues to the presence of invasion are stromal reaction and changes in cytoplasm. Glands surrounded by an edematous or inflamed swirling stroma or an increase in cytoplasmic volume with eosinophilia (*arrow*) are highly associated with invasive disease (hematoxylin and eosin stain, high magnification)

In clearly identifiable invasive endocervical adenocarcinoma, the abnormal glands are small to medium sized and penetrate the cervical wall to various levels, inciting an obvious stromal response (Fig. 8.12). Gland lumina, when discernible, may show necrotic debris. Occasional cystic glands may be present with mucin within the gland lumen or occasionally free in the stromal tissue. The abnormal cells retain a columnar appearance, with either pseudostratified, solid/cribriform, or occasionally papillary tufted growth pattern. The individual cells have high nucleus to cytoplasm ratios with granular "mucin-poor" cytoplasm. Nuclei are enlarged at greater than two times the size of normal endocervical cell nuclei and can range in shape from tall fusiform to oval. Nuclear contours are irregular. The nuclei are hyperchromatic having dense granular cytoplasm and areas of chromatin heterogeneity (so-called chromatin "clearing") (Fig. 8.13). Numerous mitotic figures are noted, often near the apex of the cells giving the appearance of "floating mitoses" (Fig. 8.14). Apoptotic debris is also commonly noted.

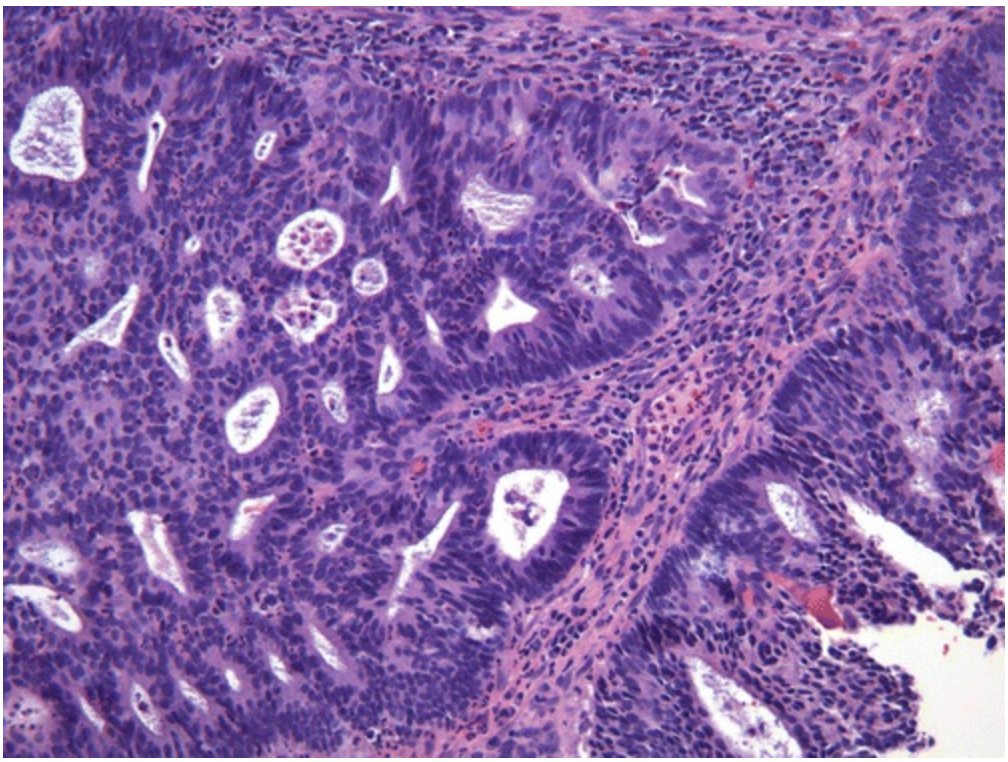

Fig. 8.12 Invasive endocervical adenocarcinoma of the usual type commonly shows cribriform architecture with some foci showing a solid growth pattern (hematoxylin and eosin stain, medium magnification)

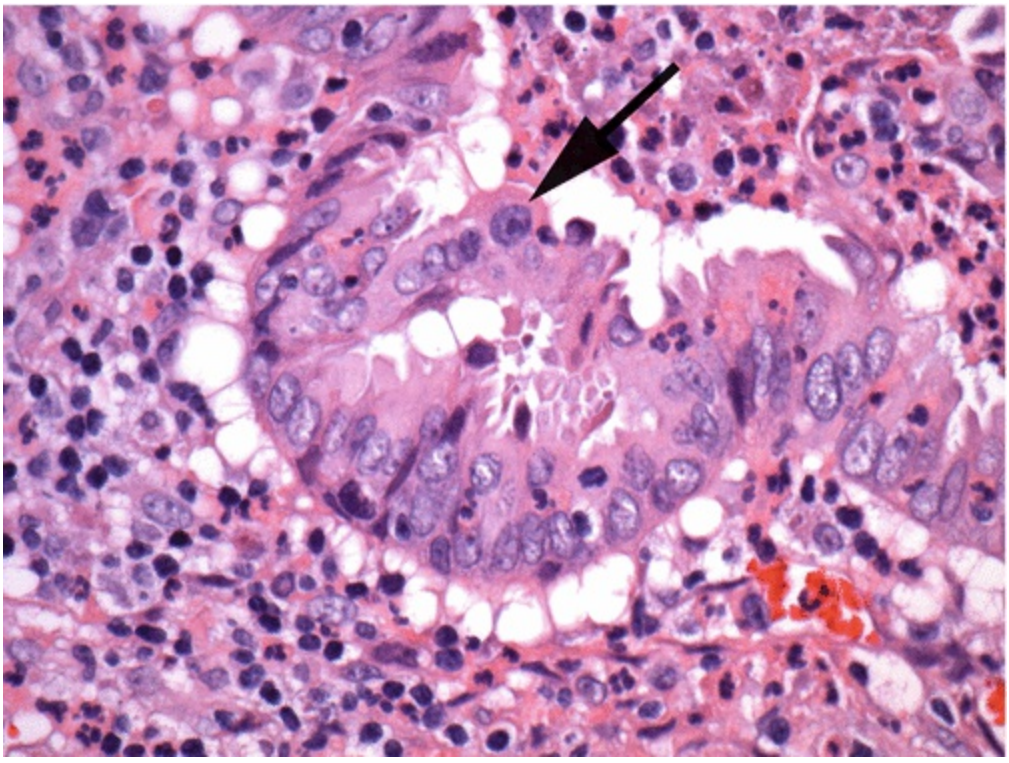

Fig. 8.13 The nuclei of endocervical adenocarcinoma of the usual type are typically pleomorphic with irregularities of size and shape. Note the coarse granularity of the chromatin which in contrast to in situ lesions shows areas of heterogeneity (*arrow*) (hematoxylin and eosin stain, high magnification)

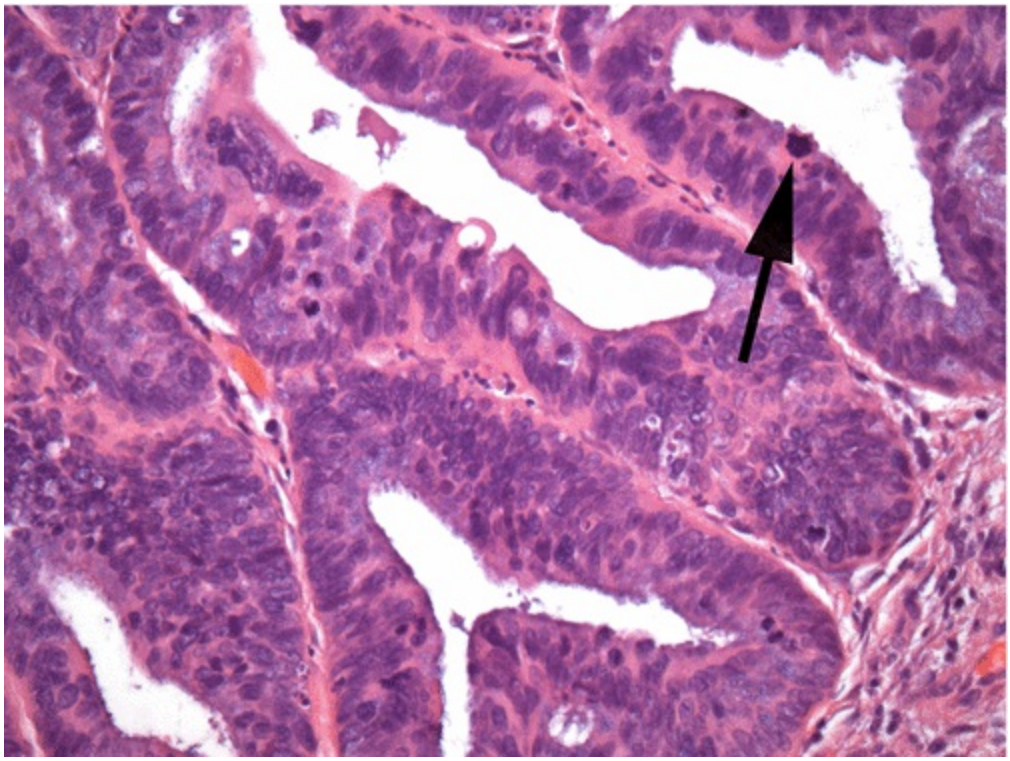

Fig. 8.14 "Floating" mitoses which are present near the luminal surface of the cells are common in endocervical adenocarcinoma of the usual type (*arrow*) (hematoxylin and eosin stain, high magnification)

Cytopathology [37–39]

The cytopathological appearance of invasive adenocarcinoma, similar to the histopathology, is dependent on the degree of differentiation of the tumor. Well-differentiated lesions may be indistinguishable from AIS. As the tumors become less differentiated, the amount of sampled neoplastic cellular material increases and the appearance of the cells becomes more atypical. Adenocarcinoma presents as isolated atypical cells, as pseudostratified strips of cells, as dense hyperchromatic crowded groups, and as two-dimensional sheets of cells (Fig. 8.15). The difference between the latter two presentations depends on how the sampling of the cells took place. Two-dimensional groupings mean the cells were directly sampled from the tumor surface and recapitulate their in situ appearance; while three-dimensional groups imply that the cells were spontaneously exfoliated prior to being sampled (Fig. 8.16). Exfoliation allows for cell groupings (and individual cells) to round up as they "float" in the cervical mucus. The individual cells of adenocarcinoma show high nucleus to cytoplasmic ratios with granular "mucin-poor" cytoplasm. The nuclei are oval to fusiform, hyperchromatic with dense coarsely granular chromatin showing areas of heterogeneity ("clearing"), and prominent macronucleoli (Fig. 8.17). Mitotic figures and apoptotic debris are commonly present. The slide background commonly shows the presence of granular cellular breakdown material intermixed with inflammatory cells (so-called tumor

diathesis) which is indicative of tissue necrosis and inflammatory response. In conventionally prepared cytology specimens, the diathesis material is spread evenly in the background of the slide, while in liquid-based cytology specimens, the diathesis material may aggregate and cling to the surface of cells (Fig. 8.18).

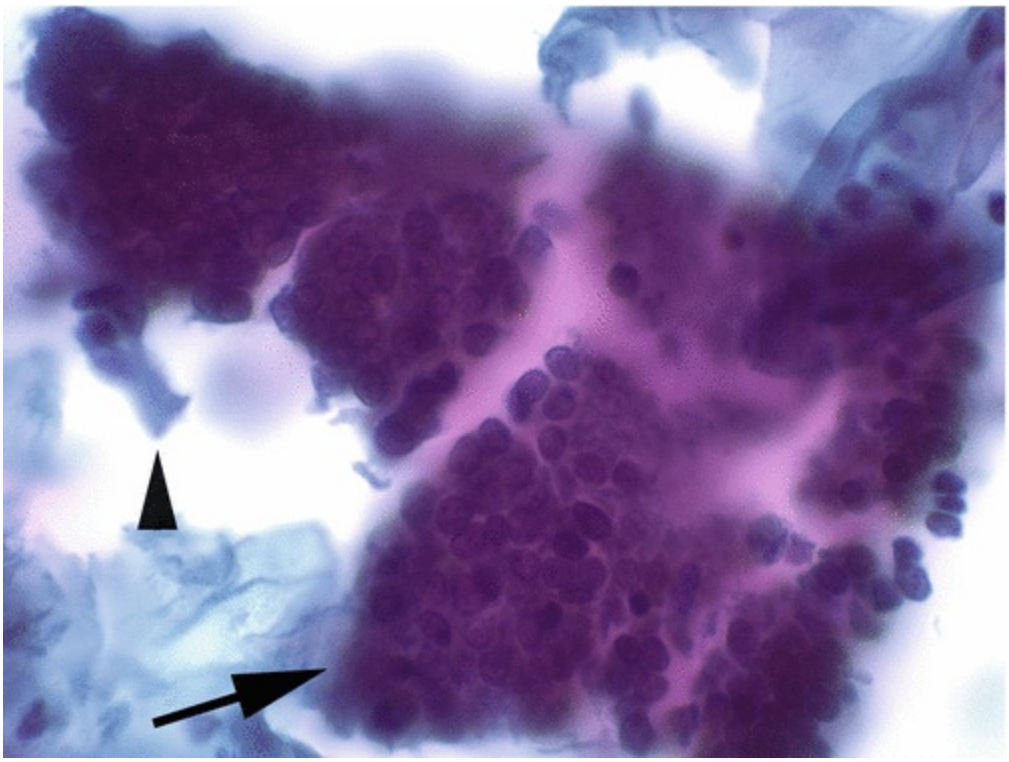

Fig. 8.15 Endocervical adenocarcinoma of the usual type presents as groups and as isolated cells. Groups retain features of columnar epithelia with "honeycomb" architecture (*arrow*), and isolated cells recapitulate the columnar configuration of normal endocervical cells (*arrowhead*) (Papanicolaou stain, high magnification)

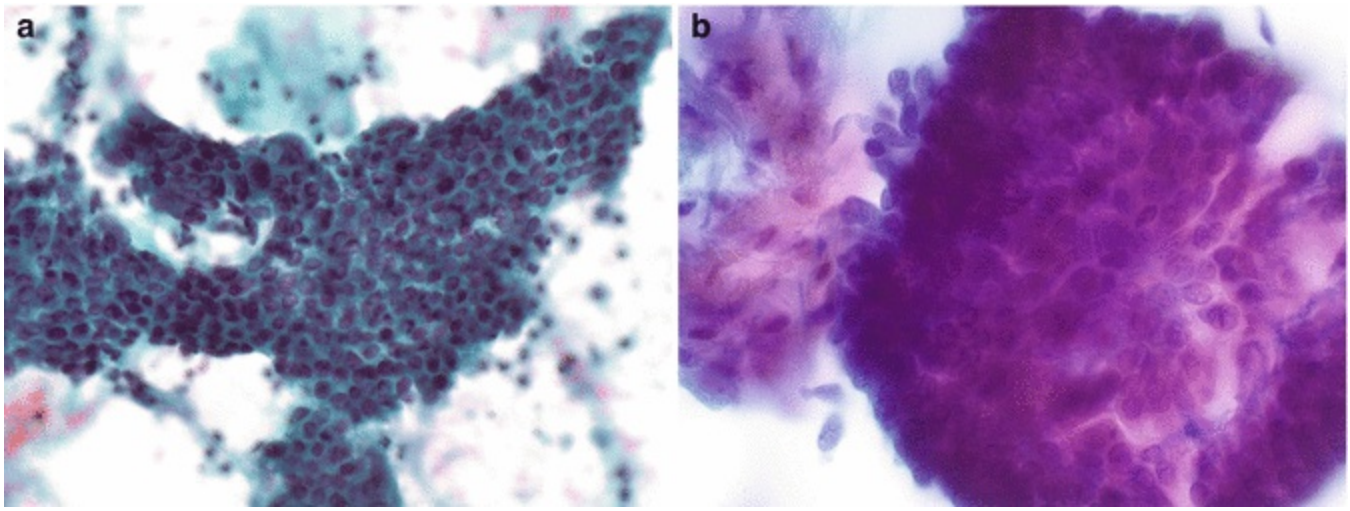

Fig. 8.16 The cytological presentation of endocervical adenocarcinoma of the usual type depends on the method of sampling. When directly sampled, a two-dimensional sheet of cells is present (**a**) (Papanicolaou stain, medium magnification). And when tumor cells exfoliate prior to sampling, three-dimensional clusters are the norm (**b**)

(Papanicolaou stain, high magnification)

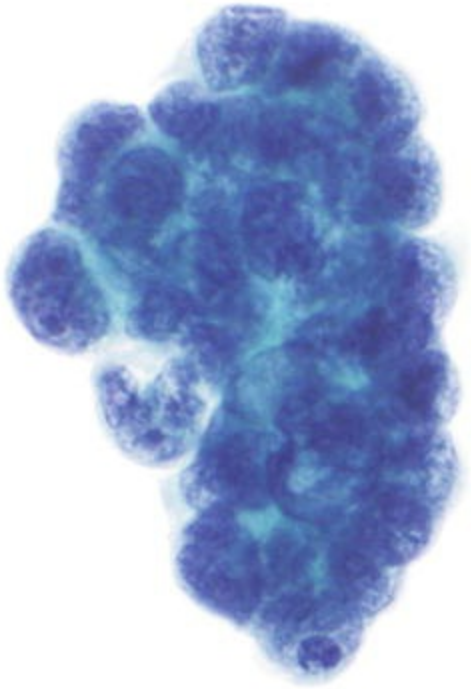

Fig. 8.17 The nuclei of endocervical adenocarcinoma of the usual type show heterogeneous coarse chromatin granularity and prominent nucleoli (Papanicolaou stain, high magnification)

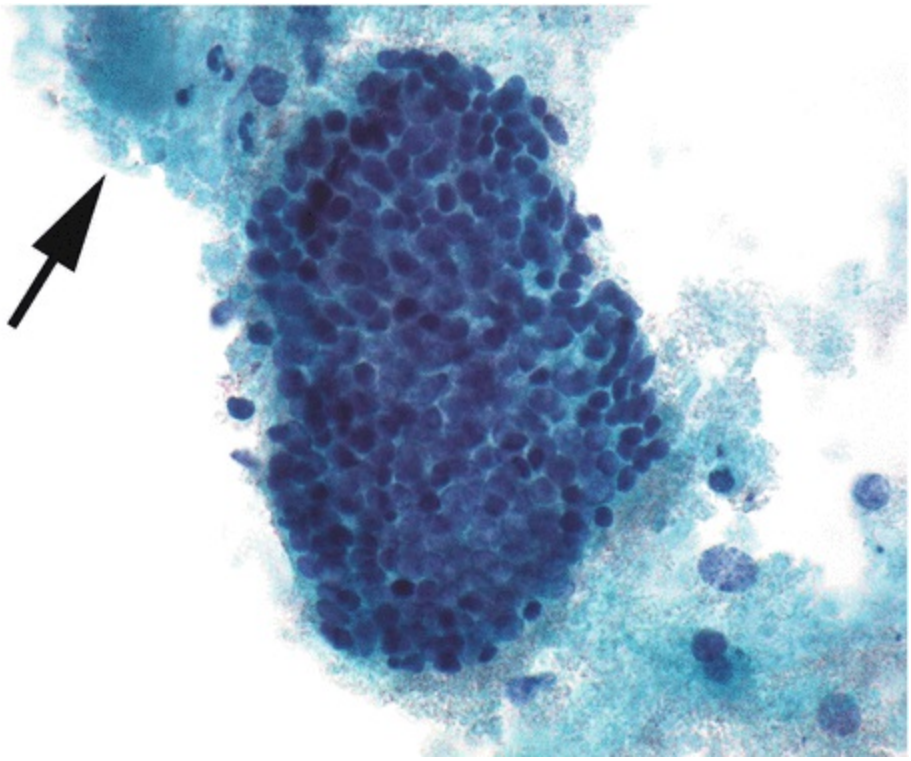

Fig. 8.18 Tumor diathesis consisting of granular amorphous debris is commonly found in the background and clinging to the surface of intact cells in invasive carcinoma (*arrow*) (Papanicolaou stain, medium magnification)

Immunohistochemistry of Endocervical Neoplasia

p16 immunohistochemistry is a useful marker for the presence of a true endocervical neoplastic lesion of the usual type. Virtually all usual type AIS and invasive endocervical adenocarcinomas are hrHPV positive and will show aberrant accumulation of p16. There are caveats for interpretation of this stain however. A positive stain should only be considered one in which the entire epithelium is diffusely positive, most often with both nuclear and cytoplasmic staining (Fig. 8.19a). This is important because several of the benign mimics noted below, such as tubal metaplasia and endometriosis, can show incomplete spotty, but sometimes strong staining for p16 (Fig. 8.19b). A marker of increased cellular proliferation (Ki67 or Mib1) can be supportive of a neoplastic process in conjunction with p16; however, studies have shown that Ki67 adds little predictive value to the p16 assay (Fig. 8.19c). Another marker IMP3 has been shown to be more specific for AIS with diffuse staining of most usual type glandular neoplasias with little staining reported in benign processes [48].

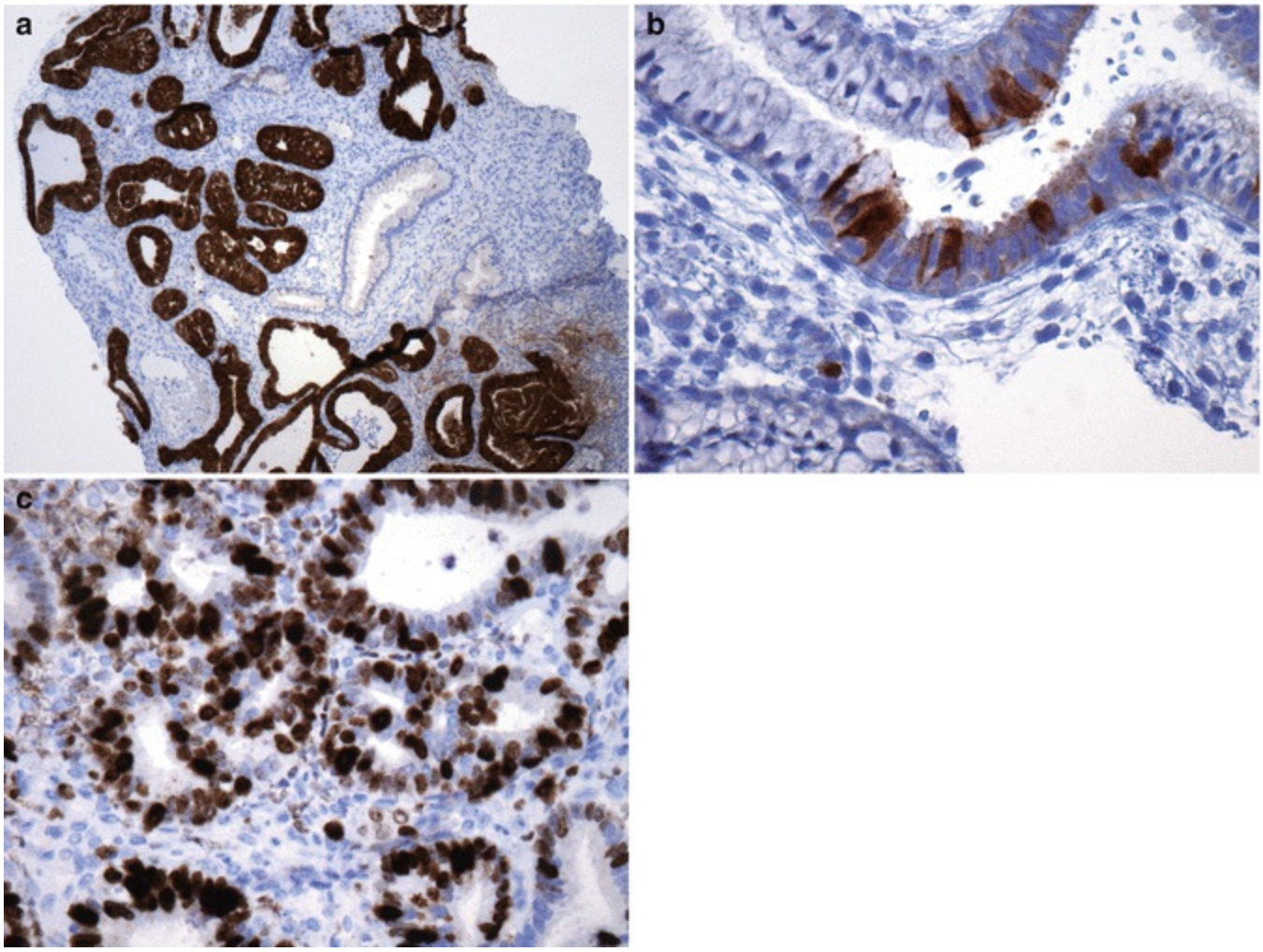

Fig. 8.19 Immunohistochemical stains can be helpful in distinguishing neoplastic endocervical lesions from benign mimics. p16 staining is strong and diffuse in neoplasia (**a**), while benign tubal metaplasia shows only focal cells which are immunoreactive (**b**). In (**a**), note the p16-positive lesional tissue in comparison to the negative residual benign endocervical glands. Increased numbers of cells show reactivity with Ki67 in neoplastic lesions (**c**)

Immunohistochemistry can be useful in distinguishing endocervical from endometrial neoplasms, particularly when the lesions are large and extend to both cervix and corpus or are present in metastatic sites. Endocervical carcinoma is diffusely p16 positive, whereas endometrial cancer is typically only focally positive. Additionally, endocervical neoplasia is positive for CEA and negative for estrogen receptor and vimentin. Endometrial neoplasia is negative for CEA and positive for estrogen receptor and vimentin [49]. Both origins are most often positive for PAX8.

Histological Mimics of In Situ and Invasive Adenocarcinoma

Tubal and Tuboendometrioid Metaplasia

In tubal metaplasia (TM), endocervical glandular epithelium is replaced by tubal-type

epithelium composed of ciliated cells, nonciliated secretory cells, and intercalated (peg) cells (Fig. 8.20). Tubal metaplasia is a common finding in the endocervical canal, being present in 21% of cone biopsies and 62% of hysterectomy specimens in a prevalence study [50] and becoming more frequent as women age [51]. Less commonly the presence of a mixture of tubal- and endometrial-type epithelia known as tuboendometrioid metaplasia (TEM) is identified. This is similar to TM but with few to rare ciliated cells (Fig. 8.21). TEM has been found in 26% of hysterectomy specimens when a prior cone biopsy had been performed, suggesting that it may be a reparative response in at least some cases [52]. Usually the glands involved by TM and TEM otherwise resemble normal endocervical glands, but one or more unusual features can occasionally be present, such as variability in size and shape, cystic dilatation, pseudostratified architecture, focal crowding, high nuclear to cytoplasmic ratio, mitotic figures, a deep location, and periglandular stromal hypercellularity or edema (Fig. 8.22) [53]. At low magnification, the dark-staining epithelia may initially raise the possibility of a well-differentiated invasive adenocarcinoma or AIS. The admixture of cell types, including the prominence of ciliated cells, as well as the lack of nuclear atypia, only rare mitotic figures without apoptotic debris, and lack of a desmoplastic stromal reaction, should allow a correct interpretation as a benign metaplastic process. Immunohistochemical staining for p16, Bcl2, and MIB1/Ki67 can help differentiate neoplastic endocervical glands from benign TM/TEM.

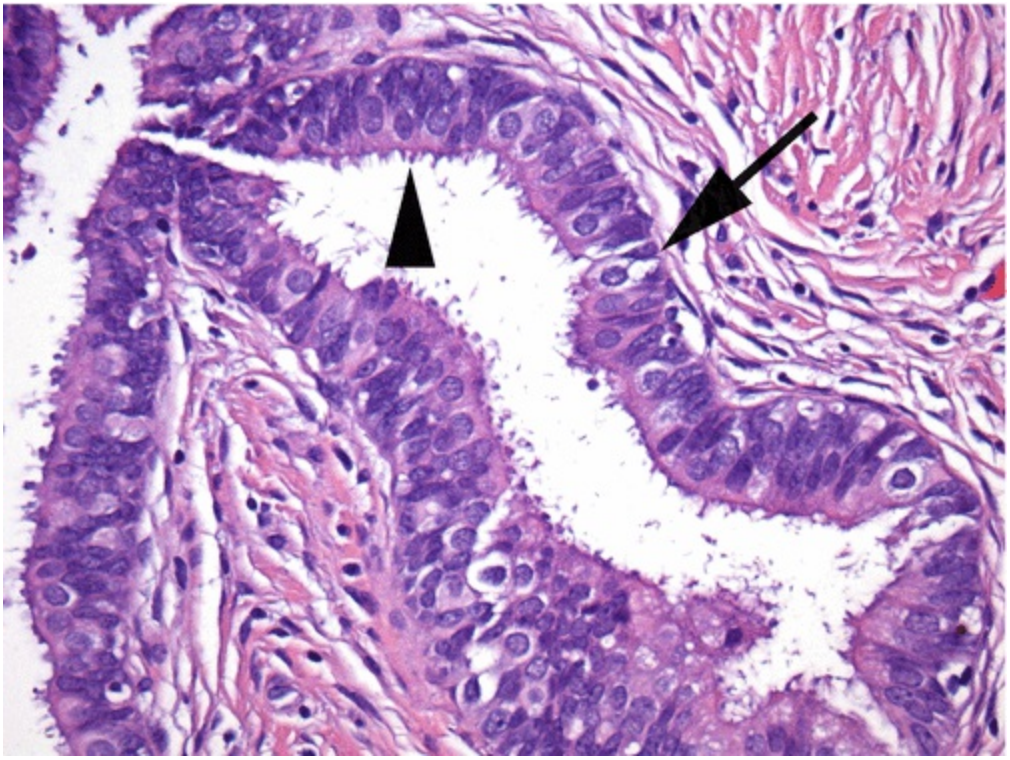

Fig. 8.20 Tubal metaplasia consists of a mucin-poor, pseudostratified epithelium which replaces normal endocervical epithelium in the upper canal as women age. Its hyperchromatic appearance gives an appearance of neoplasia at low

magnification. Note the heterogeneity of epithelial cell types vacuolated (*arrow*) and ciliated (*arrowhead*), which are the key to a correct benign interpretation (hematoxylin and eosin stain, high magnification)

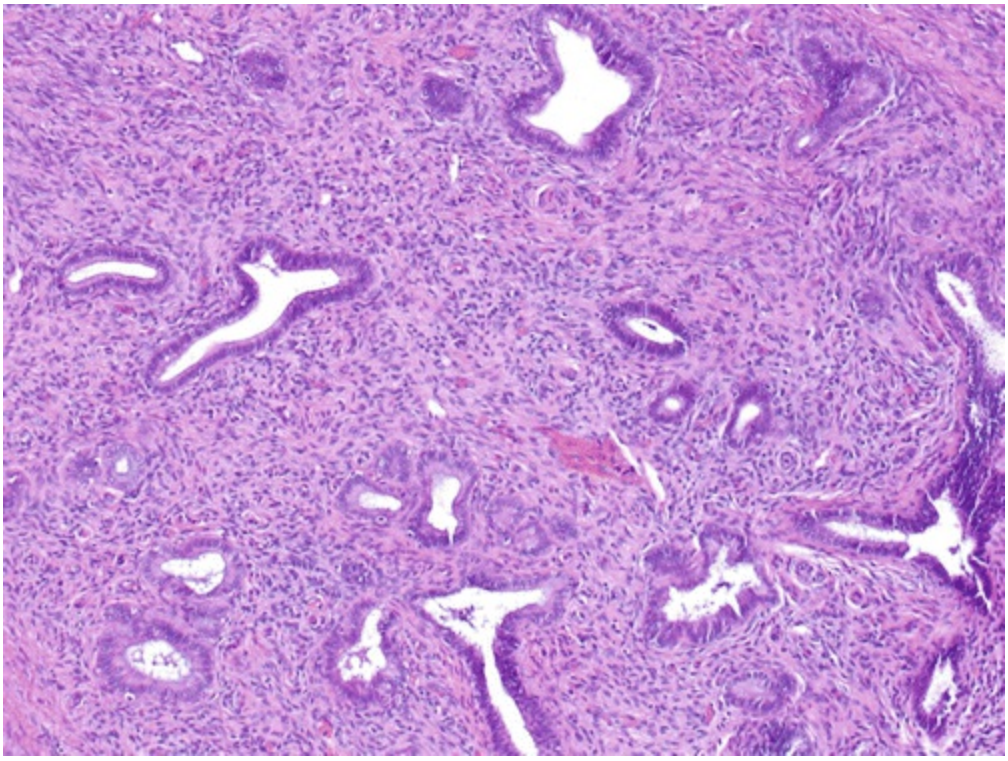

Fig. 8.21 Tuboendometrioid metaplasia is diagnosed only on histology. The glands have a mucin-depleted appearance with oval, elongate nuclei; the glands usually are surrounded by stroma with slightly increased cellularity (hematoxylin and eosin stain, low magnification)

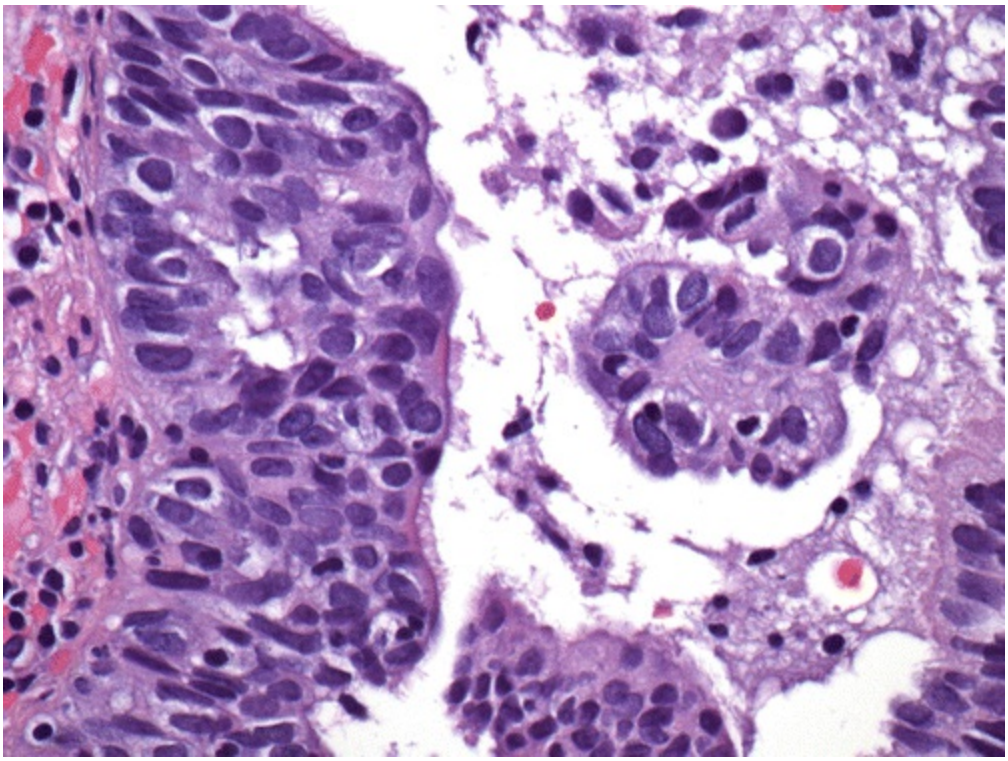

Fig. 8.22 Tubal metaplasia can present in a crowded/solid pattern with rosette-like structures which can mimic endocervical neoplasia. The presence of luminal cilia is a key benign feature

Oxyphilic Metaplasia

Oxyphilic metaplasia is an incidental finding in cervical specimens having no clinical significance. It manifests as focal replacement of endocervical epithelium with cuboidal cells having dense, eosinophilic, and focally vacuolated cytoplasm. The nuclei are usually large, hyperchromatic, and somewhat degenerate which may give rise to erroneous considerations of in situ adenocarcinoma (Fig. 8.23). Although a certain degree of epithelial atypia has been described, unlike adenocarcinoma, oxyphilic metaplasia lacks stratified cells, marked atypia, and mitotic activity which should allow for a correct interpretation [54].

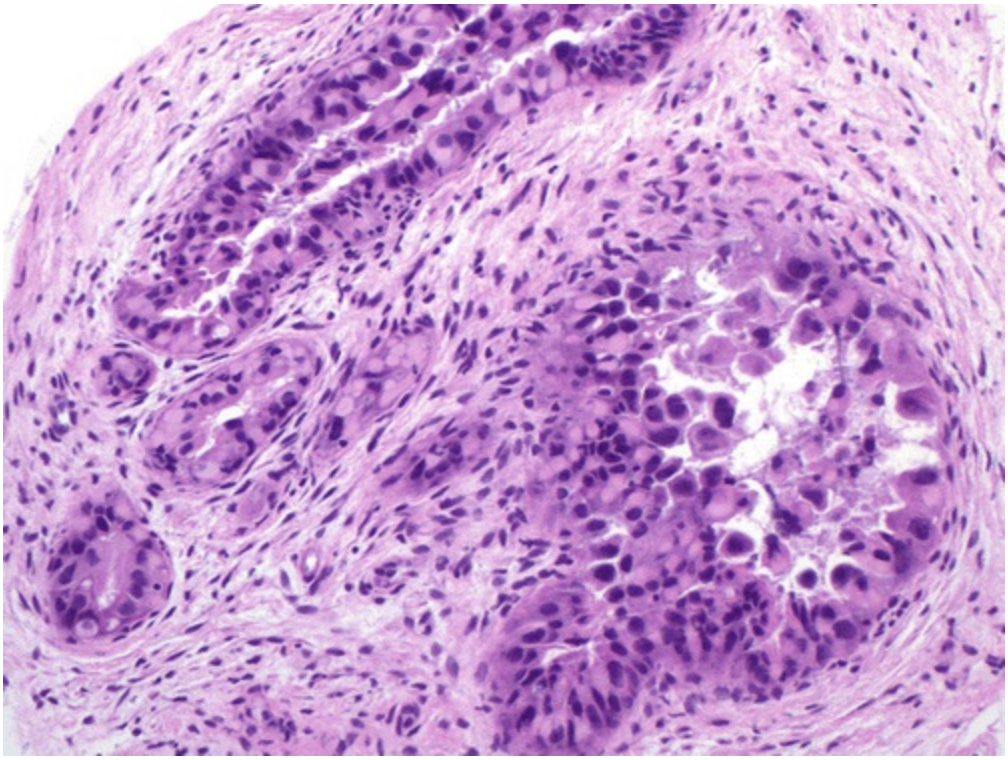

Fig. 8.23 Oxyphilic metaplasia shows cells within a metaplastic endocervical epithelium with voluminous amounts of eosinophilic granular cytoplasm. Nuclear atypia, relating to degenerative change, can be associated which can give a false impression of a neoplastic process (hematoxylin and eosin stain, high magnification)

Endometriosis

Similar to tubal and tuboendometrioid metaplasia discussed above, endometriosis consists of ectopic endometrial-type glandular epithelium with the addition of endometrial stroma (Fig. 8.24). Endometriosis can occur either superficially involving the endocervical mucosa and glands or more deeply in the cervical wall. The superficial form may result following trauma (e.g., cone biopsy) or as a result of

implantation due to menstruation [55]. Deep endometriosis often occurs as a part of more widespread pelvic endometriosis. Most often endometriosis resembles proliferative phase endometrium. Correct recognition is not difficult if the stroma and glands are in the usual proportion, but if endometrial glands predominate, the diagnosis of adenocarcinoma may be entertained due to the less abundant cytoplasm, nuclear stratification, and scattered mitotic figures that are a normal part of the proliferative cycle. A diagnostic clue in favor of nonneoplastic endometriosis is the presence of small arterioles hugging the glands within the scant stroma. Immunohistochemical stains may also help; endometrial stromal cells are positive for CD10 and negative for CD34, while the reverse is the case for endocervical stromal cells. In addition, endometriotic glands are positive for Bcl2, while endocervical glands are negative [56]. Occasionally only endometrial stroma will be identified and should not be misinterpreted as a far less common sarcoma [57].

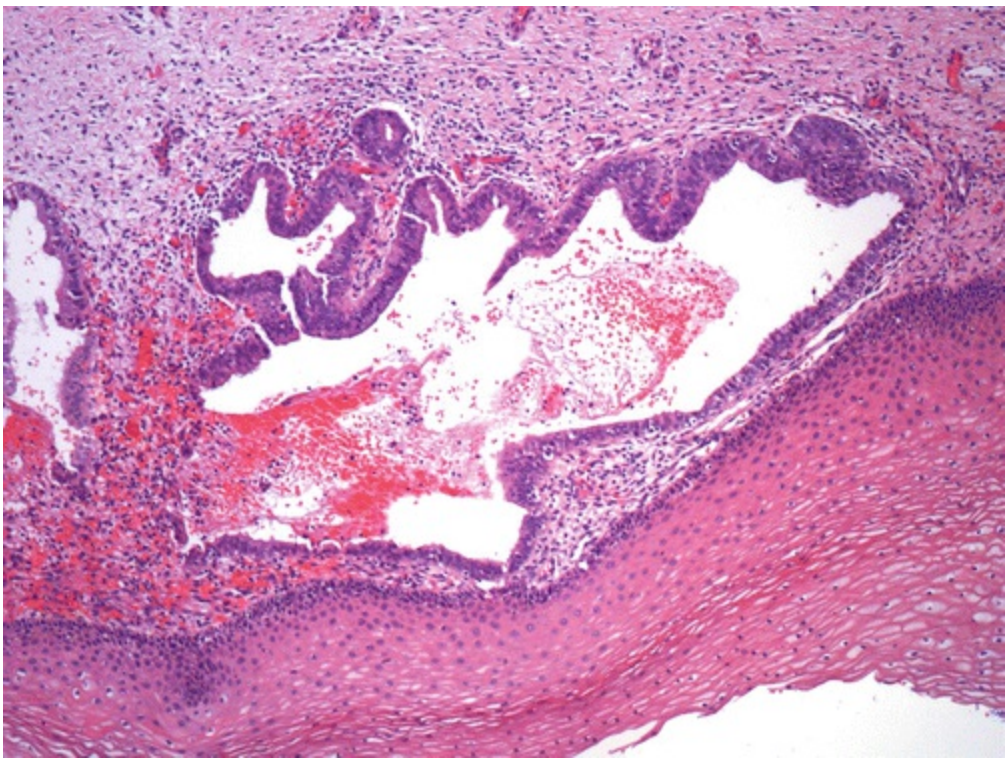

Fig. 8.24 Endometriosis involving the cervix consists of endometrial-type glands and stroma, often with hemorrhage. The pseudostratified architecture of endometrial epithelium, along with mitoses, and occasional apoptotic debris makes it a good mimic of endocervical neoplasia (hematoxylin and eosin stain, medium magnification)

Endocervicosis

Benign-appearing endocervical glands may rarely be located deep in the cervical wall (Fig. 8.25). This condition most commonly gives rise to a diagnostic consideration of well-differentiated mucinous adenocarcinoma because of the normal apical mucus. If the deep endocervical glands show any features of tubal or tuboendometrioid

metaplasia, a consideration of the usual type of endocervical adenocarcinoma may also be considered, primarily due to the inherent pseudostratification with hyperchromasia [58]. The absence of malignant nuclear features and an in situ component at the mucosal surface provides solid evidence against malignancy.

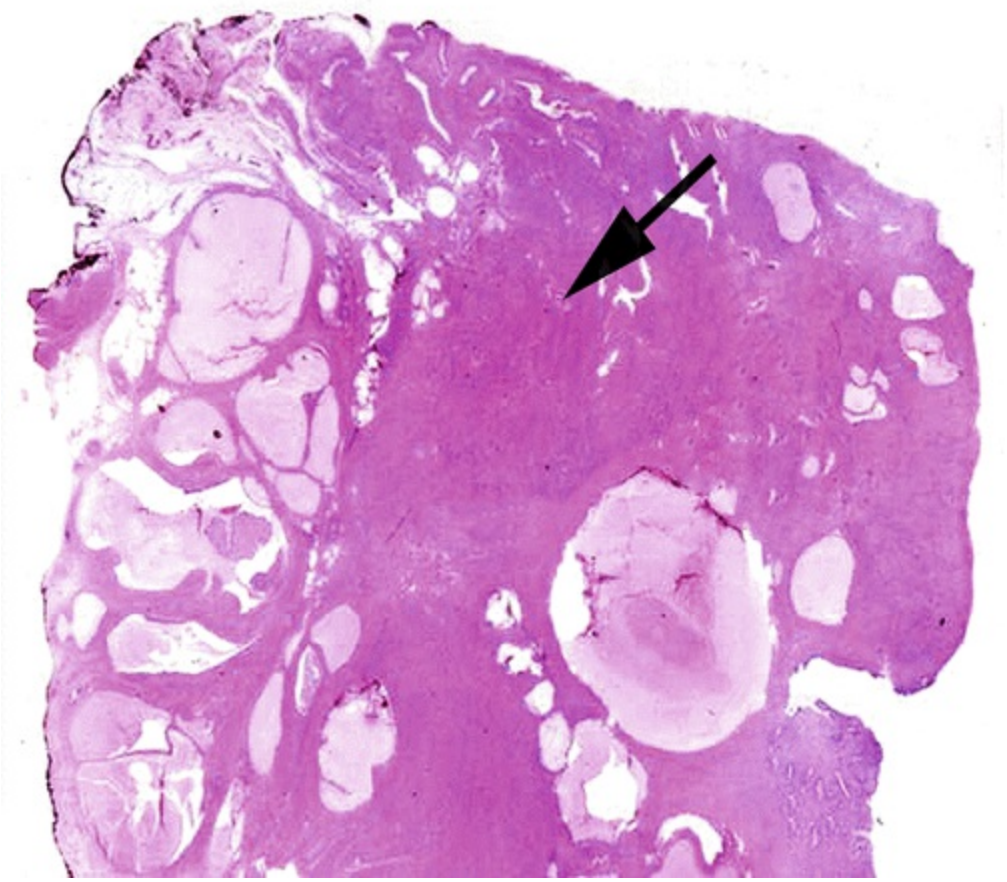

Fig. 8.25 Endocervicosis on histology; note the typical band of uninvolved cervical stroma (*arrow*) separating the endocervical mucosa from the deep band of benign endocervical glands (hematoxylin and eosin stain, low magnification)

Endosalpingiosis

Rarely endosalpingiosis, an ectopic proliferation of glands lined by tubal-type epithelium, will involve the wall of the cervix causing gross thickening and even a mass-like lesion [59]. As with endocervicosis, the differential diagnosis is with adenocarcinoma; however, a connection to a mucosal component as well as marked nuclear atypia is absent. The presence of ciliated, goblet, and peg cells should allow for a correct interpretation.

Endocervical Gland Hyperplasias

Generally hyperplasias of the endocervical glands do not fall into the differential

diagnosis of the usual type of endocervical adenocarcinoma because these types of hyperplasia are typically not "mucin-poor" but show apical caps with abundant mucus. They therefore are better considered under mimics of mucinous carcinoma. They will be briefly mentioned here because they are common and because the abundance of glands that can be present can lead to a consideration of endocervical neoplasia.

Tunnel Clusters

Tunnel clusters occur as an incidental finding in the cervix of approximately 10% of adult women. The term "tunnel cluster" was coined by Fluhmann who subdivided the entity into two categories: type A is defined as a group of noncystic endocervical glands lined with columnar epithelium and type B is defined as a group of cystic glands lined with cuboidal or flattened epithelium (Fig. 8.26) [60]. The cystic form of tunnel clusters is often visible grossly [61]. Microscopically both types of tunnel cluster are typically discrete, rounded foci composed of 20–50 closely packed tubules. The shape of the glands may be oval, round, or irregular and of varying size. Multiple foci are often identified, and occasionally several discrete foci of tunnel clusters may become confluent. The tubules, which usually contain mucin, are separated by scant connective tissue, while the entire tunnel cluster is surrounded by normal endocervical stroma. The low magnification lobulated arrangement of tunnel clusters is a helpful diagnostic feature not observed in malignancy. In addition, tunnel clusters also lack the infiltrative pattern and frequent stromal desmoplasia present in mucinous adenocarcinoma [62]. Like other benign disorders of endocervical glands, tunnel clusters may occasionally extend deeply into the cervical wall.

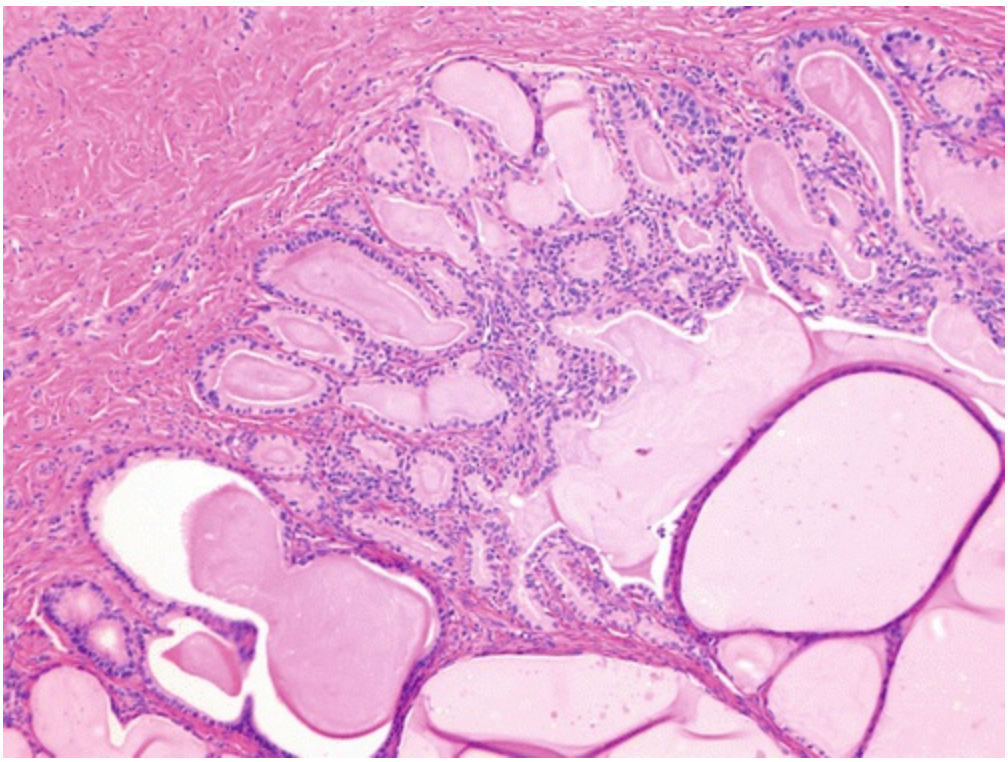

Fig. 8.26 Tunnel clusters are proliferations of benign-appearing endocervical epithelium. They are not usually mistaken for usual types of endocervical neoplasia, but their expansile growth pattern can sometimes be concerning. They are usually well circumscribed with a bland cytological appearance. Type A tunnel clusters show prominent cystic spaces, and type B show noncystic closely packed glands. The present single illustration shows both patterns in a single cluster (hematoxylin and eosin stain, medium magnification)

Microglandular Hyperplasia

Microglandular hyperplasia (MGH) was originally described as a benign lesion of the cervix resulting from oral contraceptive use [63, 64]. Although MGH is usually related to exogenous hormone administration or pregnancy in reproductive aged women, a minority of patients have no such history or are postmenopausal [65–67]. In most instances, MGH is an incidental microscopic finding, but occasionally it has the clinical appearance of an erosion, an ordinary cervical polyp, or a polypoid mass that can be friable and which may be clinically suspicious for carcinoma [68]. MGH consists of closely packed glands that vary from small and round to large, irregular, and cystically dilated (Fig. 8.27). The lumina usually contain a basophilic or eosinophilic mucinous secretion. There may be an extensive infiltrate of acute and chronic inflammatory cells in the mucin and intervening stroma. The stroma is occasionally extensively hyalinized mimicking tumor desmoplasia. The cells lining the glands and cysts are usually low columnar, cuboidal, or flat, with faintly basophilic or granular cytoplasm, and may have a hobnail appearance. Subnuclear vacuoles, which stain positively for mucin, are almost always present and may be conspicuous (Fig. 8.28). This feature is often the most important morphological clue to the correct interpretation. Rarely some cells form

solid foci and have pale mucinous cytoplasm and eccentric nuclei, simulating the signet ring cells of an adenocarcinoma. While the usual type of endocervical adenocarcinoma may enter into the differential diagnosis, in rare cases showing prominent glandular or solid features, clear cell adenocarcinoma is the neoplasm most likely to be confused with MGH. Like MGH, clear cell carcinoma may have tubular, cystic, and solid patterns, associated with a hyalinized stroma. The solid foci of clear cell carcinoma usually consist of cells with abundant, clear, glycogen-rich cytoplasm, whereas the constituent cells in solid foci of MGH never have conspicuous clear cytoplasm. In addition, the degree of nuclear atypicality and mitotic activity in clear cell carcinoma greatly exceeds that of MGH. In distinction to cervical neoplasia, MGH is CEA negative, and hence this immunohistochemical stain may be useful in difficult cases.

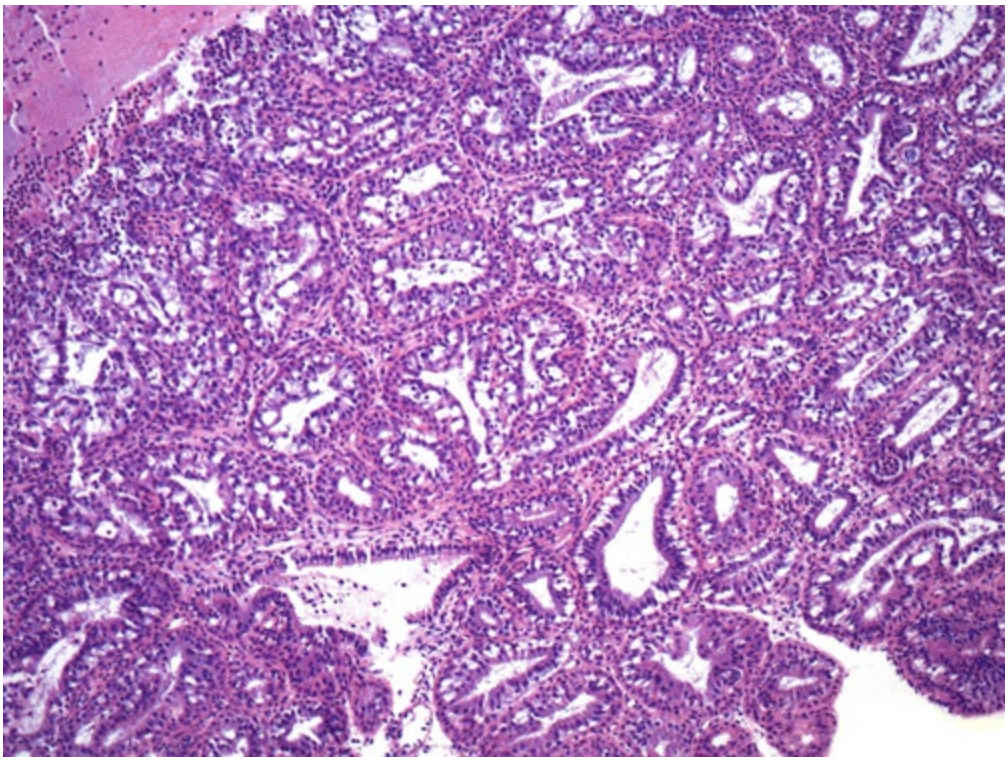

Fig. 8.27 Microglandular hyperplasia is a benign endocervical proliferation commonly associated with exogenous hormone use. It can be concerning for endocervical neoplasia at low magnification because it consists of closely packed small- to medium-sized glands (hematoxylin and eosin stain, low magnification)

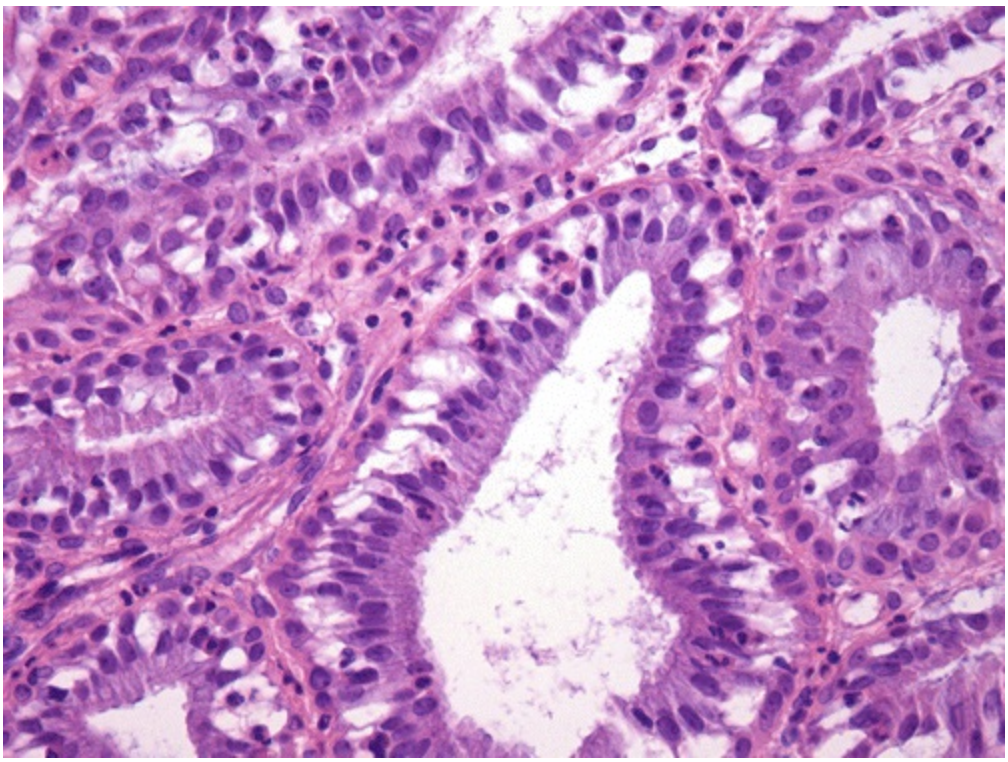

Fig. 8.28 The cells of microglandular hyperplasia have a characteristic pattern of diffuse subnuclear vacuolation below bland, non-enlarged nuclei (hematoxylin and eosin stain, high magnification)

Diffuse Laminar and Lobular Endocervical Glandular Hyperplasias

Both of these endocervical glandular hyperplasias are rare and are incidental findings with no definitive clinical symptoms. Diffuse laminar hyperplasia presents as a subsurface distribution of crowded benign-appearing endocervical glands, usually as a discrete layer which is sharply demarcated from the underlying cervical stroma and confined to the inner third of the cervical wall (Fig. 8.29) [69]. A marked inflammatory response and focal stromal edema have been present in most of the cases. Mild reactive cytological atypia can be present. Like the diffuse form, lobular endocervical glandular hyperplasia (LEGH) is characterized by lobular aggregates of small- to medium-sized glands often with a large gland in the center of the lobule (Fig. 8.30) [70]. The tall columnar glandular cells lack atypia or mitotic activity and may have the blue cytoplasm of endocervical cells or more eosinophilic cytoplasm similar to gastric pyloric cells. The eosinophilic cells have been reported to be positive by immunohistochemical staining for gastric epithelial markers. LEGH is generally thought to be a neoplastic precursor in the spectrum of endocervical lesions showing gastric differentiation, including gastric-type mucinous adenocarcinoma of the cervix [40]. Therefore, the differential diagnosis of LEGH is most commonly with mucinous adenocarcinoma. Differentiating between benign and malignant endocervical proliferations of any type on

small cervical biopsies can be a challenge and may require the clinician to procure a larger sample for a definitive assessment.

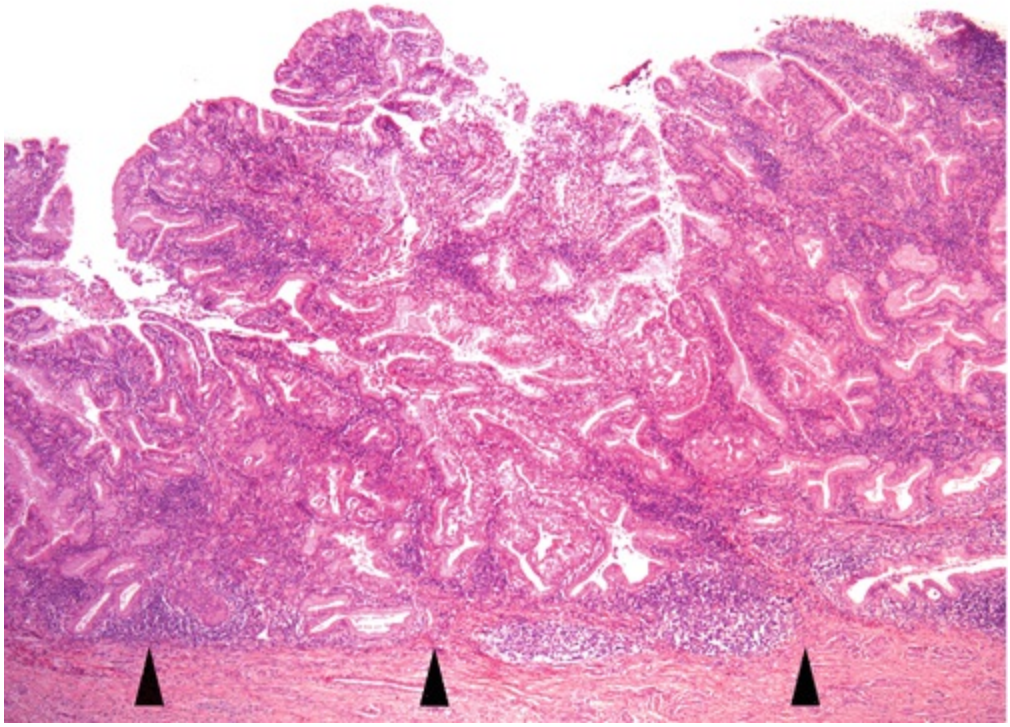

Fig. 8.29 Diffuse laminar hyperplasia consists of a densely packed aggregation of irregular endocervical glands, which on closer examination show bland nuclear features and mucin-rich caps of cytoplasm. A sharply demarcated deep edge is typical of this benign proliferation (*arrowheads*) (hematoxylin and eosin stain, low magnification)

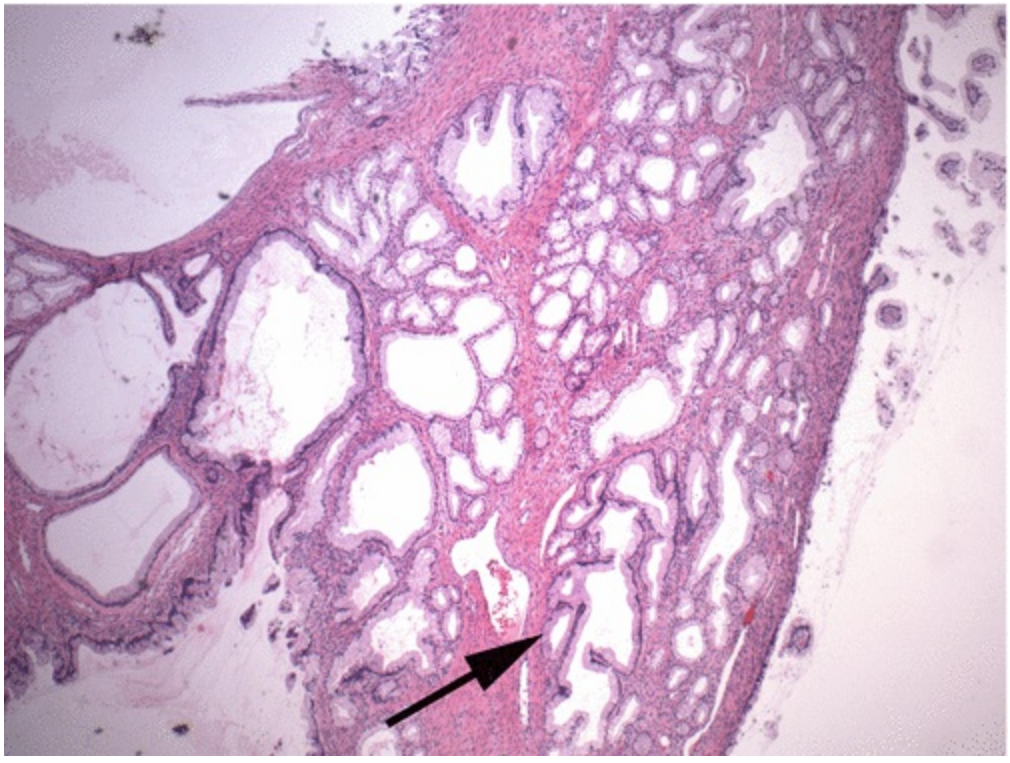

Fig. 8.30 Lobular endocervical hyperplasia is a proliferation of benign-appearing endocervical glands in a nested configuration with some lobules showing a central dilated gland (*arrow*). This proliferation is thought to be a precursor to mucinous adenocarcinoma (hematoxylin and eosin stain, low magnification)

Arias-Stella Reaction

In pregnancy, endocervical glandular cells can become hypervacuolated, hobnailed, or oxyphilic. The gland lumina may contain tufts of endocervical cells or filiform papillae. The nuclei are often are of variable size, enlarged, and intranuclear inclusions may be identified [71]. Nuclei often show degenerative changes. This constellation of changes is known as the Arias-Stella reaction and is similar to changes of the same name found in endometrial glands (Fig. 8.31). Patients with these findings are virtually always pregnant or on hormonal contraception. The small biopsies showing Arias-Stella changes can be of concern if the patient's age and history are not known, and a diagnosis of adenocarcinoma (particularly clear cell carcinoma) may be entertained. The clinical history, patient age, absence of a mass, and absence of the typical histological patterns of carcinoma support a benign interpretation.

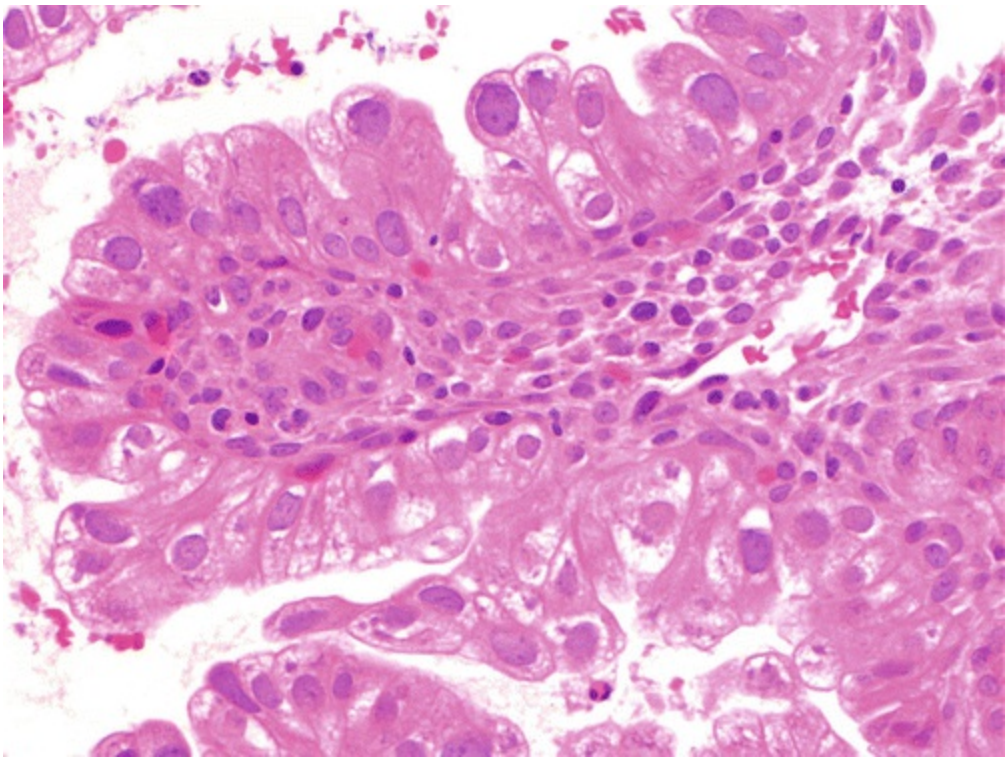

Fig. 8.31 Arias-Stella change is a benign endocervical proliferation seen in pregnancy and with hormone treatment. It consists of tufted epithelium with prominent nuclear atypia thought to be secondary to degenerative change. Although rare in the cervix, it is analogous to its more common endometrial counterpart (hematoxylin and eosin stain, high magnification)

Equivocal Lesions

Lesions which are abnormal, but which do not meet histopathological or cytopathological criteria for AIS, have in the past been classified as endocervical "dysplasia." In the British system of nomenclature, the term "low-grade glandular intraepithelial lesion (LG-CGIN)" has been used for histological lesions which are abnormal but which do not meet the criteria for AIS or invasive carcinoma. For both endocervical "dysplasia" and LG-CGIN, the interobserver reproducibility is poor and true endocervical neoplasia on follow-up is not common [35, 72–76]. In histological specimens, equivocal cases may have some of the features of neoplasia, including gland proliferation and crowding, some degrees of pseudostratification, mild nuclear atypia, and evidence of proliferation. As noted above in the section on histological mimics of endocervical neoplasia, these features can be seen in some benign proliferations. In the assessment of these lesions, p16 immunohistochemistry can be very useful as HPV-associated endocervical AIS and invasive carcinoma will be diffusely positive [77, 78]. Equivocal histological lesions which are diffusely positive for p16 are most consistent with a true neoplastic lesion, and current convention indicates that such lesions should be classified as AIS (HG-CGIN). The caveat for the use of this test (as well as HPV testing in general) is that mucinous adenocarcinoma, which may also be in the differential diagnosis of these benign mimics, is not HPV associated and will not always show diffuse p16 reactivity. In the Bethesda system for cytology nomenclature, abnormal presentations of endocervical glandular cells less than AIS have been designated as simply "atypical endocervical (or glandular) cells" to indicate the equivocal nature of such presentations [38]. As will be discussed below, a significant percentage of cases designated as such will show nonneoplastic results on follow-up procedures. Again, preliminary results of testing with p16/Ki67 immunocytochemical combinations have shown significant discriminatory power between benign mimics and true neoplastic lesions [79].

Cytological Mimics of Endocervical Neoplasia (Atypical Glandular Cells in Cytological Preparations) [38, 39]

"Atypical glandular cells (AGC)" is the preferred broad terminology in cytological specimens to denote uncertain or equivocal appearances which are abnormal but which lack sufficient cytological features to allow for a definitive interpretation. AGC can be subclassified into "atypical endocervical cells (AEC)" where features are most consistent with cells of an endocervical origin and yet further subdivided into "not otherwise specified" or "favor neoplasia" based on the confidence of the observer that a neoplastic lesion may be present. "Atypical endometrial cells" is used when an endometrial origin is most likely. The more generic AGC should be used when the origin of the cells is uncertain. The definition of AEC is "endocervical-type cells that

display nuclear (or architectural) atypia that exceeds obvious reactive or reparative changes but lack unequivocal features of endocervical AIS or invasive adenocarcinoma" [38]. AEC can include any combination of architectural abnormalities such as HCGs, pseudostratification, feathering, or rosette formations, or cellular features such as nuclear enlargement and/or irregularity, chromatin abnormalities, or mitotic activity (Fig. 8.32). All of these features have been described above as cytological appearances of AIS and invasive endocervical adenocarcinoma; however, the presence of some, but a lack of an overall adequate composite of features, may leave the observer unsure regarding a definitive diagnosis. The use of the AGC family of interpretations is entirely appropriate in this circumstance. Close monitoring of AGC rates in a laboratory practice is important. According to surveys from the College of American Pathologists, AGC interpretation rates should represent about 0.3% of all cervical cytology specimens. AGC rates which are at significantly higher levels should be scrutinized to avoid overuse of this category, which routinely leads to significant clinical intervention [34, 80].

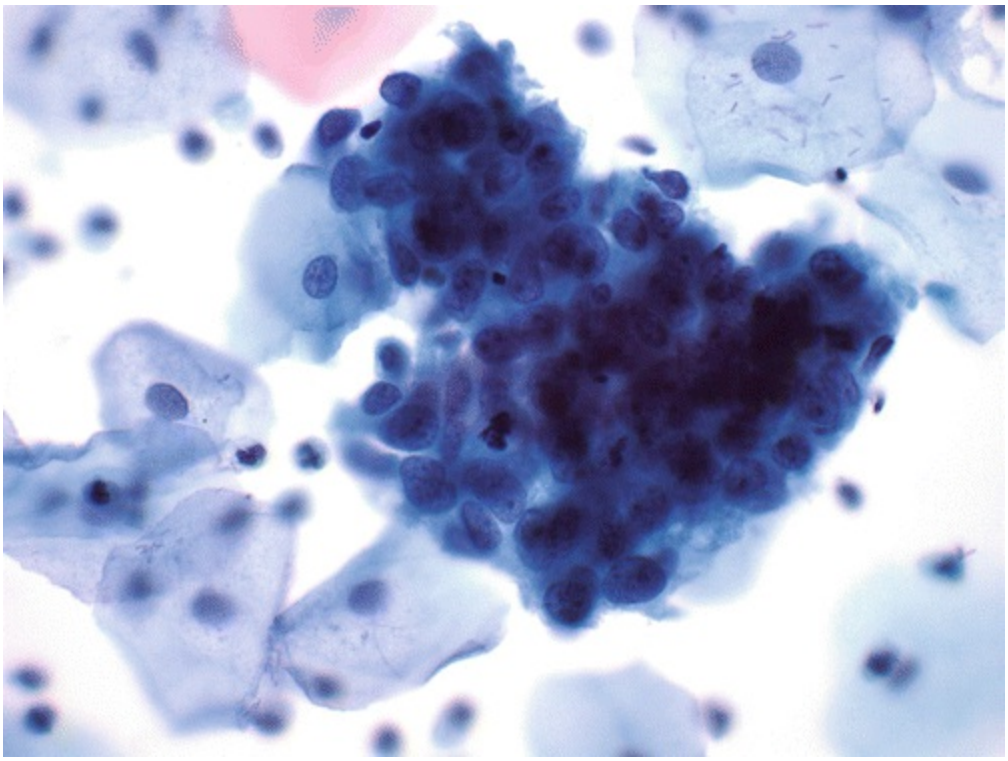

Fig. 8.32 "Atypical endocervical cells" is a designation used in cytological specimens when cells or cell groups are abnormal but not definitive for neoplasia. In this example, a hyperchromatic crowded group of cells shows some features suggesting an endocervical origin (nuclear polarity, nucleoli) but falls short of a definitive interpretation of neoplasia. In such cases, patients should be thoroughly evaluated. About 30–40% of such cases do not show neoplasia on follow-up examinations and may be secondary to tubal metaplasia, benign endocervical hyperplasias, direct sampling of endometrium, or changes associated with irritated endocervical polyps (Papanicolaou stain, high magnification)

There are several important specific benign conditions that lead to interpretations of

AGC, because they can present with some of the features of endocervical glandular neoplasia. Each has, however, specific features that, if recognized, can allow a proper benign interpretation. It is well recognized that these benign entities can make up a significant proportion of the follow-up histological outcomes in cases of AGC, in some series up to 30–40%. Overall, a good approximation is that only about 10–20% of specimens interpreted as AGC will show a true endocervical neoplastic outcome [76, 81–84].

Tubal Metaplasia

Tubal metaplasia (TM) is a benign reactive condition that commonly involves the endometrium and the upper regions of the endocervical canal, particularly as women age [50, 85]. TM replaces the normal cervical simple mucinous epithelium with a mucin-poor, pseudostratified epithelium which recapitulates the fallopian tube lining. In one study, TM was found in 100% of high endocervical samples in women over the age of 30 years [51]. When newer cytological sampling devices, which collect cells from the upper regions of the canal, were first introduced in the 1980s and 1990s, a significant increase in interpretations of AGC was initially noted, though due to the increased sampling of TM [86, 87]. Fortunately, recognition of this issue promptly ensued with a decline in AGC prevalence to a new baseline. The samples containing TM show hyperchromatic crowded groups, often with some degree of feathering, and pseudostratified strips of cells. Nuclei are oval to fusiform and can be enlarged above that of normal endocervical cell nuclei. Mitotic figures can rarely be noted with TM. Features that indicate a benign origin include the presence of multiple cell types within the groups, including ciliated cells and goblet cells, nuclei showing smooth nuclear contours, and evenly distributed, finely granular chromatin (Fig. 8.33). Often, this chromatin pattern appears “washed-out” similar to the classic appearance of papillary carcinoma of the thyroid. This appearance is in sharp distinction to the densely granular chromatin of AIS (evenly distributed) and invasive carcinoma (heterogeneously distributed). TM also lacks the apoptotic debris commonly found in neoplasia. The presence of cilia is indicative of a benign process with a very high degree of certainty. However, cytologists should be very aware that TM is a very common finding and therefore can exist in association with endocervical neoplasia. Hence, a finding of cilia in one group should not mitigate against an abnormal interpretation for other abnormal groups found on a slide. Ciliated endocervical neoplasias have been reported, but are extremely rare, and most likely represent lesions of serous origin.

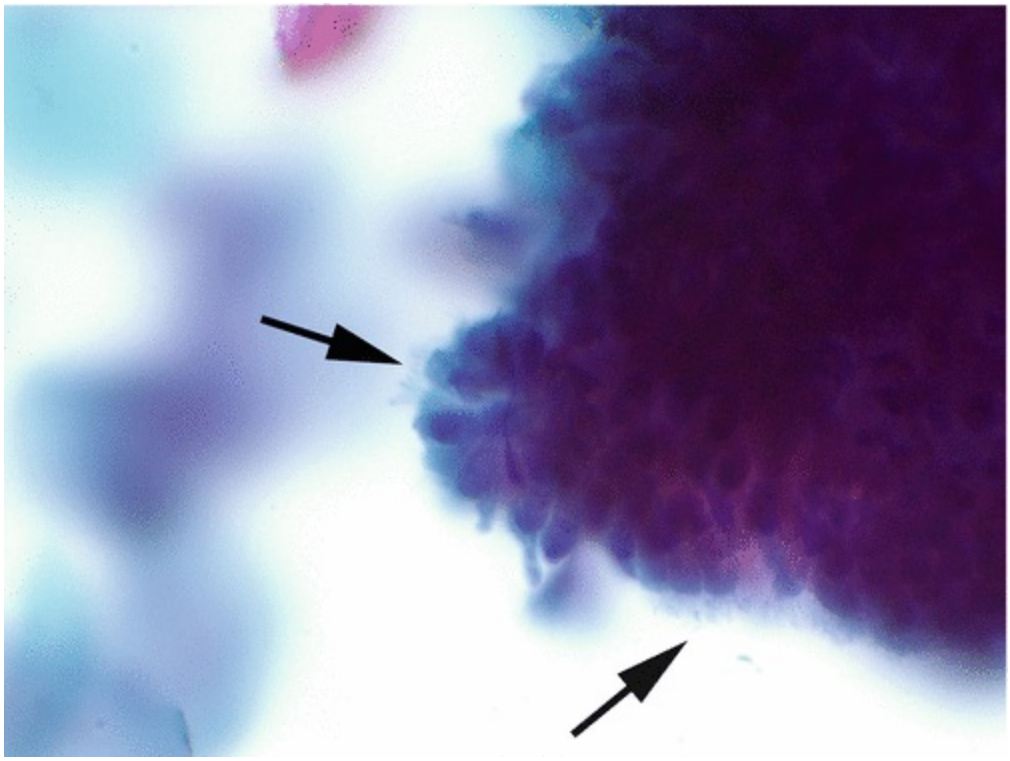

Fig. 8.33 Just as in histologic preparations (see Figs. 8.20 and 8.22), tubal metaplasia presents in cytologic specimens as pseudostratified strips of columnar cells that can mimic the architecture of AIS. The presence of cilia (*arrows*) and the finely granular nuclear chromatin pattern should allow for differentiation (Papanicolaou stain, high magnification)

Directly Sampled Endometrium (Abraded Endometrium)

Direct sampling of the endometrium takes place when the cytological sample is taken either from the uterine corpus or from an area of cervical endometriosis. In the former circumstance, the patient is likely to have had a prior cervical excision, which can lead to a shortening of the canal [88–91]. The native endometrial epithelium is pseudostratified and therefore can present as pseudostratified strips of cells that mimic AIS. The classic cytological appearance of endometrium is in the exfoliated form, in which endometrial cells are shed from the surface of the uterine corpus and round up into three-dimensional structures as they travel in the mucus of the endocervical canal. Directly sampled endometrium is abraded from the surface and therefore retains the typical two-dimensional sheeting of a columnar epithelium and therefore mimics the appearance of directly sampled endocervical epithelium. There are important differences to appreciate when considering the differential diagnosis. Endometrial epithelial cells are much smaller than endocervical cells, having nuclei which are consistently rounder and smaller than their counterparts in the endocervix. In addition, abraded endometrium will retain organoid structures characteristic of the endometrium, including long well-defined tubules with central lumens (Fig. 8.34). In addition, samples containing abraded endometrium will also contain endometrial stromal cells, present as pure groups showing characteristic mesenchymal spindled forms, with

delicate cytoplasmic appendages, or present attached to the surface of the dense tubular epithelial structures (Fig. 8.35). Spindled stromal cells attached to the surface of the groups may give a low magnification appearance similar to feathering, but close attention to the cytoplasm should allow for a correct classification. If the endometrium is sampled during proliferative phase, mitotic activity can be brisk in the tubular structures, and, in addition, endometrial nuclei can sometimes have degenerative changes leading to coarse chromatin granularity or even apoptotic debris (e.g., as in disordered proliferative endometrium). Such changes may cause concern for an endocervical neoplasia and should be assessed in the context of the other features of abraded endometrium as noted above.

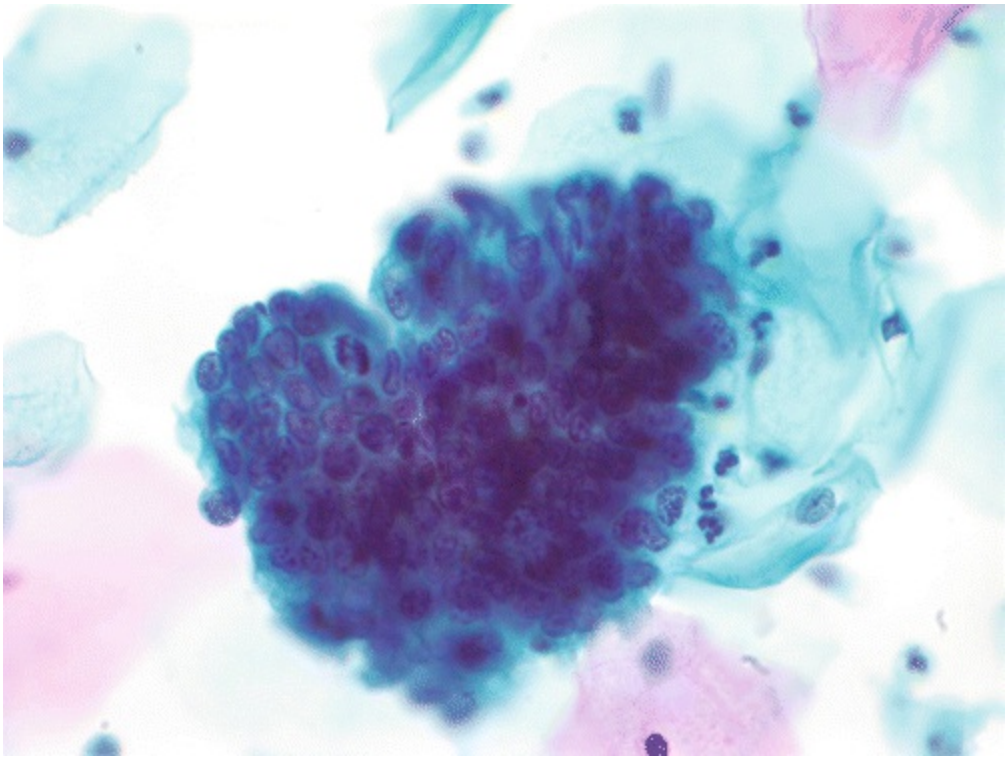

Fig. 8.34 Direct sampling of endometrium yields three-dimensional structures which represent intact endometrial glands. The tight-packing, well-delineated margins and the presence of a well-defined central "tube" are key features to recognize. In comparison to endocervical neoplasia, the nuclei are much smaller and more uniform (Papanicolaou stain, high magnification)

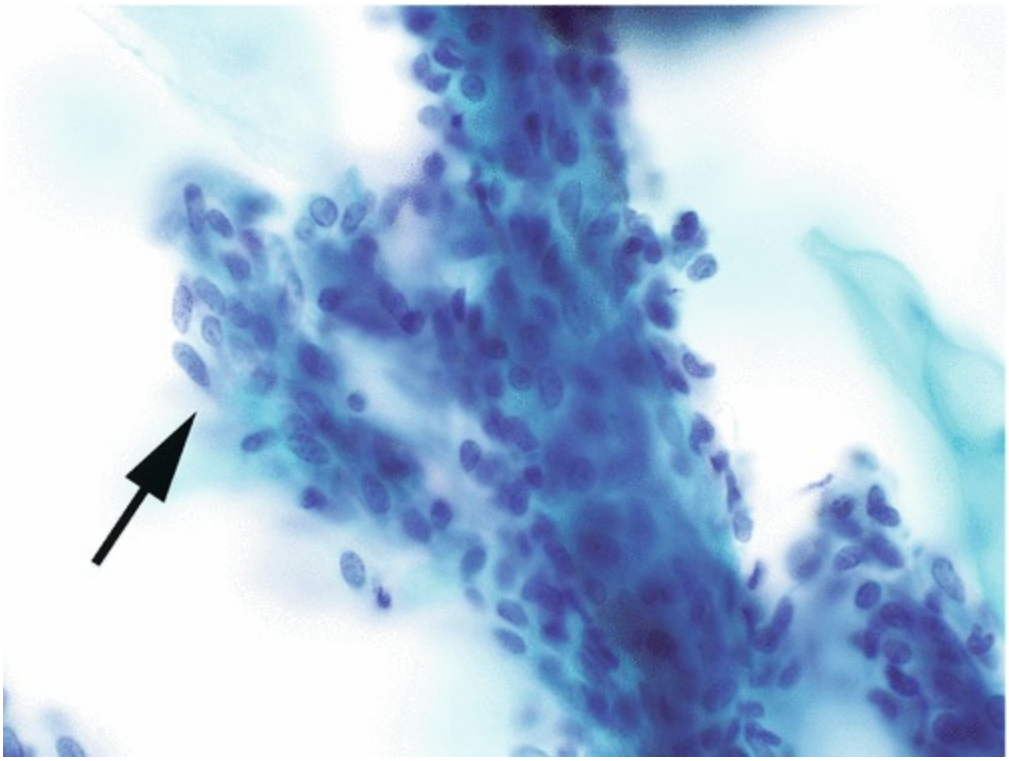

Fig. 8.35 When endometrium is abraded from the lower uterine segment, endometrial stromal groups can be prominent in cytological preparations. The margins of these groups can show protrusion of mesenchymal tapered stromal cell cytoplasm mimicking the "feathering" of AIS (*arrow*) (Papanicolaou stain, high magnification)

Reactive Changes Associated with Intrauterine Device

The presence of an intrauterine device (IUD) can cause significant irritation of the high endocervical canal/lower uterine segment surface epithelium leading to cytological features that can mimic a neoplasm [92, 93]. Most commonly, the so-called IUD change mimics endometrial lesions because the abnormal cells present as three-dimensional structures similar to an exfoliated endometrial carcinoma. The cells present in the groups show enlarged nuclei with chromatin density and coarse granularity secondary to degenerative change. In addition, prominent cytoplasmic vacuoles are present, referred to as "bubble gum vacuoles" due to their large size and protrusion from the group margins (Fig. 8.36). In approximately 25% of cases, the background of the slide will show *Actinomyces* organisms and their presence should alert the cytologist to be aware that an IUD may be present, even in the absence of a history [94]. In distinction from classic endocervical neoplasia, IUD changes do not include pseudostratified strips of cells, feathered group edges, or rosette type structures. IUD changes can also show reactive rounded up endocervical cells with very large nuclei having a high nucleus to cytoplasm ratio closely mimicking HSIL, which can be found in association with many cases of AIS (Fig. 8.37). The presence of a prominent nucleolus and degenerative chromatin are clues to the benign nature of this finding.

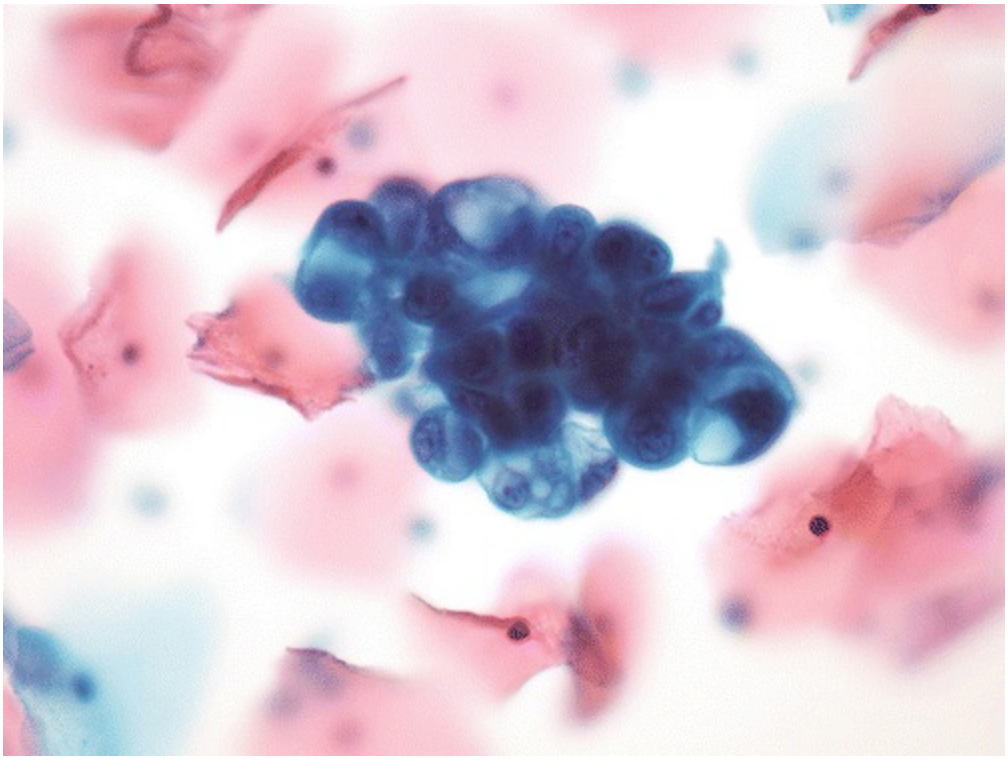

Fig. 8.36 Irritation of the high endocervical canal by an intrauterine device can lead to the exfoliation of reactive endocervical cells with prominent degenerative cytoplasmic vacuoles (*arrow*). These changes can be concerning for glandular neoplasia, although they more closely mimic an endometrial as opposed to an endocervical lesion (Papanicolaou stain, high magnification)

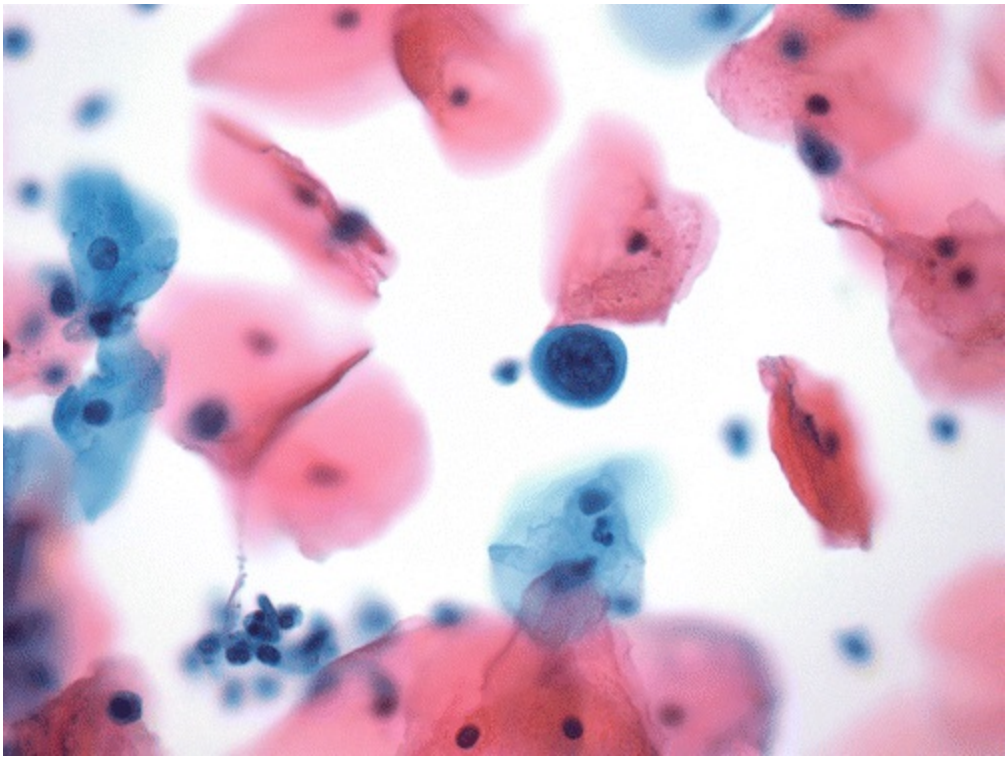

Fig. 8.37 Isolated cells with rounded cytoplasm and high nucleus to cytoplasm ratio can be present in the cervical cytology specimens from patients with an intrauterine device. These cells closely mimic high-grade squamous

intraepithelial lesions. The presence of prominent nucleoli and a degenerative chromatin pattern (sometimes with "cracks" in the nuclear material) are key features of this benign process (Papanicolaou stain, high magnification)

Reactive Changes Associated with Endocervical Polyps

Endocervical polyps are exceedingly common benign growths of endocervical epithelium covering stromal tissue. They typically form mass lesions that are identifiable at the time of colposcopy and can present with symptoms of bleeding, altogether leading to a clinical concern for the presence of a malignant process. The mass lesion and bleeding are the consequence of a reactive proliferation caused by trauma, which can cause erosion of the surface epithelium with the exposure of underlying stromal vessels. Hence, the cytologist may already be primed by the history with a concern for neoplasia when they initially examine the slide. Fortunately reactive/reparative endocervical epithelium shows a characteristic pattern that is generally easy to identify as a benign process. Reparative changes present as two-dimensional sheets of cells that are tightly cohesive. Few of any isolated cells of similar appearance are found in the background. The cells show abundant dense cytoplasm which has prominent cell boundaries and appendages which extend from the edges of the group ("taffy-pull cytoplasm"). The groups maintain polarity with cells arranged in a streaming pattern ("school of fish" appearance). The nuclei are enlarged with prominent macronucleoli indicative of their increased metabolic state. The chromatin structure remains finely granular and evenly distributed within the nucleus. Inflammatory cells (generally neutrophils) are present within the cell groupings (Fig. 8.38). If all the features noted above are present in a case, a benign interpretation is reliable and warranted. However, in severely traumatized reparative reactions, nuclei within the groups can have significant pleomorphism of size and shape with associated degenerative heterogeneity of the chromatin. In this circumstance, an interpretation of AGC ("atypical repair") may be warranted as in such cases the differential diagnosis can include invasive adenocarcinoma [95, 96]. Cases where it is virtually impossible to discern between atypical repair and cancer are well known to any experienced cytologist, and it is always best to refer the patient for additional work-up in such circumstances.

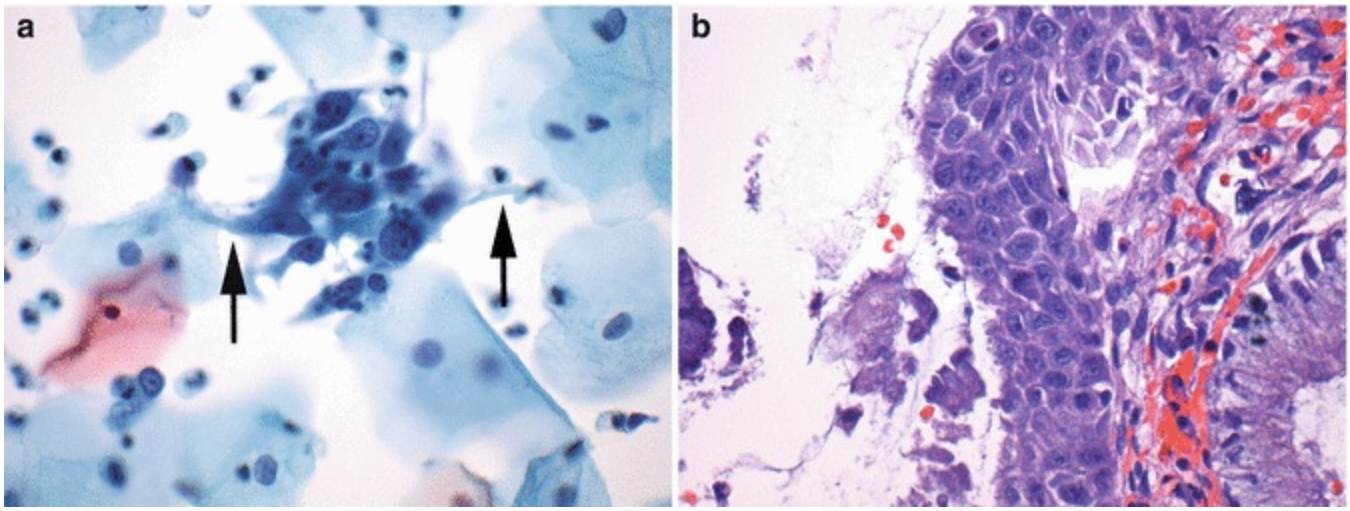

Fig. 8.38 The endocervical epithelium on the surface of irritated polyps can show marked atypia secondary to epithelial repair. In cytological specimens, (**a**) reparative reactions show cell groupings with two-dimensional architecture, prominent intracytoplasmic boundaries, abundant dense cytoplasm with tapered appendages ("taffy-pull" cytoplasm – *arrows*) (Papanicolaou stain, high magnification). Nuclei show uniform prominent nucleoli, and neutrophils commonly infiltrate the groups. Histological specimens show identical types of cells on the surfaces of polyps (**b**) (hematoxylin and eosin stain, medium magnification)

High-Grade Squamous Intraepithelial Lesions Involving Endocervical Glands

Squamous neoplastic precursor lesions (HSIL) are commonly present in association with endocervical neoplasia, particularly in the case of AIS. Up to 50% of patients with AIS have been shown to also harbor HSIL [23]. As endocervical neoplasia is relatively rare compared to its squamous counterpart, the opposite is not true – HSILs only rarely show accompanying AIS. Interestingly, HSIL can also mimic the appearance of glandular neoplasia, particularly when it involves the endocervical glands of the mid- to upper regions of the canal [97, 98] (Fig. 8.39). HCGs of HSIL can be collected from solid areas within the gland necks, and, in such circumstances, the groups can take on a different appearance than the classic syncytial groups of HSIL (described elsewhere – see Chap. 6), which lack architectural polarity and nucleoli. HSIL involving glands typically maintains group polarity in a manner that can give the appearance of a columnar configuration. In addition, nuclei maintain their coarse chromatin but show prominent nucleoli, more reminiscent of the classic nuclear features of neoplastic glandular cells (Fig. 8.40). Clues to the squamous nature of the process include lack of a luminal surface to the pseudocolumnar groups, a dense "glassy" cytoplasmic texture (as opposed to the granular/frothy texture of endocervical cells), and, most importantly, the presence of classic isolated HSIL cells in the slide background. This look-alike is well documented as many follow-up studies have noted that HSIL remains the most common neoplastic outcome of an original interpretation of AGC [81, 82]. A recent study

suggests that the use of PAX8 and PAX 2 immunostaining may be helpful in the discrimination of benign glandular, AIS, and squamous intraepithelial lesions. PAX2 reactivity is lost in a majority of neoplasias compared to its presence in nearly all benign proliferations; and PAX8 negativity favors a squamous neoplasia [99].

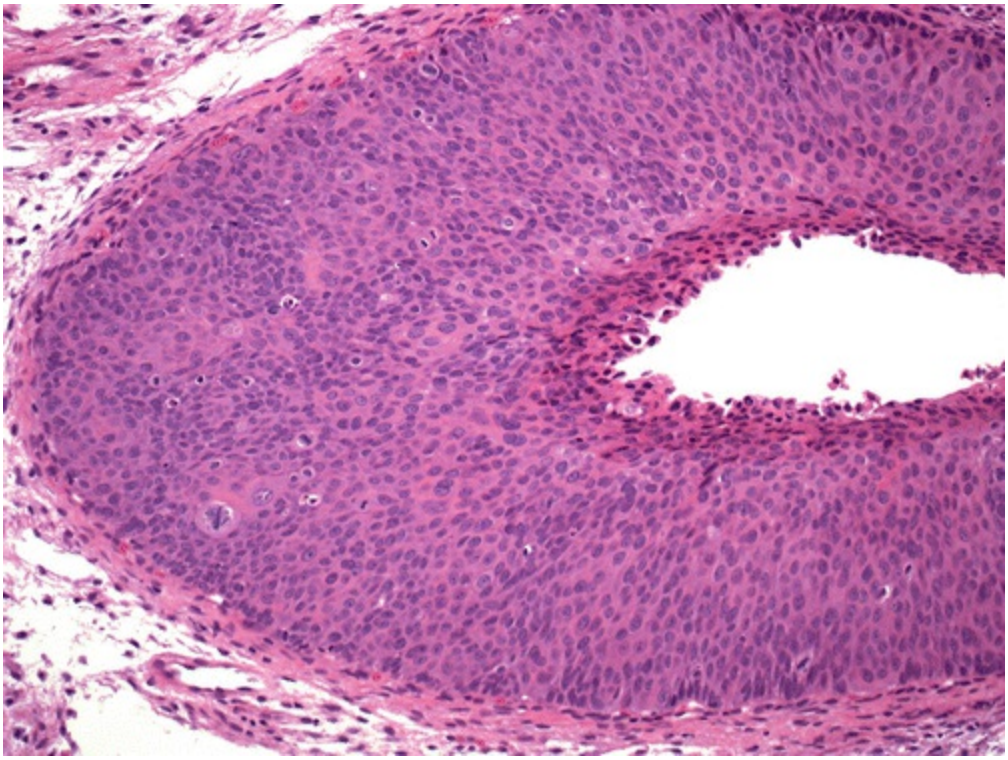

Fig. 8.39 High-grade squamous intraepithelial lesions often replace the endocervical epithelium within gland necks and crypts. In such cases, the cytological appearance of the cells can be very "gland-like" (hematoxylin and eosin stain, medium magnification)

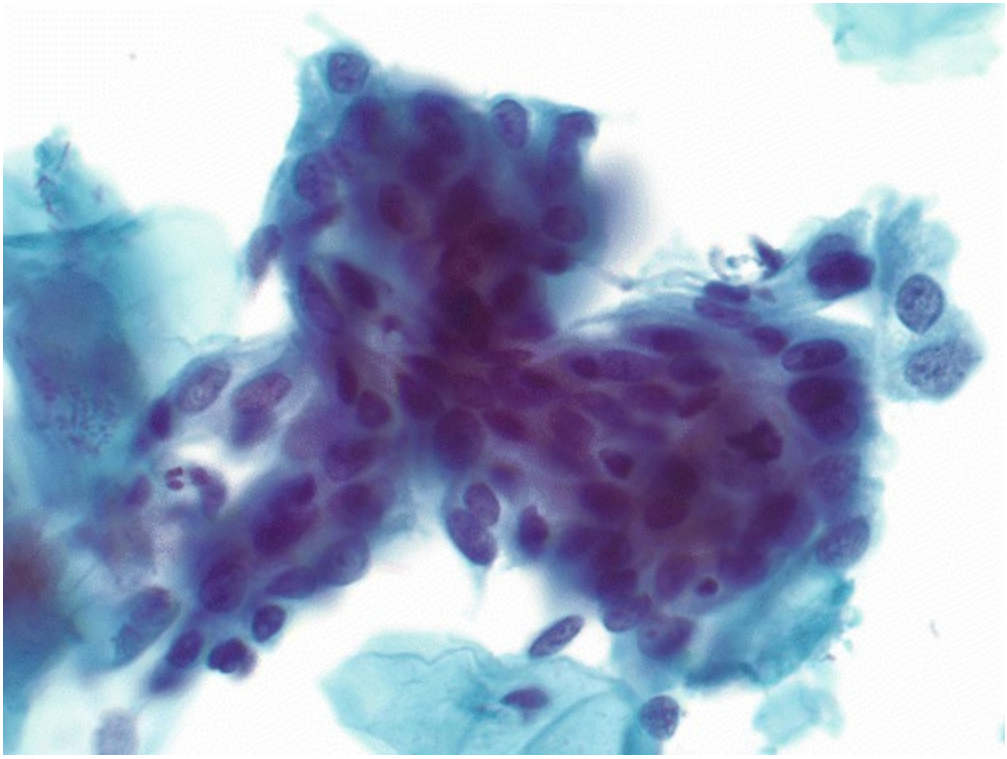

Fig. 8.40 High-grade squamous intraepithelial lesions (HSILs) present within endocervical glands present commonly in cytological specimens as "atypical endocervical cells." Hyperchromatic crowded groups with retained polarity and columnar-like tapering are commonly seen. Nucleoli can be present in distinction to their absence in the majority of surface HSILs (Papanicolaou stain, high magnification)

Conclusion Endocervical neoplasia is still a rare disease, making up a minority of cervical cancers when compared to its squamous counterparts. However, as opposed to squamous carcinomas, whose overall incidence has dramatically decreased in the last several decades, consistent with the effects of organized screening programs, endocervical adenocarcinoma has shown a small increase in overall incidence. AIS has shown an even more pronounced increase in incidence, and this is undoubtedly due to a combination of absolute increase of disease, improved sampling, and better recognition of the cytological and histological features leading to more sensitive detection. Combinations of cytology and HPV testing (co-testing), or primary HPV testing alone (see Chap. 2), both share the promise of continued improvements in detection and, with improved detection, earlier identification and improved outcomes.

References

1. Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Parkin DM. International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. Int J Cancer. 1998;75(4):536–45.

[Crossref][PubMed]

2. Bulk S, Visser O, Rozendaal L, Verheijen RH, Meijer CJ. Cervical cancer in the Netherlands 1989–1998: decrease of squamous cell carcinoma in older women, increase of adenocarcinoma in younger women. Int J Cancer. 2005;113(6):1005–9.
[Crossref][PubMed]

3. Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States – a 24-year population-based study. Gynecol Oncol. 2000;78(2):97–105.
[Crossref][PubMed]

4. Wang SS, Sherman ME, Hildesheim A, Lacey Jr JV, Devesa S. Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976–2000. Cancer. 2004;100(5):1035–44.
[Crossref][PubMed]

5. Ward KK, Shah NR, Saenz CC, McHale MT, Alvarez EA, Plaxe SC. Changing demographics of cervical cancer in the United States (1973–2008). Gynecol Oncol. 2012;126(3):330–3.
[Crossref][PubMed]

6. Bray F, Carstensen B, Moller H, Zappa M, Zakelj MP, Lawrence G, et al. Incidence trends of adenocarcinoma of the cervix in 13 European countries. Cancer Epidemiol Biomarkers Prev. 2005;14(9):2191–9.
[Crossref][PubMed]

7. Pirog EC, Kleter B, Olgac S, Bobkiewicz P, Lindeman J, Quint WG, et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. Am J Pathol. 2000;157(4):1055–62.
[Crossref][PubMed][PubMedCentral]

8. Castellsague X, Diaz M, de Sanjose S, Munoz N, Herrero R, Franceschi S, et al. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. J Natl Cancer Inst. 2006;98(5):303–15.
[Crossref][PubMed]

9. Lacey Jr JV, Brinton LA, Abbas FM, Barnes WA, Gravitt PE, Greenberg MD, et al. Oral contraceptives as risk factors for cervical adenocarcinomas and squamous cell carcinomas. Cancer Epidemiol Biomarkers Prev. 1999;8(12):1079–85.
[PubMed]

10. Lacey Jr JV, Brinton LA, Barnes WA, Gravitt PE, Greenberg MD, Hadjimichael OC, et al. Use of hormone replacement therapy and adenocarcinomas and squamous cell carcinomas of the uterine cervix. Gynecol Oncol. 2000;77(1):149–54.
[Crossref][PubMed]

11. Appleby P, Beral V, Berrington de Gonzalez A, Colin D, Franceschi S, Goodill A, et al. Carcinoma of the cervix and tobacco smoking: collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. Int J Cancer. 2006;118(6):1481–95.
[Crossref][PubMed]

12. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093–101.
[Crossref][PubMed]

13. Lacey Jr JV, Frisch M, Brinton LA, Abbas FM, Barnes WA, Gravitt PE, et al. Associations between smoking and adenocarcinomas and squamous cell carcinomas of the uterine cervix (United States). Cancer Causes Control. 2001;12(2):153–61.
[Crossref][PubMed]

14. An HJ, Kim KR, Kim IS, Kim DW, Park MH, Park IA, et al. Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. Mod Pathol. 2005;18(4):528–34.

15. Clifford G, Franceschi S. Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. Int J Cancer. 2008;122(7):1684–5.
[Crossref][PubMed]

16. Tornesello ML, Losito S, Benincasa G, Fulciniti F, Botti G, Greggi S, et al. Human papillomavirus (HPV) genotypes and HPV16 variants and risk of adenocarcinoma and squamous cell carcinoma of the cervix. Gynecol Oncol. 2011;121(1):32–42.
[Crossref][PubMed]

17. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128(4):927–35.
[Crossref][PubMed]

18. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048–56.
[Crossref][PubMed]

19. Kurman RJ, Carcangiu M-L, Herrington CS, Young RH. WHO classification of tumours of female reproductive organs. Lyon: IARC; 2014.

20. Friedell GH, Mc KD. Adenocarcinoma in situ of the endocervix. Cancer. 1953;6(5):887–97.
[Crossref][PubMed]

21. Krumins I, Young Q, Pacey F, Bousfield L, Mulhearn L. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri. Acta Cytol. 1977;21(2):320–9.
[PubMed]

22. Bousfield L, Pacey F, Young Q, Krumins I, Osborn R. Expanded cytologic criteria for the diagnosis of adenocarcinoma in situ of the cervix and related lesions. Acta Cytol. 1980;24(4):283–96.
[PubMed]

23. Ayer B, Pacey F, Greenberg M, Bousfield L. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions I. Adenocarcinoma in situ. Acta Cytol. 1987;31(4):397–411.
[PubMed]

24. Qizilbash AH. In-situ and microinvasive adenocarcinoma of the uterine cervix. A clinical, cytologic and histologic study of 14 cases. Am J Clin Pathol. 1975;64(2):155–70.
[Crossref][PubMed]

25. Boon ME, Baak JP, Kurver PJ, Overdiep SH, Verdonk GW. Adenocarcinoma in situ of the cervix: an underdiagnosed lesion. Cancer. 1981;48(3):768–73.
[Crossref][PubMed]

26.

Costales AB, Milbourne AM, Rhodes HE, Munsell MF, Wallbillich JJ, Brown J, et al. Risk of residual disease and invasive carcinoma in women treated for adenocarcinoma in situ of the cervix. Gynecol Oncol. 2013;129(3):513–6. [Crossref][PubMed]

27. Zielinski GD, Snijders PJ, Rozendaal L, Daalmeijer NF, Risse EK, Voorhorst FJ, et al. The presence of high-risk HPV combined with specific p53 and p16INK4a expression patterns points to high-risk HPV as the main causative agent for adenocarcinoma in situ and adenocarcinoma of the cervix. J Pathol. 2003;201(4):535–43. [Crossref][PubMed]

28. Quint KD, de Koning MN, Geraets DT, Quint WG, Pirog EC. Comprehensive analysis of Human Papillomavirus and Chlamydia trachomatis in in-situ and invasive cervical adenocarcinoma. Gynecol Oncol. 2009;114(3):390–4. [Crossref][PubMed]

29. Hurt WG, Silverberg SG, Frable WJ, Belgrad R, Crooks LD. Adenocarcinoma of the cervix: histopathologic and clinical features. Am J Obstet Gynecol. 1977;129(3):304–15. [Crossref][PubMed]

30. Shingleton HM, Gore H, Bradley DH, Soong SJ. Adenocarcinoma of the cervix. I. Clinical evaluation and pathologic features. Am J Obstet Gynecol. 1981;139(7):799–814. [Crossref][PubMed]

31. Saigo PE, Cain JM, Kim WS, Gaynor JJ, Johnson K, Lewis Jr JL. Prognostic factors in adenocarcinoma of the uterine cervix. Cancer. 1986;57(8):1584–93. [Crossref][PubMed]

32. Tobon H, Dave H. Adenocarcinoma in situ of the cervix. Clinicopathologic observations of 11 cases. Int J Gynecol Pathol. 1988;7(2):139–51. [Crossref][PubMed]

33. Mulvany N, Ostor A. Microinvasive adenocarcinoma of the cervix: a cytohistopathologic study of 40 cases. Diagn Cytopathol. 1997;16(5):430–6. [Crossref][PubMed]

34. 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. J Low Genit Tract Dis. 2013;17(5 Suppl 1):S1–S27. [Crossref][PubMed]

35. McCluggage WG. New developments in endocervical glandular lesions. Histopathology. 2013;62(1):138–60. [Crossref][PubMed]

36. Young RH, Clement PB. Endocervical adenocarcinoma and its variants: their morphology and differential diagnosis. Histopathology. 2002;41(3):185–207. [Crossref][PubMed]

37. Wilbur DC. Practical issues related to uterine pathology: in situ and invasive cervical glandular lesions and their benign mimics: emphasis on cytology-histology correlation and interpretive pitfalls. Mod Pathol. 2016;29(Suppl 1):S1–S11. [Crossref][PubMed]

38. Nayar R, Wilbur DC. The Bethesda system for reporting cervical cytology: definitions, criteria and explanatory notes. 3rd ed. New York: Springer; 2015.

39.

Tambouret RT, Wilbur DC. In: Rosenthal DL, editor. Glandular lesions of the uterine cervix. Cytopathology with Histologic Correlates. New York: Springer; 2015.

40. Mikami Y, McCluggage WG. Endocervical glandular lesions exhibiting gastric differentiation: an emerging spectrum of benign, premalignant, and malignant lesions. Adv Anat Pathol. 2013;20(4):227–37. [Crossref][PubMed]

41. Kastritis E, Bamias A, Efstathiou E, Gika D, Bozas G, Zorzou P, et al. The outcome of advanced or recurrent non-squamous carcinoma of the uterine cervix after platinum-based combination chemotherapy. Gynecol Oncol. 2005;99(2):376–82. [Crossref][PubMed]

42. Lee KB, Lee JM, Park CY, Cho HY, Ha SY. What is the difference between squamous cell carcinoma and adenocarcinoma of the cervix? A matched case-control study. Int J Gynecol Cancer. 2006;16(4):1569–73. [Crossref][PubMed]

43. Katanyoo K, Sanguanrungsirikul S, Manusirivithaya S. Comparison of treatment outcomes between squamous cell carcinoma and adenocarcinoma in locally advanced cervical cancer. Gynecol Oncol. 2012;125(2):292–6. [Crossref][PubMed]

44. Macdonald OK, Chen J, Dodson M, Lee CM, Gaffney DK. Prognostic significance of histology and positive lymph node involvement following radical hysterectomy in carcinoma of the cervix. Am J Clin Oncol. 2009;32(4):411–6. [Crossref][PubMed]

45. Davy ML, Dodd TJ, Luke CG, Roder DM. Cervical cancer: effect of glandular cell type on prognosis, treatment, and survival. Obstet Gynecol. 2003;101(1):38–45. [PubMed]

46. Shimada M, Kigawa J, Nishimura R, Yamaguchi S, Kuzuya K, Nakanishi T, et al. Ovarian metastasis in carcinoma of the uterine cervix. Gynecol Oncol. 2006;101(2):234–7. [Crossref][PubMed]

47. Eifel PJ, Morris M, Oswald MJ, Wharton JT, Delclos L. Adenocarcinoma of the uterine cervix. Prognosis and patterns of failure in 367 cases. Cancer. 1990;65(11):2507–14. [Crossref][PubMed]

48. Li C, Rock KL, Woda BA, Jiang Z, Fraire AE, Dresser K. IMP3 is a novel biomarker for adenocarcinoma in situ of the uterine cervix: an immunohistochemical study in comparison with p16(INK4a) expression. Mod Pathol. 2007;20(2):242–7. [Crossref][PubMed]

49. Han CP, Lee MY, Tyan YS, Kok LF, Yao CC, Wang PH, et al. p16 INK4 and CEA can be mutually exchanged with confidence between both relevant three-marker panels (ER/Vim/CEA and ER/Vim/p16 INK4) in distinguishing primary endometrial adenocarcinomas from endocervical adenocarcinomas in a tissue microarray study. Virchows Arch. 2009;455(4):353–61. [Crossref][PubMed]

50. Jonasson JG, Wang HH, Antonioli DA, Ducatman BS. Tubal metaplasia of the uterine cervix: a prevalence study in patients with gynecologic pathologic findings. Int J Gynecol Pathol. 1992;11(2):89–95. [Crossref][PubMed]

51.

Babkowski RC, Wilbur DC, Rutkowski MA, Facik MS, Bonfiglio TA. The effects of endocervical canal topography, tubal metaplasia, and high canal sampling on the cytologic presentation of nonneoplastic endocervical cells. Am J Clin Pathol. 1996;105(4):403–10.
[Crossref][PubMed]

52. Ismail SM. Cone biopsy causes cervical endometriosis and tubo-endometrioid metaplasia. Histopathology. 1991;18(2):107–14.
[Crossref][PubMed]

53. Oliva E, Clement PB, Young RH. Tubal and tubo-endometrioid metaplasia of the uterine cervix. Unemphasized features that may cause problems in differential diagnosis: a report of 25 cases. Am J Clin Pathol. 1995;103(5):618–23.
[Crossref][PubMed]

54. Jones MA, Young RH. Atypical oxyphilic metaplasia of the endocervical epithelium: a report of six cases. Int J Gynecol Pathol. 1997;16(2):99–102.
[Crossref][PubMed]

55. Baker PM, Clement PB, Bell DA, Young RH. Superficial endometriosis of the uterine cervix: a report of 20 cases of a process that may be confused with endocervical glandular dysplasia or adenocarcinoma in situ. Int J Gynecol Pathol. 1999;18(3):198–205.
[Crossref][PubMed]

56. Cameron RI, Maxwell P, Jenkins D, McCluggage WG. Immunohistochemical staining with MIB1, bcl2 and p16 assists in the distinction of cervical glandular intraepithelial neoplasia from tubo-endometrial metaplasia, endometriosis and microglandular hyperplasia. Histopathology. 2002;41(4):313–21.
[Crossref][PubMed]

57. Clement PB, Young RH, Scully RE. Stromal endometriosis of the uterine cervix. A variant of endometriosis that may simulate a sarcoma. Am J Surg Pathol. 1990;14(5):449–55.
[Crossref][PubMed]

58. Young RH, Clement PB. Endocervicosis involving the uterine cervix: a report of four cases of a benign process that may be confused with deeply invasive endocervical adenocarcinoma. Int J Gynecol Pathol. 2000;19(4):322–8.
[Crossref][PubMed]

59. Clement PB, Young RH. Florid cystic endosalpingiosis with tumor-like manifestations: a report of four cases including the first reported cases of transmural endosalpingiosis of the uterus. Am J Surg Pathol. 1999;23(2):166–75.
[Crossref][PubMed]

60. Fluhmann CF. Focal hyperplasis (tunnel clusters) of the cervix uteri. Obstet Gynecol. 1961;17:206–14.
[PubMed]

61. Segal GH, Hart WR. Cystic endocervical tunnel clusters. A clinicopathologic study of 29 cases of so-called adenomatous hyperplasia. Am J Surg Pathol. 1990;14(10):895–903.
[Crossref][PubMed]

62. Gilks CB, Young RH, Aguirre P, DeLellis RA, Scully RE. Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix. A clinicopathological and immunohistochemical analysis of 26 cases. Am J Surg Pathol. 1989;13(9):717–29.
[Crossref][PubMed]

63. Taylor HB, Irey NS, Norris HJ. Atypical endocervical hyperplasia in women taking oral contraceptives. JAMA. 1967;202(7):637–9.
[Crossref][PubMed]

64. Kyriakos M, Kempson RL, Konikov NF. A clinical and pathologic study of endocervical lesions associated with oral contraceptives. Cancer. 1968;22(1):99–110.
[Crossref][PubMed]

65. Candy J, Abell MR. Progesten-induced adenomatous hyperplasia of the uterine cervix. JAMA. 1968;203(5):85–8.
[Crossref]

66. Govan AD, Black WP, Sharp JL. Aberrant glandular polypi of the uterine cervix associated with contraceptive pills: pathology and pathogenesis. J Clin Pathol. 1969;22(1):84–9.
[Crossref][PubMed][PubMedCentral]

67. Chumas JC, Nelson B, Mann WJ, Chalas E, Kaplan CG. Microglandular hyperplasia of the uterine cervix. Obstet Gynecol. 1985;66(3):406–9.
[PubMed]

68. Wilkinson E, Dufour DR. Pathogenesis of microglandular hyperplasia of the cervix uteri. Obstet Gynecol. 1976;47(2):189–95.
[PubMed]

69. Jones MA, Young RH, Scully RE. Diffuse laminar endocervical glandular hyperplasia. A benign lesion often confused with adenoma malignum (minimal deviation adenocarcinoma). Am J Surg Pathol. 1991;15(12):1123–9.
[Crossref][PubMed]

70. Nucci MR, Clement PB, Young RH. Lobular endocervical glandular hyperplasia, not otherwise specified: a clinicopathologic analysis of thirteen cases of a distinctive pseudoneoplastic lesion and comparison with fourteen cases of adenoma malignum. Am J Surg Pathol. 1999;23(8):886–91.
[Crossref][PubMed]

71. Nucci MR, Young RH. Arias-Stella reaction of the endocervix: a report of 18 cases with emphasis on its varied histology and differential diagnosis. Am J Surg Pathol. 2004;28(5):608–12.
[Crossref][PubMed]

72. Laverty CR, Farnsworth A, Thurloe J, Bowditch R. The reliability of a cytological prediction of cervical adenocarcinoma in situ. Aust N Z J Obstet Gynaecol. 1988;28(4):307–12.
[Crossref][PubMed]

73. Lee KR, Minter LJ, Granter SR. Papanicolaou smear sensitivity for adenocarcinoma in situ of the cervix. A study of 34 cases. Am J Clin Pathol. 1997;107(1):30–5.
[Crossref][PubMed]

74. Ashfaq R, Gibbons D, Vela C, Saboorian MH, Iliya F. ThinPrep Pap Test. Accuracy for glandular disease. Acta Cytol. 1999;43(1):81–5.
[Crossref][PubMed]

75. Ramsaroop R, Chu I. Accuracy of diagnosis of atypical glandular cells – Conventional and ThinPrep. Diagn Cytopathol. 2006;34(9):614–9.
[Crossref][PubMed]

76. DeSimone CP, Day ME, Tovar MM, Dietrich 3rd CS, Eastham ML, Modesitt SC. Rate of pathology from atypical

glandular cell Pap tests classified by the Bethesda 2001 nomenclature. Obstet Gynecol. 2006;107(6):1285–91.
[Crossref][PubMed]

77. Negri G, Bellisano G, Carico E, Faa G, Kasal A, Antoniazzi S, et al. Usefulness of p16ink4a, ProEX C, and Ki-67 for the diagnosis of glandular dysplasia and adenocarcinoma of the cervix uteri. Int J Gynecol Pathol. 2011;30(4):407–13.
[Crossref][PubMed]

78. Ravarino A, Nemolato S, Macciocu E, Fraschini M, Senes G, Faa G, et al. CINtec PLUS immunocytochemistry as a tool for the cytologic diagnosis of glandular lesions of the cervix uteri. Am J Clin Pathol. 2012;138(5):652–6.
[Crossref][PubMed]

79. Singh M, Mockler D, Akalin A, Burke S, Shroyer A, Shroyer KR. Immunocytochemical colocalization of P16(INK4a) and Ki-67 predicts CIN2/3 and AIS/adenocarcinoma. Cancer Cytopathol. 2012;120(1):26–34.
[Crossref][PubMed]

80. College of American Pathologists Accreditation Program, Cytopathology Checklist 2016, pages 27–29, CYP.07600 Statistical Records, revised 7/28/2015, Northfield, IL, USA

81. Bose S, Kannan V, Kline TS. Abnormal endocervical cells. Really abnormal? Really endocervical? Am J Clin Pathol. 1994;101(6):708–13.
[Crossref][PubMed]

82. Chhieng DC, Elgert PA, Cangiarella JF, Cohen JM. Clinical significance of atypical glandular cells of undetermined significance. A follow-up study from an academic medical center. Acta Cytol. 2000;44(4):557–66.
[Crossref][PubMed]

83. Zhao C, Austin RM, Pan J, Barr N, Martin SE, Raza A, et al. Clinical significance of atypical glandular cells in conventional pap smears in a large, high-risk U.S. west coast minority population. Acta Cytol. 2009;53(2):153–9.
[Crossref][PubMed]

84. Ajit D, Gavas S, Joseph S, Rekhi B, Deodhar K, Kane S. Identification of atypical glandular cells in pap smears: is it a hit and miss scenario? Acta Cytol. 2013;57(1):45–53.
[Crossref][PubMed]

85. Suh KS, Silverberg SG. Tubal metaplasia of the uterine cervix. Int J Gynecol Pathol. 1990;9(2):122–8.
[Crossref][PubMed]

86. Novotny DB, Maygarden SJ, Johnson DE, Frable WJ. Tubal metaplasia. A frequent potential pitfall in the cytologic diagnosis of endocervical glandular dysplasia on cervical smears. Acta Cytol. 1992;36(1):1–10.
[PubMed]

87. Van Le L, Novotny D, Dotters DJ. Distinguishing tubal metaplasia from endocervical dysplasia on cervical Papanicolaou smears. Obstet Gynecol. 1991;78(5 Pt 2):974–6.
[PubMed]

88. de Peralta-Venturino MN, Purslow MJ, Kini SR. Endometrial cells of the “lower uterine segment” (LUS) in cervical smears obtained by endocervical brushings: a source of potential diagnostic pitfall. Diagn Cytopathol. 1995;12(3):263–8; discussion 8–71.

89. Heaton Jr RB, Harris TF, Larson DM, Henry MR. Glandular cells derived from direct sampling of the lower uterine segment in patients status post-cervical cone biopsy. A diagnostic dilemma. Am J Clin Pathol. 1996;106(4):511–6.

[Crossref][PubMed]

90. Lundeen SJ, Horwitz CA, Larson CJ, Stanley MW. Abnormal cervicovaginal smears due to endometriosis: a continuing problem. Diagn Cytopathol. 2002;26(1):35–40.
[Crossref][PubMed]

91. Sauder K, Wilbur DC, Duska L, Tambouret RH. An approach to post-radical trachelectomy vaginal-isthmus cytology. Diagn Cytopathol. 2009;37(6):437–42.
[Crossref][PubMed]

92. Gupta PK. Intrauterine contraceptive devices: vaginal cytology, pathologic changes and clinical implications. Acta Cytol. 1982;26(5):571–613.
[PubMed]

93. Kobayashi TK, Casslen B, Stormby N. Cytologic atypias in the uterine fluid of intrauterine contraceptive device users. Acta Cytol. 1983;27(2):138–41.
[PubMed]

94. Mali B, Joshi JV, Wagle U, Hazari K, Shah R, Chadha U, et al. Actinomyces in cervical smears of women using intrauterine contraceptive devices. Acta Cytol. 1986;30(4):367–71.
[PubMed]

95. Geirsson G, Woodworth FE, Patten Jr SF, Bonfiglio TA. Epithelial repair and regeneration in the uterine cervix. I. An analysis of the cells. Acta Cytol. 1977;21(3):371–8.
[PubMed]

96. Yelverton CL, Bentley RC, Olenick S, Krigman HR, Johnston WW, Robboy SJ. Epithelial repair of the uterine cervix: assessment of morphologic features and correlations with cytologic diagnosis. Int J Gynecol Pathol. 1996;15(4):338–44.
[Crossref][PubMed]

97. Selvaggi SM. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in endocervical cytobrush specimens. Acta Cytol. 1994;38(5):687–92.
[PubMed]

98. Kumar N, Bongiovanni M, Molliet MJ, Pelte MF, Egger JF, Pache JC. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. Cytopathology. 2009;20(6):351–8.
[Crossref][PubMed]

99. Shukla A, Thomas D, Roh MH. PAX8 and PAX2 expression in endocervical adenocarcinoma in situ and high-grade squamous dysplasia. Int J Gynecol Pathol. 2013;32(1):116–21.
[Crossref][PubMed]

C. Simon Herrington (ed.), Pathology of the Cervix, Essentials of Diagnostic Gynecological Pathology 3,
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9. Non-Human-Papillomavirus (HPV)-Related Adenocarcinomas and Their Precursors

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Abstract
Many recent studies of cervical carcinoma have focused on unusual subtypes of endocervical adenocarcinoma that arise independently of high-risk HPV infection. This chapter summarizes the clinicopathological features of HPV-negative endocervical adenocarcinomas and their precursors, particularly gastric-type adenocarcinoma, an emerging entity, as well as clear cell, serous, and mesonephric carcinomas.

Keywords Non-human papillomavirus (HPV)-related adenocarcinomas – Human papillomavirus (HPV) infection – Cervix – Adenocarcinoma – Gastric – Endometrioid – Serous – Clear cell – Mesonephric

Introduction

High-risk human papillomavirus (HPV) infection is implicated in the carcinogenesis of endocervical adenocarcinomas as well as squamous cell carcinomas. However, the reported prevalence of HPV detection in adenocarcinomas varies significantly from 62% to 97% in the English-language literature [1–5]. This variation was considered to be caused largely by differences in detection assays, specimen types (i.e., biopsy, conization, or hysterectomy specimens), patient population, or socioeconomic status. However, recent studies have focused on unusual subtypes of endocervical

adenocarcinoma, which arise independently of high-risk HPV, as represented by gastric-type adenocarcinoma (GAS), an emerging entity, as well as clear cell, serous, and mesonephric carcinomas [6–9]. This chapter summarizes the clinicopathological features of HPV-negative endocervical adenocarcinomas and their precursors.

Non-HPV-Related Invasive Adenocarcinomas

The recently revised WHO Classification of Tumors of Female Reproductive Organs (2014) separates true mucinous carcinoma to distinguish it from usual-type endocervical adenocarcinoma. The latter accounts for approximately 90% of all endocervical adenocarcinomas [10], which were mostly designated as endocervical-type mucinous adenocarcinomas in the previous version of the WHO classification published in 2003 [11]. The 2014 classification describes GAS as a variant of mucinous carcinoma. GAS is a distinct entity that arises independently of HPV and shows aggressive clinical behavior. Other unusual variants include villoglandular, clear cell, serous, endometrioid, and mesonephric carcinomas. Recent studies have shown that the latter four, as well as GAS, are also non-HPV related [1, 2, 6, 9, 12] and thus are a pitfall of HPV DNA testing as an adjunctive tool for screening and HPV vaccination for prevention of cervical cancer. Importantly, *TP53* mutation is more common in HPV-negative tumors [13–15], and some studies have reported the aggressive nature of p53-positive/*TP53*-mutated or HPV-negative endocervical adenocarcinomas [14–16]. From the clinical point of view, differences between squamous cell carcinoma and adenocarcinoma of the uterine cervix have been controversial, but some authors suggest an unfavorable outcome in patients with adenocarcinoma. These differences may result from the higher incidence of HPV-negative tumors in adenocarcinomas by comparison with squamous cell carcinomas, which are almost always HPV related. Therefore, pathologists, cytologists, and gynecologists should be aware of the existence of non-HPV-related endocervical adenocarcinomas (Tables 9.1 and 9.2).

Table 9.1 HPV-positive and negative adenocarcinomas

HPV positive	HPV negative
Usual type	Clear cell carcinoma
Intestinal-type mucinous carcinoma[a]	Mesonephric carcinoma
Serous carcinoma[b]	Serous carcinoma
Endometrioid carcinoma[b]	Endometrioid carcinoma
	Gastric-type mucinous carcinoma

[a]Absence of HPV detection reported by some studies [1, 2, 7]

[b]Some studies have implicated high-risk HPV infection [1, 2, 6, 9, 12], possibly as a function of different diagnostic criteria

Table 9.2 Detection rate of high-risk HPV in cases of unusual endocervical adenocarcinoma

Histological type	% (*n*)	Authors
Gastric-type mucinous carcinoma (including MDA)	0% (0/1) 0% (0/3) 0% (0/6) 0% (0/2) 0% (0/3) 0% (0/7) 0% (0/6) 0% (0/14) 0% (0/7) 0% (0/20) 8.3% (1/12) 25% (1/4)	Fukushima et al. [17] Ferguson et al. [18] Toki et al. [19] Pirog et al. [1] Xu et al. [20] Kusanagi et al. [8] Houghton et al. [7] Park et al. [21] Holl et al. [22] Molijn et al. [23] Pirog et al. [9] An et al. [2]
	Total 2.4% (2/85)	
Clear cell carcinoma	0% (0/4) 0% (0/3) 0% (0/9) 0% (0/13) 13% (2/15)[a] 20% (6/30) 27.6% (8/29)	Pirog et al. [1] Houghton et al. [7] Park et al. [21] Ueno et al. [24] Molijn et al. [23] Pirog et al. [9] Holl et al. [22]
	Total 15.5% (16/103)	
Mesonephric carcinoma	0% (0/1) 0% (0/2) 0% (0/1) 0% (0/7)	Pirog et al. [1] Houghton et al. [7] Park et al. [21]Kenny et al. [25]
	Total 0% (0/11)	
Serous carcinoma	0% (0/1) 0% (0/9) 25% (6/24) 30.4% (7/23) 33.3% (4/12) 100% (1/1) 100% (1/1)	Pirog et al. [1] Molijn et al. [23] Pirog et al. [9] Holl et al. [22] Togami et al. [26] Houghton et al. [7] Park et al. [21]
	Total 28.2% (20/71)	
Endometrioid carcinoma	12.9% (4/31) 13% (1/8)[a] 27.3% (3/11) 80% (8/10) 100% (4/4)	Holl et al. [22] Molijn et al. [23] Pirog et al. [9] An et al. [2] Pirog et al. [1]

	Total 31.3% (20/64)	
Intestinal-type mucinous carcinoma	0% (0/3) 83.3% (5/6) 76.7% (18/21)	An et al. [2] Houghton et al. [7] Pirog et al. [1]
	Total 76.7% 23/30	

[a]Cases positive for HPV by whole-tissue section polymerase chain reaction (PCR) further analyzed by laser capture microdissection and PCR

Authors and HPV types examined in each study

Fukushima et al. [17]	6, 16, 18, 31, 33
Ferguson et al. [18]	16,18, 45
Toki et al. [19]	16, 18
Pirog et al. [1]	6,11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74
Xu et al. [20]	6,11, 16, 18, 31, 33, 35, 52b, 58,
Kusanagi et al. [8]	6, 11, 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66
Houghton et al. [7]	High risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 Low risk: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39, CP108
Park et al. [21]	High risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 Low risk: 6, 11, 34, 40, 42, 43, 44, 53, 70, 74
Holl et al. [22]	High risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 Low risk; 6, 11,34, 40, 42, 43, 44, 53, 54, 70, 74
Molijn et al. [23]	High risk: 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 Low risk; 6, 11,34, 40, 42, 43, 44, 53, 54, 70, 74
Pirog et al. [9]	6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 32, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74
An et al. [2]	High risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69 Low risk: 6, 11, 34, 40, 42, 43, 44
Ueno et al. [24]	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68
Kenny et al. [25]	High risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 Low risk: 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39, CP108

Gastric-Type Adenocarcinoma

The revised WHO classification strictly defines mucinous carcinoma to include tumors composed of cells with intracytoplasmic mucin and includes gastric-type adenocarcinoma (GAS) in this group, as well as intestinal and signet-ring cell types.

The concept of GAS was first proposed by Kojima et al. in 2007 and included minimal deviation adenocarcinoma (MDA) (Fig. 9.1) in its morphological spectrum as an extremely well-differentiated variant, based on common gastric morphology and phenotype and aggressive clinical behavior [27]. Before the introduction of the concept

and diagnostic criteria for GAS, the less-differentiated form was designated as endocervical-type mucinous adenocarcinoma [27, 28], which was in fact a wastebasket diagnostic category in the WHO 2003 classification. GAS is common in Japan, accounting for 20% to 25% of all endocervical adenocarcinomas [27], and limited data and personal communications indicate that it is less frequent in western countries [29, 30]. A European multinational epidemiological study demonstrated that GAS accounted for 1.5% (7/461) of all endocervical adenocarcinomas [22]. Microscopically, GAS is characterized by a proliferation of columnar cells with abundant pale or pale eosinophilic cytoplasm and distinct cell borders, arranged in glandular or cribriform patterns (Fig. 9.2) [27]. A recent study has described a foamy gland variant characterized by microvesicles in the cytoplasm [31], as seen in a variant of pancreatic adenocarcinoma with a deceptively benign appearance [32].

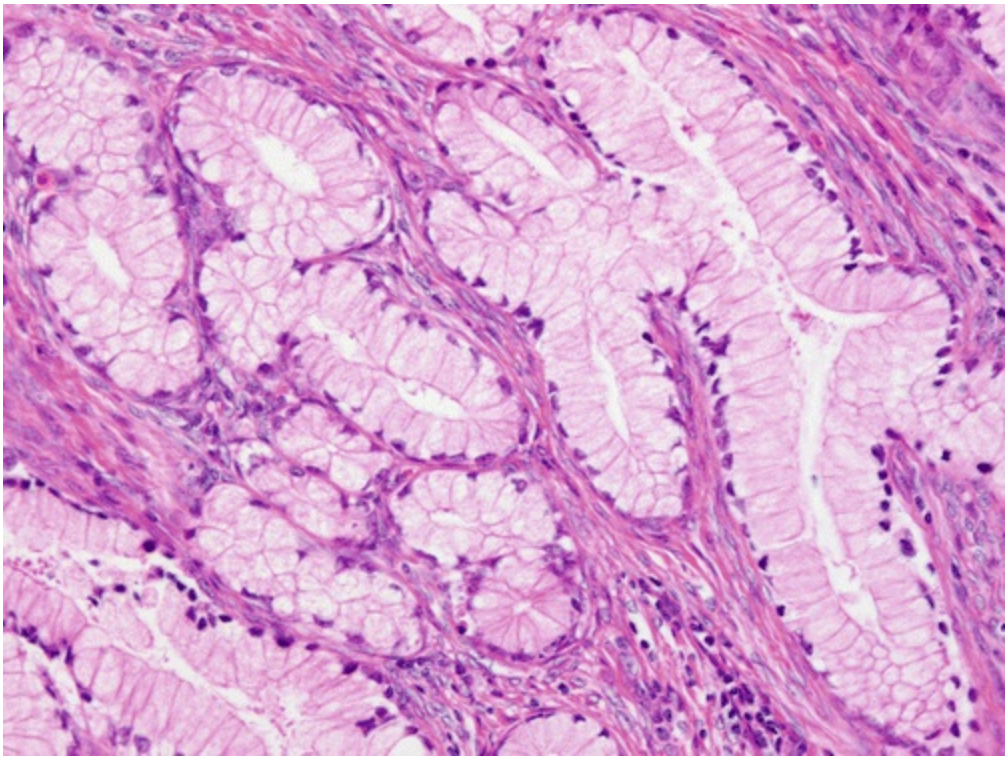

Fig. 9.1 Minimal deviation adenocarcinoma (MDA). Highly differentiated neoplastic glands with abundant pale intracytoplasmic mucin and basally located bland nuclei, infiltrating into the stroma. Limited sampling may be challenging for making a definite diagnosis because of the absence of nuclear anaplasia or desmoplastic stromal reaction

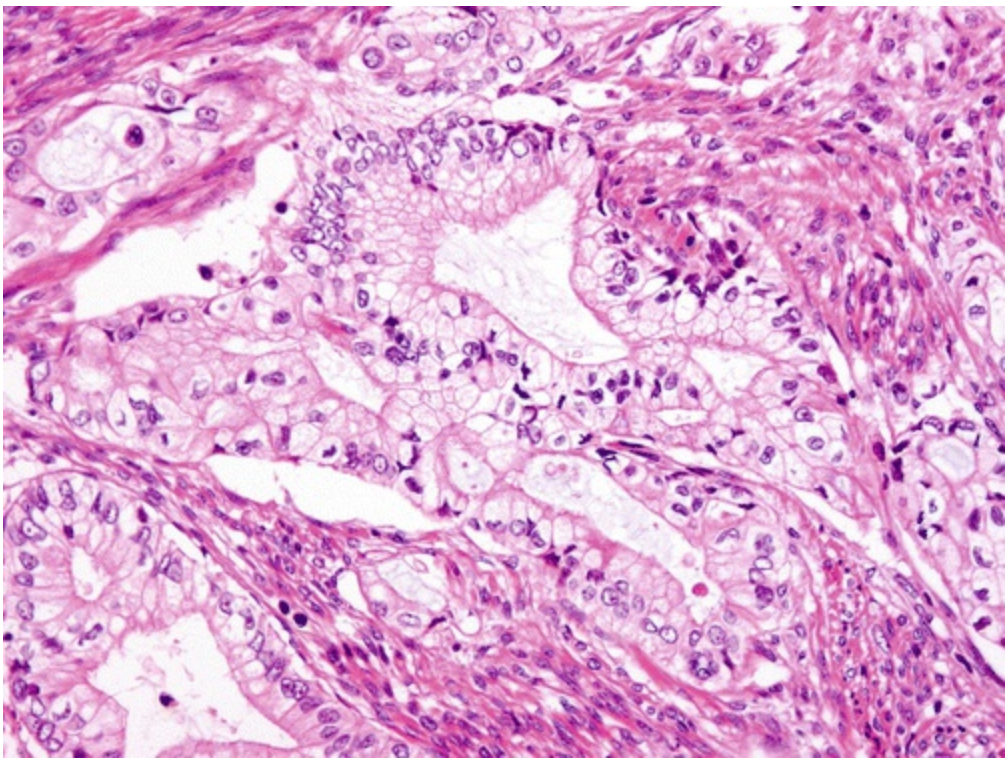

Fig. 9.2 Gastric-type adenocarcinoma. Well-differentiated but angulated or occasionally fused neoplastic glands composed of cells with abundant pale or pale eosinophilic cytoplasm and distinct cell borders, infiltrating into the stroma. Desmoplastic reaction is easily discernible

GAS characteristically has a gastric immunophenotype, as shown by positive staining with HIK1083 and/or anti-MUC6 antibody, two representative antibodies that recognize pyloric gland mucin of the stomach, and it is usually negative for $p16^{INK4a}$ [27]. A comprehensive immunohistochemical study revealed that this tumor is frequently positive for p53 (mutation pattern), paired box gene-8 protein (PAX8), and carbonic anhydrase type IX [33]. HER2 expression is only rarely seen, in contrast to prototypical gastric adenocarcinoma [33]. In addition, $p16^{INK4a}$, a marker of HPV-driven neoplasms, is usually negative [21, 27], and subsequently Park et al. [6], Houghton et al. [7] and Kusanagi et al. [8] independently demonstrated the absence of high-risk HPV in cases of GAS.

Clinical presentation of GAS is similar to usual-type adenocarcinoma, but a notable finding is mucoid or watery discharge, as in cases of MDA. Magnetic resonance imaging features are distinctive and characterized by highly infiltrating and endophytic growth without forming well-demarcated masses associated with occasional cystic spaces, location in the upper cervix, and frequent vaginal or parametrial invasion in more than 60% of cases [34]. Importantly, Kojima et al. demonstrated that patients with GAS had significantly decreased overall survival of 30%, compared with 77% in those with usual-type tumors [27]. This aggressive nature was also demonstrated in a larger study. Based on a review of 40 cases of GAS, Karamurzin et al. reported frequent

lymph node and distant metastasis with a 5-year-disease-specific survival of 42% compared with 91% in usual-type adenocarcinoma [35]. A subset of HPV-negative and *TP53*-mutated or p53-immunopositive endocervical adenocarcinomas, which show aggressive clinical behavior [14–16], might represent GAS. Omori et al. recently demonstrated that alteration of cyclin-dependent kinase inhibitors, including p16, p14, p27 and p21, and p53 overexpression, is closely related to frequent lymph node metastasis and worse prognosis in HPV-negative endocervical adenocarcinomas, including GAS [36].

A unique clinical presentation of GAS is an association with synchronous multifocal mucinous metaplasia and neoplasia of the female genital tract, involving the endometrium, fallopian tubes, ovaries, and uterine cervix. It may be associated with Peutz-Jeghers syndrome (PJS) [37–40]. Recently, a case of GAS arising in a patient with Lynch syndrome has been described [41].

Endometrioid Carcinoma

Some studies have shown that endometrioid carcinoma is an HPV-driven neoplasm. However, the detection rate of high-risk HPV, as well as the incidence of this particular subtype of endocervical adenocarcinoma, varies significantly, ranging from 12.9% to 100%, with a total of 31.3% (20/64) in the English-language literature [1, 2, 9, 22, 23]. Endometrioid carcinoma is defined as showing features similar to endometrioid carcinoma of the uterine corpus. With strict criteria of the absence or paucity of intracytoplasmic mucin, and existence of ciliated cells, squamous differentiation, or morules (Fig. 9.3) and exclusion of extension from an endometrial carcinoma, endometrioid carcinoma of the cervix appears to be a rare neoplasm, with an incidence of less than 10% of all endocervical adenocarcinomas [42], and most cases are considered to be HPV negative. A significant number of HPV-related, usual-type endocervical adenocarcinomas may have been regarded as endometrioid carcinoma because of the paucity of intracytoplasmic mucin, resulting in a higher incidence of up to 30% in some series [42, 43].

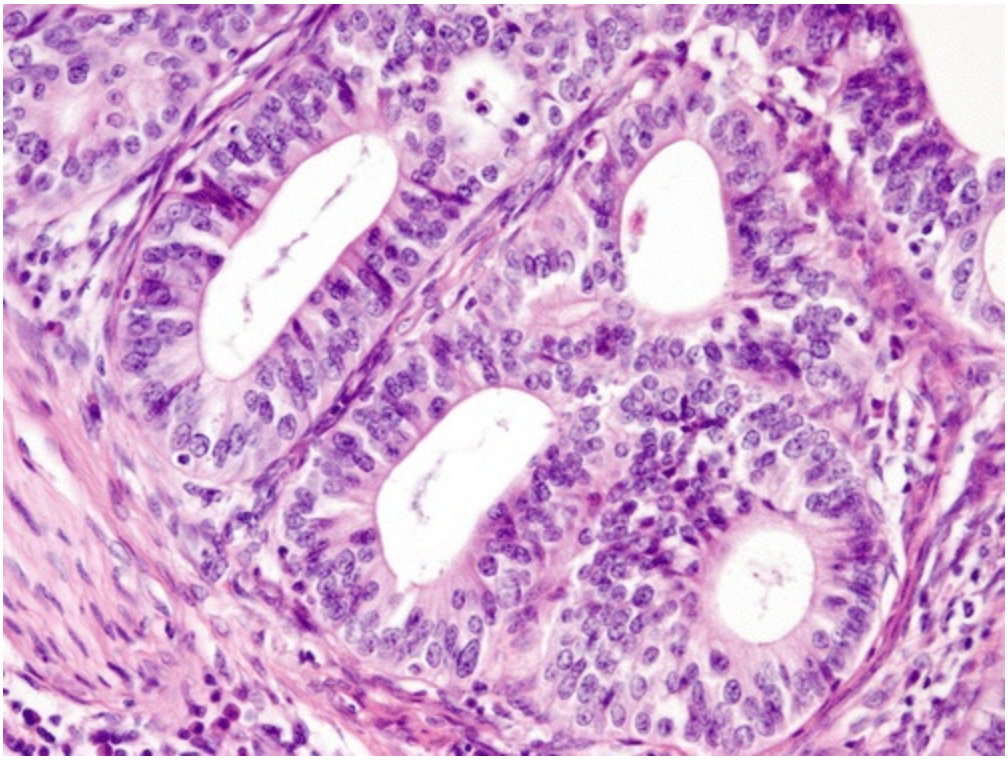

Fig. 9.3 Endometrioid carcinoma. Cribriform or fused glands composed of tall columnar cells without intracytoplasmic mucin

Serous Carcinoma

Diagnosis of serous carcinoma of the uterine cervix should be established by diligent imaging studies and microscopic examination to eliminate secondary cervical involvement by serous carcinoma of other sites including the endometrium, fallopian tubes, ovaries, or peritoneum. Serous carcinoma shows proliferation of highly anaplastic cells arranged in papillary, micropapillary, or solid growth with occasional slit-like spaces (Fig. 9.4). Strictly defined primary serous carcinoma of the cervix is rare and accounts for less than 1% of all endocervical adenocarcinomas. It should be kept in mind that usual-type endocervical adenocarcinoma occasionally shows a micropapillary pattern of growth and nuclear anaplasia, imparting a close resemblance to serous carcinoma (Fig. 9.5) [44]. Such tumors arising in young patients may have been designated as HPV-positive serous carcinomas of the cervix in the English-language literature [7, 10]. In addition, serous carcinoma shows immunoreactivity for $p16^{INK4a}$ as a result of non-HPV-related mechanisms [45]. Some investigators have suggested that serous carcinoma is HPV driven, although the detection rate of high-risk HPV ranges from 0% to 100%, with a total of 28.2% (20/71) in the literature [1, 7, 9, 21–23, 26].

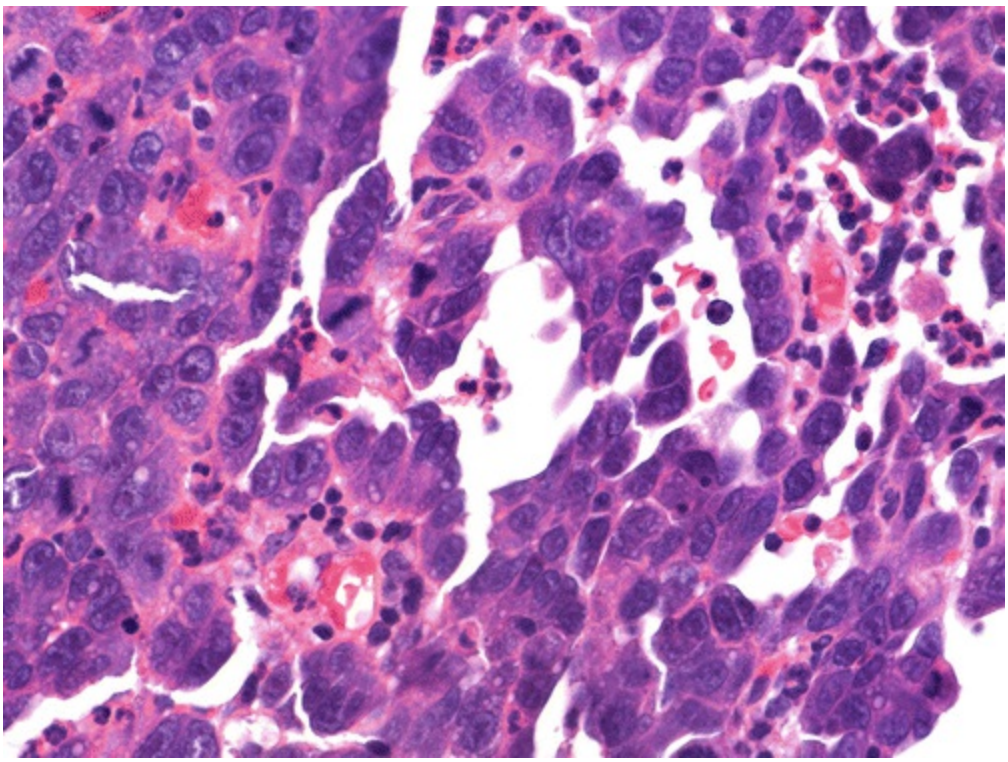

Fig. 9.4 Serous carcinoma, composed of highly anaplastic cells showing papillary or micropapillary architecture

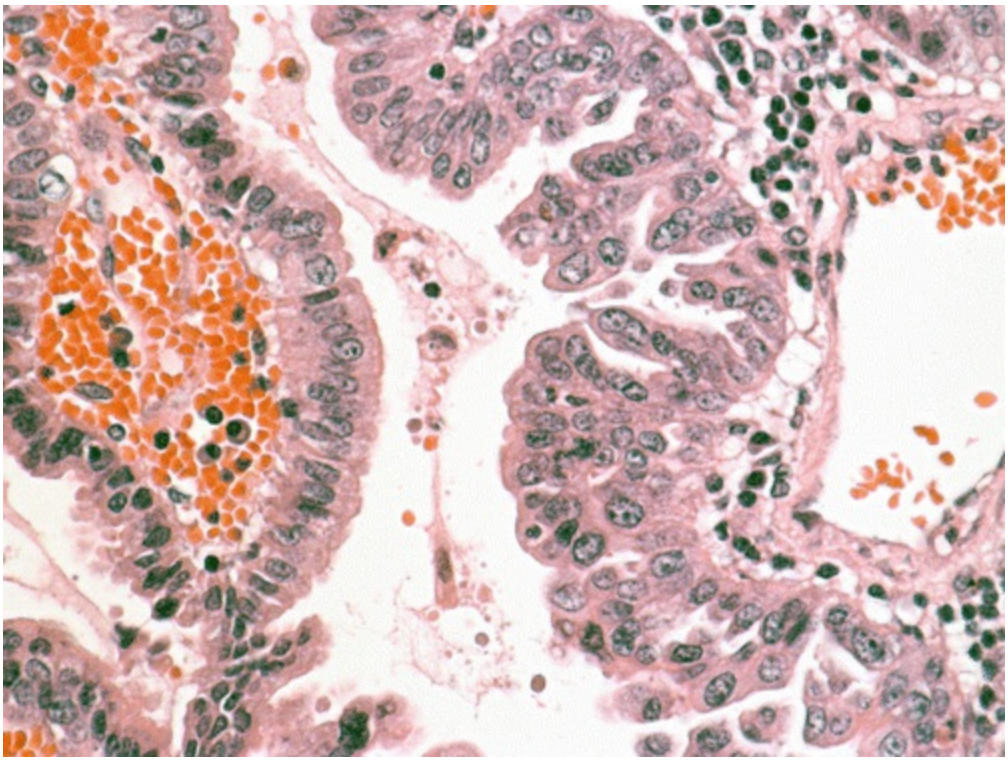

Fig. 9.5 Usual-type adenocarcinoma with micropapillary pattern. Piling up of anaplastic cells without stromal core, imparting a close resemblance to the characteristic architectural pattern of serous carcinoma (*right side*). In this particular case, usual-type endocervical adenocarcinoma composed of columnar cells was also seen (*left side*)

Clear Cell Carcinoma

Clear cell carcinoma is characterized by neoplastic cells with abundant clear cytoplasm caused by accumulation of glycogen, arranged in tubular, tubulocystic, papillary, micropapillary, and solid patterns, as well as a hobnail appearance of cells (Fig. 9.6). Stromal hyalinization resulting from deposition of basement membrane material is a common finding. As in serous carcinoma, diagnosis of clear cell carcinoma is made after excluding secondary involvement by endometrial or ovarian tumors. It is well known that this particular tumor was commonly found among young women who had a history of in utero exposure to diethylstilbestrol (DES), a synthetic estrogenic agent that was approved by the US Food and Drug Administration in 1941 [46]. However, patients are now mostly postmenopausal, and clear cell carcinoma appears to be independent of exposure to DES. Recent studies have shown that clear cell carcinoma is mostly unrelated to HPV [1, 7, 21, 24], although three studies detected HPV in 13% [23], 20% [9], and 27.3% [22] of cases. Clear cell carcinoma frequently shows increased epidermal growth factor receptor (EGFR) or HER2 expression or activation of AKT or mammalian target of rapamycin (mTOR). Therefore, tyrosine kinase inhibitor blockade of the AKT-mTOR pathway might be an effective treatment strategy for this tumor [24].

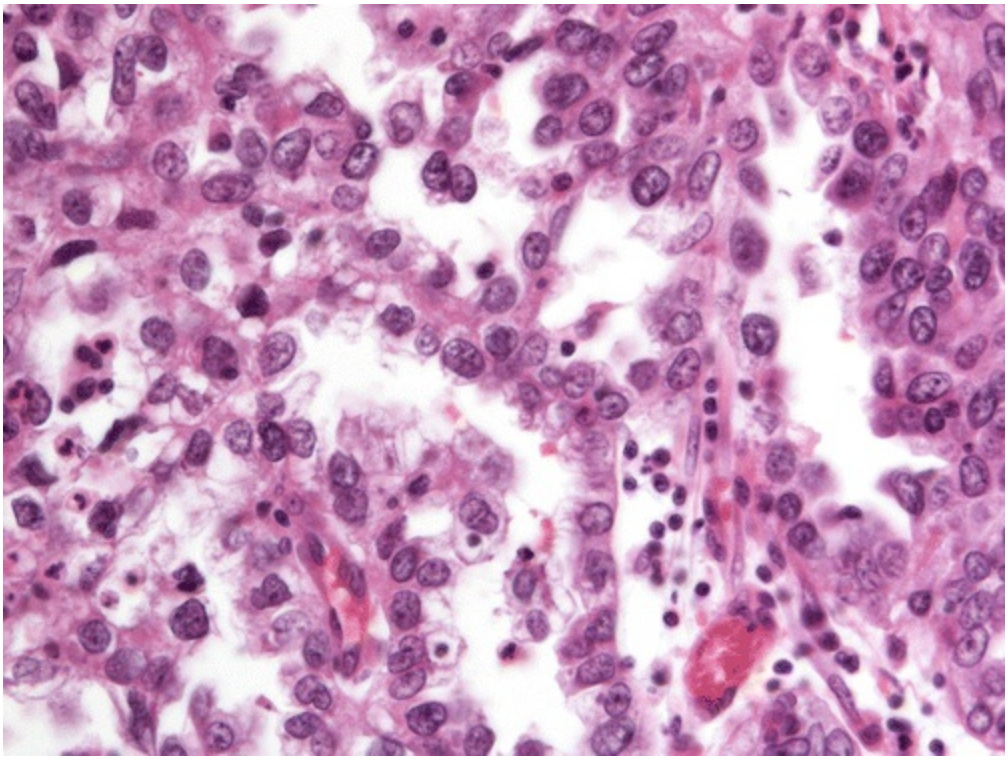

Fig. 9.6 Clear cell carcinoma, composed of cells with abundant clear cytoplasm

Mesonephric Carcinoma

Mesonephric duct remnants and hyperplasia are the putative origins of mesonephric carcinoma, because of morphology, topographic relationship, and immunophenotype. Mesonephric carcinoma is characterized by proliferation of cuboidal or columnar cells with pale eosinophilic cytoplasm, arranged in tubular, trabecular, papillary, reticular, or solid patterns, and diastase-resistant periodic acid-Schiff (PAS)-positive luminal secretion (Fig. 9.7). Well-differentiated mesonephric carcinoma can be misinterpreted as endometrioid carcinoma. Immunohistochemically, it is positive for CD10, inhibin, and calretinin and negative for estrogen receptor and $p16^{INK4a}$. More recent studies have shown that it is positive for HMGA2 [25], PAX8 [25, 47], and GATA3 [48] and occasionally can be positive for hepatocyte nuclear factor 1-β (beta) and thyroid transcription factor 1 (TTF-1) [25]. Detection of high-risk HPV has not hitherto been reported in cases of this subtype of endocervical adenocarcinomas [1, 7, 21, 25].

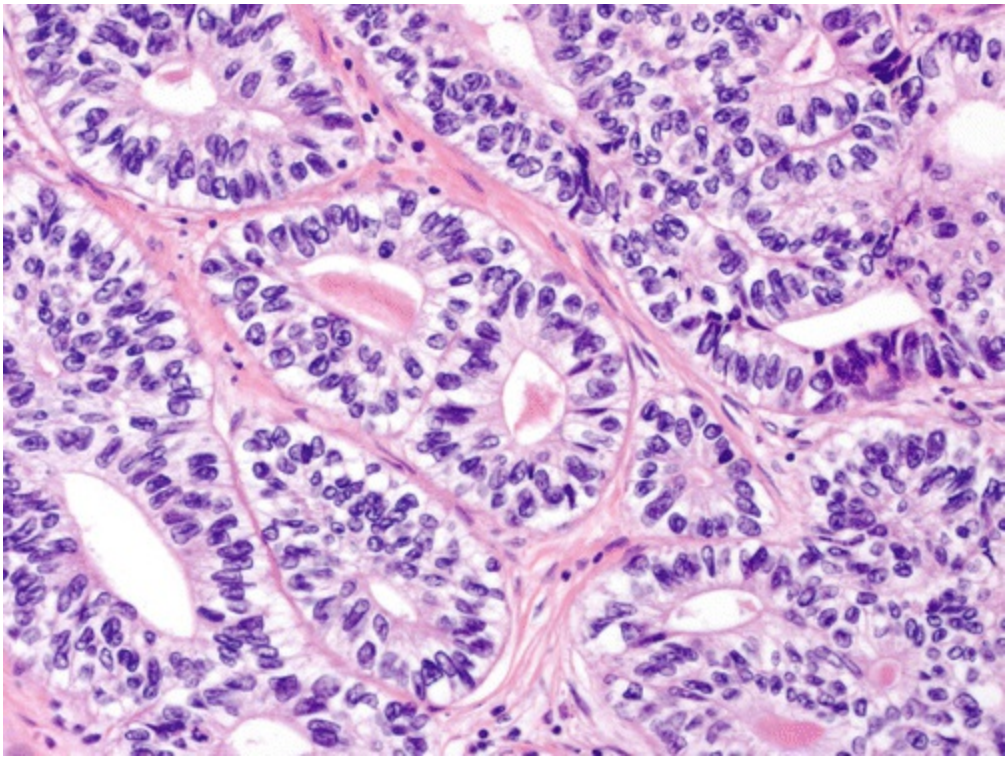

Fig. 9.7 Mesonephric carcinoma, composed of columnar cells arranged in a tubular pattern, with eosinophilic intraluminal secretion

Histogenesis and Precursors of Non-HPV-Related Endocervical Adenocarcinomas

Better understanding of precursor lesions of endocervical adenocarcinomas is mandatory to establish the strategy for early detection of this particular tumor and cancer prevention. For this purpose HPV- and non-HPV-related pathways of

carcinogenesis should be separated because HPV-targeted adjunctive tools and strategies appear to be a pitfall for early detection and prevention.

Lobular Endocervical Glandular Hyperplasia (LEGH) as a Precursor of GAS

There is a lot of evidence indicating a link between LEGH, also known as pyloric gland metaplasia (PGM), and GAS/MDA. LEGH was first described by Nucci et al. in 1999 as a worrisome benign mimic of MDA, characterized by proliferation of small glands arranged in a lobular fashion around cystically dilated endocervical glands (Fig. 9.8) [49]. Simultaneously, an identical lesion was described as PGM of the uterine cervix by Mikami et al. [50, 51]. LEGH shows a gastric immunophenotype, as demonstrated by positive staining with HIK1083, an antibody that recognizes pyloric gland mucin. Although LEGH/PGM was originally considered to be a benign condition, it shows a spectrum of cytological atypia, ranging from reactive changes or nuclear abnormalities of uncertain significance to significant atypia considered to be intraepithelial carcinoma (atypical LEGH) (Fig. 9.9). It has also been shown to coexist with invasive adenocarcinoma including MDA [52, 53]. In addition, Kawauchi et al. and Mikami et al. demonstrated that atypical LEGH has copy number abnormalities that are shared by MDA [54] and p53 immunoreactivity (mutant pattern) and high Ki-67 labeling index similar to MDA [55]. Xu et al. reported the absence of HPV in LEGH [20]. These facts suggest an HPV-independent pathway of carcinogenesis linking LEGH and GAS/MDA (LEGH-GAS/MDA sequence). Therefore, it appears reasonable to consider that a subset of LEGH, in particular, with atypical features (atypical LEGH), represents a neoplastic condition and thus is a precursor of GAS/MDA. Kuragaki et al. demonstrated abnormalities of *STK11/LKB1*, a gene responsible for PJS, in sporadic cases of MDA unrelated to PJS. Recently, Ito et al. reported a single case with an identical *KRAS* gene mutation in LEGH associated with GAS [56]. Matsubara et al. demonstrated frequent *GNAS*, *KRAS*, or *STK11/LKB1* mutations, which are mutually exclusive, in 42% (8/19) of cases of LEGH [57]. However, it should be kept in mind that LEGH is not uncommon in the general population, and otherwise prototypical LEGH without any cytological atypia is a benign condition, although it can be a risk factor for GAS/MDA. It appears that a subset of cases in the series examined by Matsubara et al. represent atypical LEGH (personal communication).

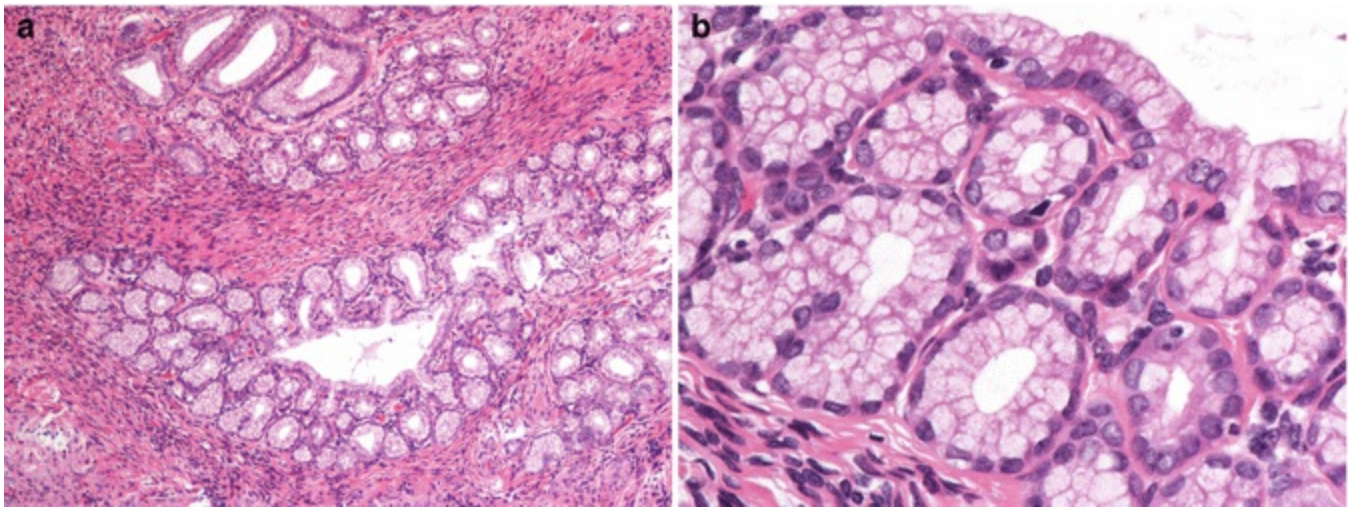

Fig. 9.8 LEGH. Clusters of small glands surrounding central cystically dilated endocervical glands with a close resemblance to pyloric glands of the stomach, justifying pyloric gland metaplasia as a synonym (**a**). The small glands are composed of columnar cells with pale eosinophilic cytoplasm and basally located bland nuclei (**b**)

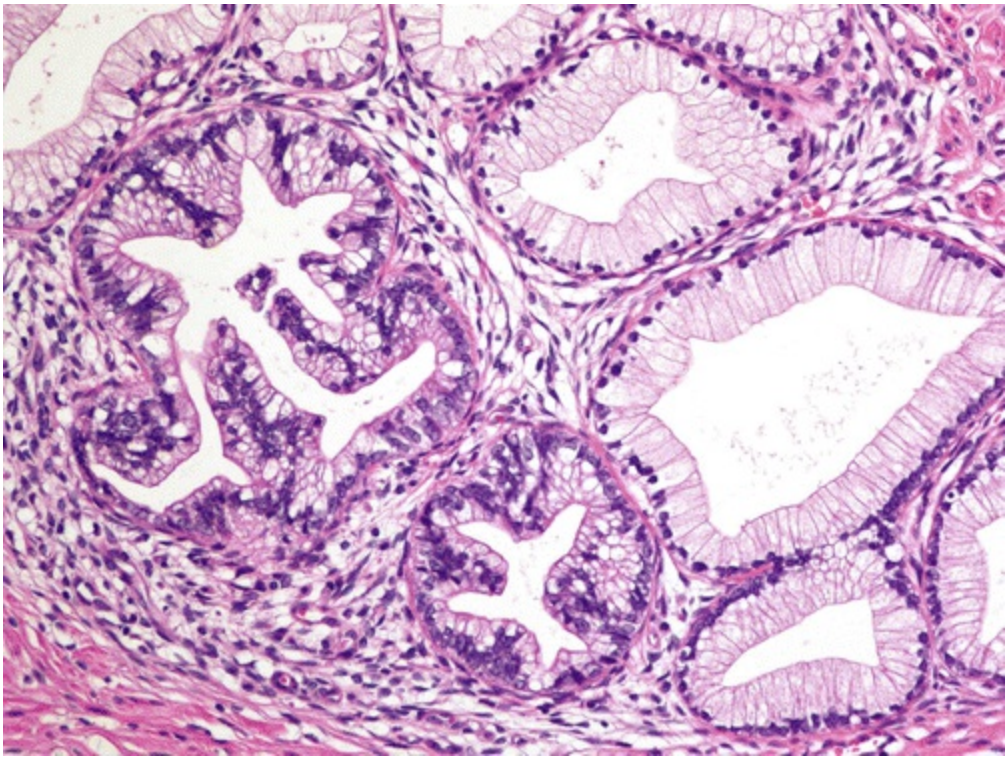

Fig. 9.9 LEGH, with intraepithelial carcinoma. In this case, the patient was diagnosed as having Peutz-Jeghers syndrome based on pigmented macules on the lips and hamartomatous intestinal polyps

Another precursor of GAS is gastric-type adenocarcinoma in situ (AIS) [29, 30, 52]. In contrast to atypical LEGH, it is defined as replacement of preexisting mucin-producing columnar cells by highly atypical cells with abundant pale or pale eosinophilic cytoplasm similar to those of GAS, with preservation of the normal endocervical gland architecture (Fig. 9.10). Gastric-type AIS may be preceded by

simple gastric (pyloric gland) metaplasia that does not show the lobular architecture characteristic of LEGH [29, 30]. In contrast to the LEGH-GAS/MDA sequence, an HPV-independent pathway of carcinogenesis, a subset of gastric-type AIS is positive for $p16^{INK4a}$ [52]. This might be a consequence of heterodifferentiation of usual-type AIS and is related to HPV, although HPV status in this condition remains undetermined.

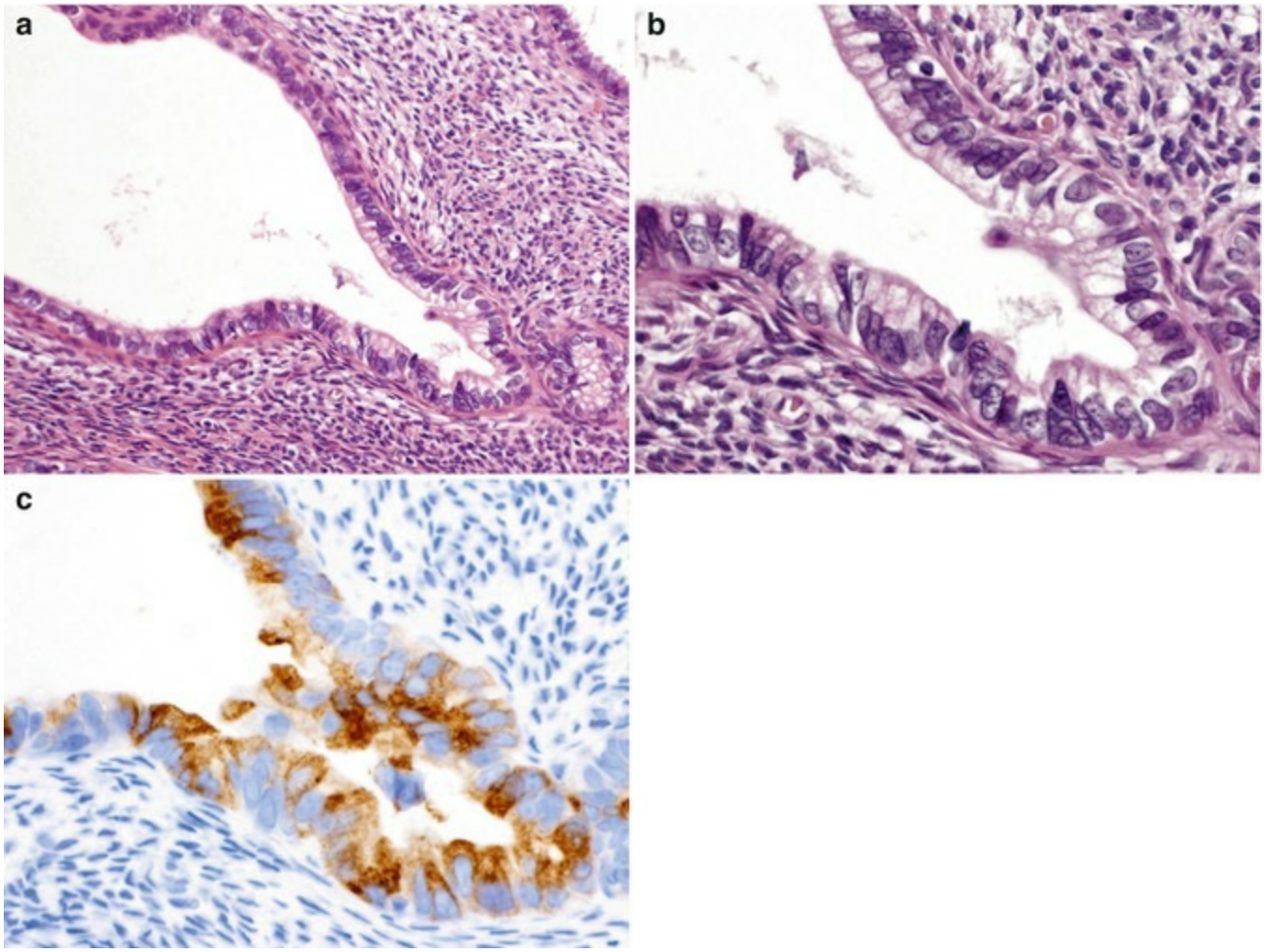

Fig. 9.10 Gastric-type AIS. Preexisting glands lined by atypical columnar cells with clear cytoplasm (**a**). Columnar cells with nuclear enlargement and overlapping and abundant cytoplasm showing distinct cell borders (**b**). Immunohistochemistry using HIK1083, a specific antibody, recognizing pyloric gland mucin, showing positive cytoplasmic staining of glandular cells (**c**)

Precursors of Clear Cell Carcinoma

DES-associated clear cell carcinoma, which typically occurs in young women, is considered to be associated with vaginal adenosis or a congenital abnormality of the lower female genital tract, independent of HPV. A rare example of clear cell-type AIS has been described [58], which can coexist with invasive clear cell carcinoma of the cervix (Fig. 9.11).

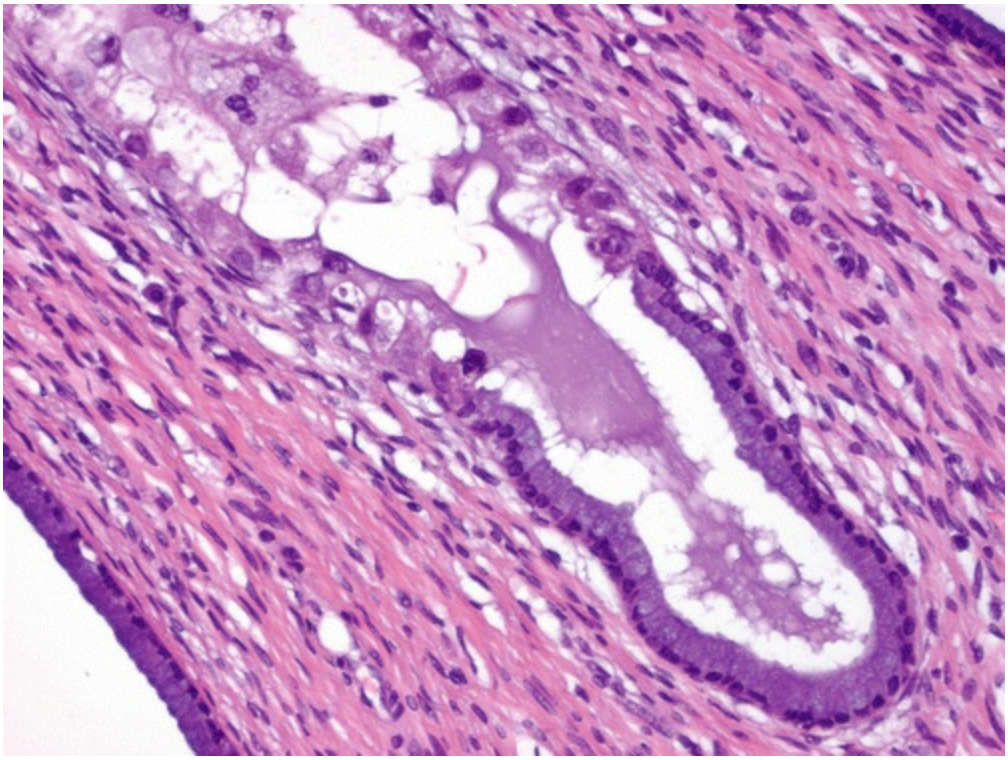

Fig. 9.11 Clear cell carcinoma in situ. The upper portion of the endocervical gland is occupied by atypical cells with abundant clear cytoplasm

Serous Carcinoma In Situ

Serous carcinoma in situ is rarely seen and is characterized by highly anaplastic cells replacing preexisting endocervical glands without destructive stromal invasion (Fig. 9.12). It should be kept in mind that serous endometrial carcinoma may be implanted throughout the endocervix, imparting a close resemblance to serous carcinoma in situ, and this possibility should be considered when examining cervical biopsy specimens.

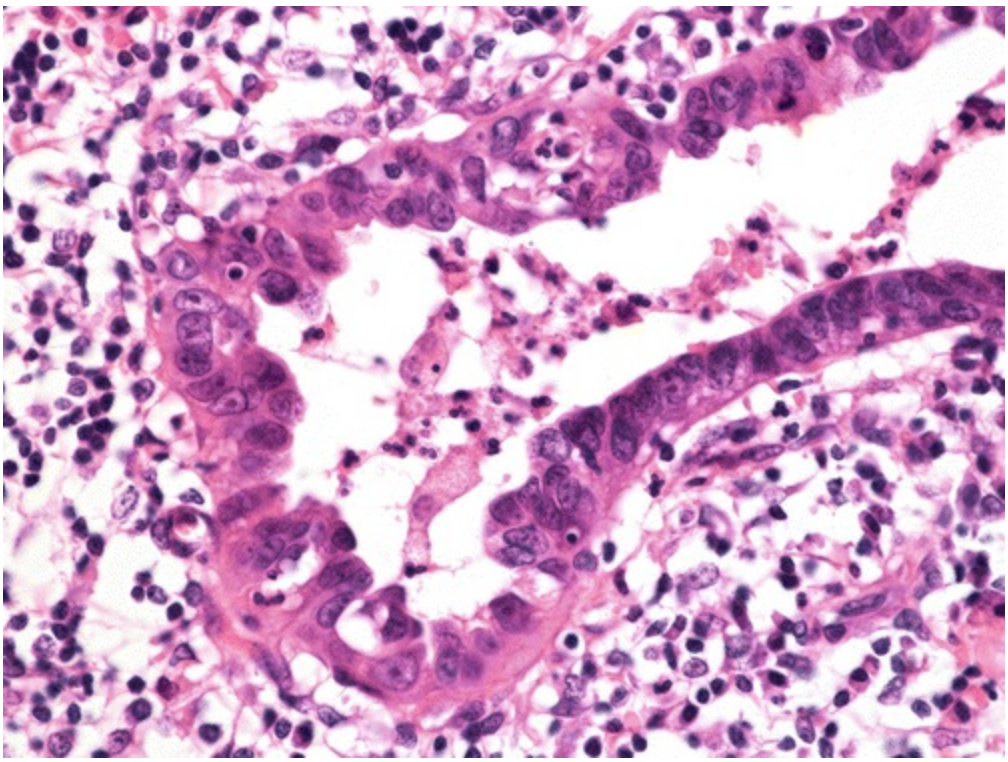

Fig. 9.12 Serous carcinoma in situ. Highly anaplastic cells replacing preexisting mucin-producing columnar cells of the endocervical glands, without destructive stromal invasion

Endometrioid Carcinoma In Situ and Tuboendometrioid Metaplasia or Endometriosis

Jaworski et al. described an endometrioid variant of AIS, and it can be identified in association with usual-type AIS, or be present alone [59]. The differences in detection rate of HPV in the literature suggest that hitherto examined endometrioid carcinomas include HPV-positive usual-type adenocarcinoma with a paucity of intracytoplasmic mucin, providing a resemblance to prototypical and true endometrioid carcinoma, which may be associated with tuboendometrioid metaplasia or endometriosis of the uterine cervix.

Mesonephric Remnants and Hyperplasia

Mesonephric duct remnants and hyperplasia are considered to be putative precursors of mesonephric carcinoma, although in the English-language literature there are no descriptions of mesonephric carcinoma *in situ*. Candidate genes implicated in the pathogenesis of mesonephric carcinoma include *KRAS*, *NRAS*, *ARID1A*, *ARID1B*, *SMARC4A*, and *IDH1* [60].

References

1. Pirog EC, Kleter B, Olgac S, Bobkiewicz P, Lindeman J, Quint WG, et al. Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma. Am J Pathol. 2000;157(4):1055–62. [Crossref][PubMed][PubMedCentral]

2. An HJ, Kim KR, Kim IS, Kim DW, Park MH, Park IA, et al. Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. Mod Pathol. 2005;18(4):528–34. [Crossref][PubMed]

3. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048–56. [Crossref][PubMed]

4. Siriaunkgul S, Utaipat U, Suthipintawong C, Tungsinmunkong K, Triratanachat S, Khunamornpong S. HPV genotyping in adenocarcinoma of the uterine cervix in Thailand. Int J Gynaecol Obstet. 2013;123(3):226–30. [Crossref][PubMed]

5. Park JS, Kim YT, Lee A, Lee Y, Kim KT, Cho CH, et al. Prevalence and type distribution of human papillomavirus in cervical adenocarcinoma in Korean women. Gynecol Oncol. 2013;130(1):115–20. [Crossref][PubMed]

6. Park KJ, Kiyokawa T, Soslow RA, Lamb CA, Oliva E, Zivanovic O, et al. Unusual endocervical adenocarcinomas: an immunohistochemical analysis with molecular detection of human papillomavirus. Am J Surg Pathol. 2011;35(5):633–46. [Crossref][PubMed]

7. Houghton O, Jamison J, Wilson R, Carson J, McCluggage WG. p16 Immunoreactivity in unusual types of cervical adenocarcinoma does not reflect human papillomavirus infection. Histopathology. 2010;57(3):342–50. [Crossref][PubMed]

8. Kusanagi Y, Kojima A, Mikami Y, Kiyokawa T, Sudo T, Yamaguchi S, et al. Absence of high-risk human papillomavirus (HPV) detection in endocervical adenocarcinoma with gastric morphology and phenotype. Am J Pathol. 2010;177(5):2169–75. [Crossref][PubMed][PubMedCentral]

9. Pirog EC, Lloveras B, Molijn A, Tous S, Guimera N, Alejo M, et al. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. Mod Pathol. 2014;27(12):1559–67. [Crossref][PubMed]

10. Wilbur DC, Colgan TJ, Ferenczy TJ, Hirschowitz L, Loening T, McCluggage WG, et al. In: Kurman RM, Carcangiu ML, Herrington CS, Young RH, editors. Glandular tumours and precursors. WHO Classification of Tumours of Female Reproductive Organs. 4th ed. Lyon: IARC; 2014.

11. Wells M, Ostor AG, Crum CP, Franceschi S, Tommasino M, Nesland JM, et al. In: Tavassoli FA, Stratton MR, editors. Epithelial tumours. WHO classification of tumours. Pathology and genetics of tumours of the breast and female genital organs. Lyon: IARCPress; 2003.

12. Togami S, Sasajima Y, Kasamatsu T, Oda-Otomo R, Okada S, Ishikawa M, et al. Immunophenotype and human papillomavirus status of serous adenocarcinoma of the uterine cervix. Pathol Oncol Res. 2014;21(2):487–94. [Crossref][PubMed]

13. Tenti P, Pavanello S, Padovan L, Spinillo A, Vesentini N, Zappatore R, et al. Analysis and clinical implications of p53 gene mutations and human papillomavirus type 16 and 18 infection in primary adenocarcinoma of the uterine cervix. Am J Pathol. 1998;152(4):1057–63. [PubMed][PubMedCentral]

14. Uchiyama M, Iwasaka T, Matsuo N, Hachisuga T, Mori M, Sugimori H. Correlation between human papillomavirus positivity and p53 gene overexpression in adenocarcinoma of the uterine cervix. Gynecol Oncol. 1997;65(1):23–9. [Crossref][PubMed]

15. Tsuda H, Jiko K, Tsugane S, Yajima M, Yamada T, Tanemura K, et al. Prognostic value of p53 protein accumulation in cancer cell nuclei in adenocarcinoma of the uterine cervix. Jpn J Cancer Res. 1995;86(11):1049–53. [Crossref][PubMed]

16. Baalbergen A, Ewing-Graham PC, Eijkemans MJ, Helmerhorst TJ. Prognosis of adenocarcinoma of the uterine cervix: p53 expression correlates with higher incidence of mortality. Int J Cancer. 2007;121(1):106–10. [Crossref][PubMed]

17. Fukushima M, Shimano S, Yamakawa Y, Yamaguchi T, Sawada Y, Hashimoto M, et al. The detection of human papillomavirus (HPV) in a case of minimal deviation adenocarcinoma of the uterine cervix (adenoma malignum) using in situ hybridization. Jpn J Clin Oncol. 1990;20(4):407–12. [PubMed]

18. Ferguson AW, Svoboda-Newman SM, Frank TS. Analysis of human papillomavirus infection and molecular alterations in adenocarcinoma of the cervix. Mod Pathol. 1998;11(1):11–8. [PubMed]

19. Toki T, Zhai YL, Park JS, Fujii S. Infrequent occurrence of high-risk human papillomavirus and of p53 mutation in minimal deviation adenocarcinoma of the cervix. Int J Gynecol Pathol. 1999;18(3):215–9. [Crossref][PubMed]

20. Xu JY, Hashi A, Kondo T, Yuminamochi T, Nara M, Hashi K, et al. Absence of human papillomavirus infection in minimal deviation adenocarcinoma and lobular endocervical glandular hyperplasia. Int J Gynecol Pathol. 2005;24(3):296–302. [Crossref][PubMed]

21. Park KJ, Lamb C, Oliva E, Soslow RA, Kiyokawa T. Unusual endocervical adenocarcinomas: an immunohistochemical analysis with molecular detection of human papillomavirus. Mod Pathol. 2008;21(January suppl-1):217A.

22. Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas De Souza S, et al. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: results from a European multinational epidemiological study. Int J Cancer. 2015;137(12):2858–68. [Crossref][PubMed][PubMedCentral]

23. Molijn A, Jenkins D, Chen W, Zhang X, Pirog E, Enqi W, et al. The complex relationship between human papillomavirus and cervical adenocarcinoma. Int J Cancer. 2016;138(2):409–16.

[Crossref][PubMed]

24. Ueno S, Sudo T, Oka N, Wakahashi S, Yamaguchi S, Fujiwara K, et al. Absence of human papillomavirus infection and activation of PI3K-AKT pathway in cervical clear cell carcinoma. Int J Gynecol Cancer. 2013;23(6):1084–91. [Crossref][PubMed]

25. Kenny SL, McBride HA, Jamison J, McCluggage WG. Mesonephric adenocarcinomas of the uterine cervix and corpus: HPV-negative neoplasms that are commonly PAX8, CA125, and HMGA2 positive and that may be immunoreactive with TTF1 and hepatocyte nuclear factor 1-beta. Am J Surg Pathol. 2012;36(6):799–807. [Crossref][PubMed]

26. Togami S, Sasajima Y, Kasamatsu T, Oda-Otomo R, Okada S, Ishikawa M, et al. Immunophenotype and human papillomavirus status of serous adenocarcinoma of the uterine cervix. Pathol Oncol Res. 2015;21(2):487–94. [Crossref][PubMed]

27. Kojima A, Mikami Y, Sudo T, Yamaguchi S, Kusanagi Y, Ito M, et al. Gastric morphology and immunophenotype predict poor outcome in mucinous adenocarcinoma of the uterine cervix. Am J Surg Pathol. 2007;31(5):664–72. [Crossref][PubMed]

28. Mikami Y, Kiyokawa T, Moriya T, Sasano H. Immunophenotypic alteration of the stromal component in minimal deviation adenocarcinoma ('adenoma malignum') and endocervical glandular hyperplasia: a study using oestrogen receptor and alpha-smooth muscle actin double immunostaining. Histopathology. 2005;46(2):130–6. [Crossref][PubMed]

29. Mikami Y, McCluggage WG. Endocervical glandular lesions exhibiting gastric differentiation: an emerging spectrum of benign, premalignant, and malignant lesions. Adv Anat Pathol. 2013;20(4):227–37. [Crossref][PubMed]

30. McCluggage WG. Recent developments in non-HPV-related adenocarcinomas of the lower female genital tract and their precursors. Adv Anat Pathol. 2016;23(1):58–69. [Crossref][PubMed]

31. Stewart CJ, Frost F, Leake R, Mohan GR, Tan J. Foamy gland changes in gastric-type endocervical neoplasia. Pathology. 2015;47(7):653–8. [Crossref][PubMed]

32. Adsay V, Logani S, Sarkar F, Crissman J, Vaitkevicius V. Foamy gland pattern of pancreatic ductal adenocarcinoma: a deceptively benign-appearing variant. Am J Surg Pathol. 2000;24(4):493–504. [Crossref][PubMed]

33. Carleton C, Hoang L, Sah S, Kiyokawa T, Karamurzin YS, Talia KL, et al. A detailed immunohistochemical analysis of a large series of cervical and vaginal gastric-type adenocarcinomas. Am J Surg Pathol. 2016;40(5):636–44. [Crossref][PubMed][PubMedCentral]

34. Kido A, Mikami Y, Koyama T, Kataoka M, Shitano F, Konishi I, et al. Magnetic resonance appearance of gastric-type adenocarcinoma of the uterine cervix in comparison with that of usual-type endocervical adenocarcinoma: a pitfall of newly described unusual subtype of endocervical adenocarcinoma. Int J Gynecol Cancer. 2014;24(8):1474–9. [Crossref][PubMed]

35. Karamurzin YS, Kiyokawa T, Parkash V, Jotwani AR, Patel P, Pike MC, et al. Gastric-type endocervical adenocarcinoma: an aggressive tumor with unusual metastatic patterns and poor prognosis. Am J Surg Pathol. 2015;39(11):1449–57.
[Crossref][PubMed][PubMedCentral]

36. Omori M, Hashi A, Kondo T, Katoh R, Hirata S. Dysregulation of CDK inhibitors and p53 in HPV-negative endocervical adenocarcinoma. Int J Gynecol Pathol. 2015;34(2):196–203.
[Crossref][PubMed]

37. Young RH, Scully RE. Mucinous ovarian tumors associated with mucinous adenocarcinomas of the cervix. A clinicopathological analysis of 16 cases. Int J Gynecol Pathol. 1988;7(2):99–111.
[Crossref][PubMed]

38. Gilks CB, Young RH, Aguirre P, DeLellis RA, Scully RE. Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix. A clinicopathological and immunohistochemical analysis of 26 cases. Am J Surg Pathol. 1989;13(9):717–29.
[Crossref][PubMed]

39. Ito M, Minamiguchi S, Mikami Y, Ueda Y, Sekiyama K, Yamamoto T, et al. Peutz-Jeghers syndrome-associated atypical mucinous proliferation of the uterine cervix: a case of minimal deviation adenocarcinoma ('adenoma malignum') in situ. Pathol Res Pract. 2012;208(10):623–7.
[Crossref][PubMed]

40. McCluggage WG, Harley I, Houghton JP, Geyer FC, MacKay A, Reis-Filho JS. Composite cervical adenocarcinoma composed of adenoma malignum and gastric type adenocarcinoma (dedifferentiated adenoma malignum) in a patient with Peutz Jeghers syndrome. J Clin Pathol. 2010;63(10):935–41.
[Crossref][PubMed]

41. Moat M, O'Donnell RL, McCluggage WG, Ralte A, Edmondson RJ. Gastric-type adenocarcinoma of the cervix in a patient with Lynch syndrome: a case report. Gynecol Oncol Rep. 2014;10:41–3.
[Crossref][PubMed][PubMedCentral]

42. Young RH, Clement PB. Endocervical adenocarcinoma and its variants: their morphology and differential diagnosis. Histopathology. 2002;41(3):185–207.
[Crossref][PubMed]

43. Witkiewicz AK, Wright TC, Ferenczy A, ROnnett BM, Kurman RJ. Carcinoma and other tumors of the cervix. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's pathology of the female genital tract. 6th ed. New York: Springer; 2011. p. 254–303.

44. Toyoda S, Kita T, Sugiura A, Itani Y, Okada H, Nakamura S, et al. Cervical adenocarcinoma with stromal micropapillary pattern. Diagn Cytopathol. 2016;44(2):133–6.
[Crossref][PubMed]

45. Milde-Langosch K, Riethdorf S, Kraus-Poppinghaus A, Riethdorf L, Loning T. Expression of cyclin-dependent kinase inhibitors p16MTS1, p21WAF1, and p27KIP1 in HPV-positive and HPV-negative cervical adenocarcinomas. Virchows Arch. 2001;439(1):55–61.
[Crossref][PubMed]

46. Scully RE, Robboy SJ, Herbst AL. Vaginal and cervical abnormalities, including clear-cell adenocarcinoma, related

to prenatal exposure to stilbestrol. Ann Clin Lab Sci. 1974;4(4):222–33.
[PubMed]

47. Goyal A, Yang B. Differential patterns of PAX8, p16, and ER immunostains in mesonephric lesions and adenocarcinomas of the cervix. Int J Gynecol Pathol. 2014;33(6):613–9.
[Crossref][PubMed]

48. Howitt BE, Emori MM, Drapkin R, Gaspar C, Barletta JA, Nucci MR, et al. GATA3 is a sensitive and specific marker of benign and malignant mesonephric lesions in the lower female genital tract. Am J Surg Pathol. 2015;39(10):1411–9.
[Crossref][PubMed]

49. Nucci MR, Clement PB, Young RH. Lobular endocervical glandular hyperplasia, not otherwise specified: a clinicopathologic analysis of thirteen cases of a distinctive pseudoneoplastic lesion and comparison with fourteen cases of adenoma malignum. Am J Surg Pathol. 1999;23(8):886–91.
[Crossref][PubMed]

50. Mikami Y, Hata S, Fujiwara K, Imajo Y, Kohno I, Manabe T. Florid endocervical glandular hyperplasia with intestinal and pyloric gland metaplasia: worrisome benign mimic of "adenoma malignum". Gynecol Oncol. 1999;74(3):504–11.
[Crossref][PubMed]

51. Mikami Y, Hata S, Melamed J, Fujiwara K, Manabe T. Lobular endocervical glandular hyperplasia is a metaplastic process with a pyloric gland phenotype. Histopathology. 2001;39(4):364–72.
[Crossref][PubMed]

52. Mikami Y, Kiyokawa T, Hata S, Fujiwara K, Moriya T, Sasano H, et al. Gastrointestinal immunophenotype in adenocarcinomas of the uterine cervix and related glandular lesions: a possible link between lobular endocervical glandular hyperplasia/pyloric gland metaplasia and 'adenoma malignum'. Mod Pathol. 2004;17(8):962–72.
[Crossref][PubMed]

53. Kondo T, Hashi A, Murata S, Nakazawa T, Yuminamochi T, Nara M, et al. Endocervical adenocarcinomas associated with lobular endocervical glandular hyperplasia: a report of four cases with histochemical and immunohistochemical analyses. Mod Pathol. 2005;18(9):1199–210.
[Crossref][PubMed]

54. Kawauchi S, Kusuda T, Liu XP, Suehiro Y, Kaku T, Mikami Y, et al. Is lobular endocervical glandular hyperplasia a cancerous precursor of minimal deviation adenocarcinoma?: a comparative molecular-genetic and immunohistochemical study. Am J Surg Pathol. 2008;32(12):1807–15.
[Crossref][PubMed]

55. Mikami Y, Kojima A, Kiyokawa T, Manabe T. Ki67 labelling index and p53 status indicate neoplastic nature of atypical lobular endocervical glandular hyperplasia (ALEGH). Histopathology. 2009;55(3):362–4.
[Crossref][PubMed]

56. Ito S, Tase T, Satoh K, Ueki M, Sato I, Sasano H. Gastric-type endocervical glandular neoplasms associated with aberrant p16 expression and K-RAS gene mutation in Peutz-Jeghers syndrome. Pathol Int. 2014;64(6):283–8.
[Crossref][PubMed]

57. Matsubara A, Sekine S, Ogawa R, Yoshida M, Kasamatsu T, Tsuda H, et al. Lobular endocervical glandular

hyperplasia is a neoplastic entity with frequent activating GNAS mutations. Am J Surg Pathol. 2014;38(3):370–6. [Crossref][PubMed]

58. Hasumi K, Ehrmann RL. Clear cell carcinoma of the uterine endocervix with an in situ component. Cancer. 1978;42(5):2435–8. [Crossref][PubMed]

59. Jaworski RC, Pacey NF, Greenberg ML, Osborn RA. The histologic diagnosis of adenocarcinoma in situ and related lesions of the cervix uteri. Adenocarcinoma in situ. Cancer. 1988;61(6):1171–81. [Crossref][PubMed]

60. Mirkovic J, Sholl LM, Garcia E, Lindeman N, MacConaill L, Hirsch M, et al. Targeted genomic profiling reveals recurrent KRAS mutations and gain of chromosome 1q in mesonephric carcinomas of the female genital tract. Mod Pathol. 2015;28(11):1504–14. [Crossref][PubMed]

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10. Mesenchymal and Mixed Epithelial-Mesenchymal Neoplasms of the Cervix

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Abstract
This chapter deals with mesenchymal and mixed epithelial-mesenchymal neoplasms of the cervix. These are, in general, uncommon neoplasms compared to their counterparts in the uterine corpus.

Keywords Cervix – Mesenchymal neoplasms – Mixed epithelial and mesenchymal neoplasms

Introduction

Mesenchymal and mixed epithelial and mesenchymal neoplasms (mixed Mullerian tumors) occasionally occur in the cervix but are much less common than in the uterine corpus. Some involve the cervix by direct spread from the corpus. In general, the morphological features are similar to the corresponding neoplasms in the uterine corpus. In this chapter, an overview of these cervical neoplasms is provided, and differences from the corresponding neoplasms in the uterine corpus are discussed when appropriate.

Mesenchymal Neoplasms

Smooth Muscle Neoplasms

Leiomyomas are by far the most common mesenchymal neoplasm to occur within the cervix but are much less common than in the uterine corpus [1]. The morphological features are usually those of a typical leiomyoma composed of bland spindle-shaped cells, but, compared to their counterparts in the corpus, cervical leiomyomas are more likely to exhibit nuclear palisading, reminiscent of a neurilemmoma ("neurilemmoma-like" leiomyoma or "schwannoma-like" leiomyoma) (Fig. 10.1). Leiomyoma variants, similar to those occurring in the corpus, also occur in the cervix. Leiomyosarcomas, including epithelioid and myxoid variants, occasionally occur as primary cervical neoplasms [2].

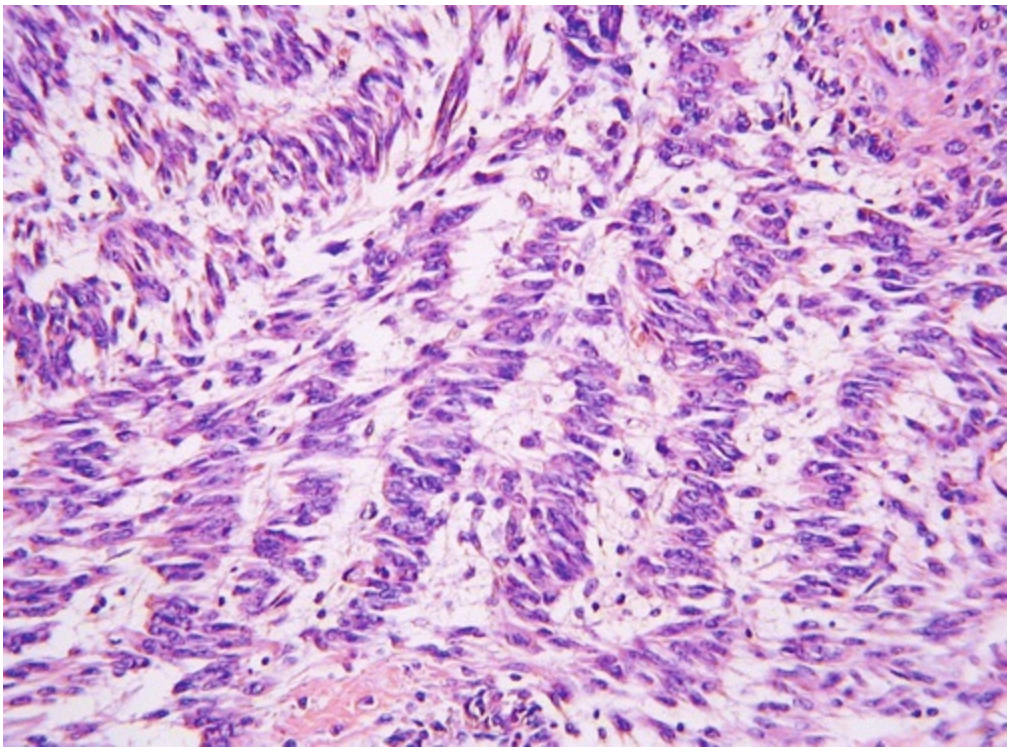

Fig. 10.1 Leiomyoma of the cervix exhibiting nuclear palisading

Embryonal Rhabdomyosarcoma

Embryonal rhabdomyosarcoma (sarcoma botryoides) occurs uncommonly, but not rarely, as a primary cervical neoplasm. Affected patients are most commonly in the late teens and early twenties (mean age 18 years), although the age range is relatively wide and much older patients can be affected [3–9]. The usual presentation is vaginal bleeding or a mass protruding from the introitus. There is an association between cervical embryonal rhabdomyosarcomas, ovarian Sertoli-Leydig cell tumors, thyroid goiters, pleuropulmonary blastomas, and some other embryonic neoplasms. This is due to underlying germline *DICER1* mutation [10, 11]. Since some studies show that a significant minority of patients (around 20%) with cervical embryonal rhabdomyosarcoma have other DICER1-associated tumors [12], the diagnosis should

prompt consideration of the DICER1 syndrome with a careful review of the patient's personal and family history with genetic studies if appropriate. It is also noteworthy that while most patients with either proven germline *DICER1* mutation or DICER1-associated cervical embryonal rhabdomyosarcoma reported in the literature are relatively young (<25 years), occasional cases arise in older women [13] such that older age is not a reliable criterion for excluding DICER1 syndrome in patients with cervical embryonal rhabdomyosarcoma.

Grossly, cervical embryonal rhabdomyosarcoma usually takes the form of a polypoid mass or multiple polyps, which may be totally removed by polypectomy. Occasionally, there is an infiltrative mass without a polypoid architecture, but this is uncommon. The cut surface may be myxoid with areas of necrosis, and some neoplasms have an overtly botryoid (grapelike) gross appearance.

Histological examination usually shows a polypoid lesion covered by a variety of types of benign glandular Mullerian-type epithelium, sometimes with focal squamous differentiation (Fig. 10.2a). Glands may also be present deep within the core of the neoplasm. The features of malignancy may be subtle in that, in large part, the stroma can be hypocellular and myxoid or edematous. However, tightly packed hypercellular foci are also present which sometimes coalesce to form large cellular aggregates. There is usually mitotic and apoptotic activity within the cellular foci (Fig. 10.2b). Characteristically there is increased cellularity around the glandular elements, resulting in a cambium layer, and here mitotic figures and apoptotic bodies are usually apparent. Most of the stromal cells have small hyperchromatic nuclei with scant cytoplasm and delicate cytoplasmic processes, but cells with larger nuclei and an almost epithelioid appearance may be present. Cells with more abundant eosinophilic cytoplasm and cytoplasmic cross striations may also be identified (Fig. 10.2c), but these are typically difficult to find, are not present in all cases, and are not necessary for the diagnosis. Islands of hyaline or cellular, but benign, cartilage are a common feature being found in approximately 50% of these neoplasms (Fig.10.2d), a much higher incidence than in embryonal rhabdomyosarcoma arising at other sites [3–9]. In occasional cases, there are foci resembling alveolar rhabdomyosarcoma, or collections of pleomorphic cells with multilobated nuclei are present; the clinical significance of these features is uncertain. Hyaline globules may be present in association with the pleomorphic cells. There are commonly areas of hemorrhage with extravasated erythrocytes or necrosis. The hemorrhage may be so extensive as to mask the underlying hypercellular areas to some extent.

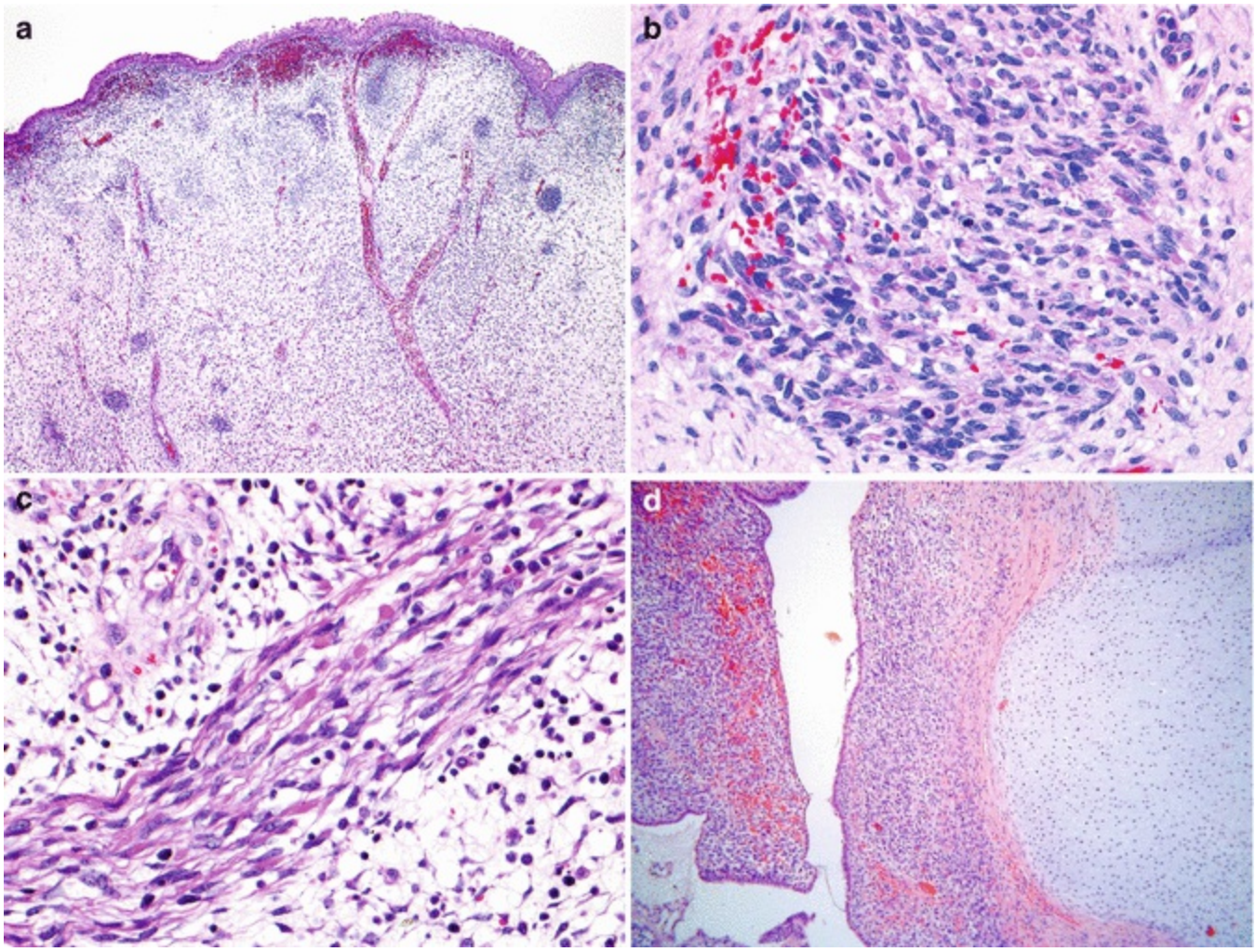

Fig. 10.2 Embryonal rhabdomyosarcoma of the cervix with low-power polypoid architecture. The lesion is covered by squamous epithelium, and the underlying stroma is somewhat edematous with cellular foci (**a**). There are cellular aggregates exhibiting mitotic activity (**b**). Collections of cells with more abundant eosinophilic cytoplasm are present in some cases (**c**). Islands of cellular cartilage are present in some cases (**d**)

Positive nuclear staining with the skeletal muscle markers myogenin and myoD1 assists in diagnosis, but typically only a minor proportion of the nuclei are immunoreactive (Fig. 10.3). Desmin is usually positive but normal cervical stroma is also desmin positive. Hormone receptors (estrogen receptor (ER) and progesterone receptor (PR)) are generally negative, as is smooth muscle actin and h-caldesmon.

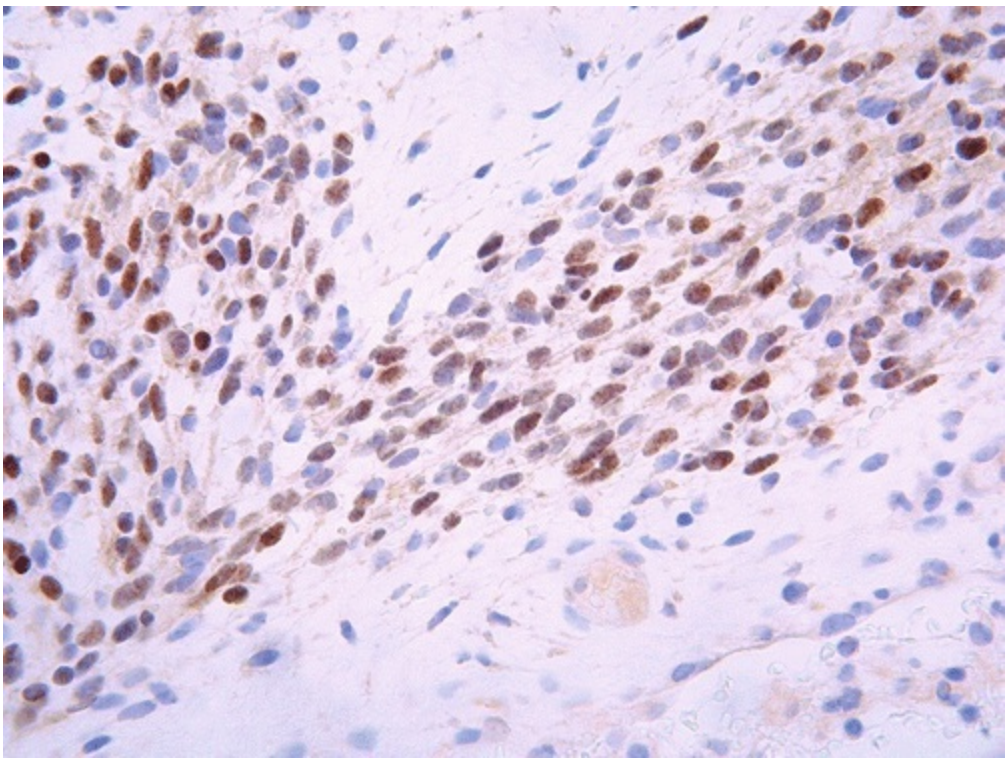

Fig. 10.3 Cervical embryonal rhabdomyosarcoma exhibiting nuclear staining with myogenin

Given the polypoid nature of the lesion and the presence of a cambium layer, often the main differential diagnosis is an adenosarcoma with heterologous stromal elements, especially in those cases where glands are present deep within the core of the neoplasm. An absence of the typical phyllodes-like (club-like or leaflike) architecture of adenosarcoma is helpful, as is the usual relative paucity of glands deep within the stroma. Adenosarcomas usually occur in an older age group. However, in some cases the distinction between cervical embryonal rhabdomyosarcoma and adenosarcoma may be arbitrary. Given the hypocellular background, an unusual benign endocervical or endometrial polyp or endometriosis may also be considered in the differential diagnosis, but these are usually easily excluded given the morphological features described above. Carcinosarcoma is excluded due to the absence of a malignant epithelial component.

Most cervical embryonal rhabdomyosarcomas are treated by a combination of surgery (which may be radical or comprise local conservative excision) and chemotherapy, and the overall prognosis is good with an approximately 80% overall survival [14]. However, there are few large series with significant follow-up. In agreement with these findings, patients with cervical embryonal rhabdomyosarcoma presented at lower stage and had a better 5-year prognosis than younger patients with vaginal rhabdomyosarcoma in a review of the SEER database [14]. The main adverse prognostic feature is deep invasion of the cervical stroma, but this is uncommon.

Myofibroblastoma of the Lower Female Genital Tract

Myofibroblastoma of the lower female genital tract was first described by Laskin et al. and was originally referred to as superficial cervicovaginal myofibroblastoma [15]. These authors reported a distinctive mesenchymal tumor arising in the superficial lamina propria of the cervix and vagina [15]. The term superficial cervicovaginal myofibroblastoma was proposed to encompass the superficial location in the cervix or vagina and presumed myofibroblastic differentiation. A subsequent series of cases involved the vagina and the vulva, and the term superficial myofibroblastoma of the lower female genital tract was proposed since some neoplasms have a vulval location [16, 17].

These neoplasms occur in premenopausal or postmenopausal women and usually present as polypoid lesions. Some patients have been on tamoxifen, raising the possibility of an association with this medication [15–17]. Based on the morphology and follow-up, superficial myofibroblastoma of the lower female genital tract is a benign lesion, although there is uncommonly local recurrence following excision [15–17]. Metastasis or malignant transformation has not been reported.

Grossly these are well circumscribed and are often, but not always, polypoid in appearance. Histological examination shows a well-circumscribed but unencapsulated lesion covered by unremarkable squamous or glandular epithelium. Deep to the surface epithelium, there is usually an uninvolved grenz zone, although sometimes the lesion extends up to the epithelial-stromal junction. There are typically areas of varying cellularity, the constituent cells having bland ovoid, spindled, or stellate nuclei, sometimes with a somewhat wavy appearance. The cells are embedded in a finely collagenous stroma, sometimes with thicker collagen bundles (Fig. 10.4a). Multiple architectural patterns, including lacelike, sievelike, and fascicular, which result in a heterogeneous appearance, are a characteristic feature. The stroma is often overtly edematous which results in the lacelike architecture. Occasionally, there is stromal myxoid change. Few or no mitoses are present.

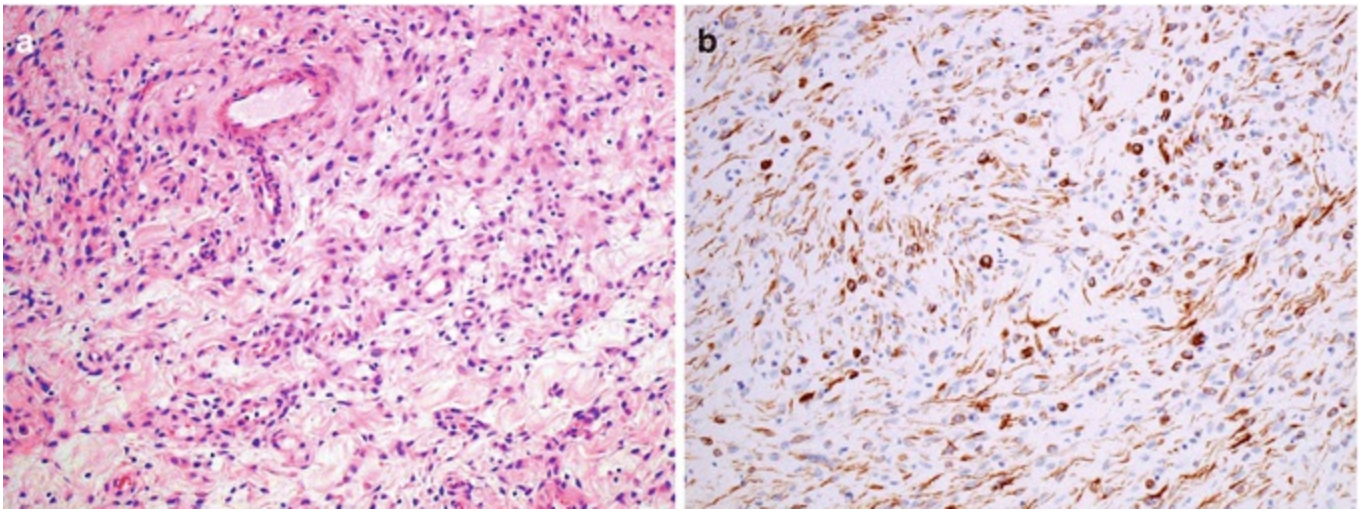

Fig. 10.4 Superficial myofibroblastoma of the lower female genital tract with bland spindle-shaped cells in an edematous stroma (**a**). There is diffuse staining with desmin which highlights dendritic cell processes (**b**)

The cells are positive with vimentin and almost always with desmin [15–17]. CD34 and smooth muscle actin (SMA) are positive in some cases, and most are ER and PR positive. S100, EMA, h-caldesmon, HMGA2, and cytokeratins are negative. Desmin staining typically highlights the ramifying dendritic processes of many of the tumor cells (Fig. 10.4b) [15–17]. The immunophenotype is nonspecific and identical to that of many of the other site-specific mesenchymal lesions which involve the lower female genital tract, especially the vulva and vagina.

The main differential diagnosis in the cervix is likely to be an unusual endocervical polyp, and focally the stroma of endocervical polyps may resemble myofibroblastoma of the lower female genital tract. However, mucinous glands are usually present throughout endocervical polyps, while more than an occasional entrapped gland is unusual in myofibroblastoma of the lower female genital tract. A fibroepithelial polyp may also enter into the differential diagnosis. The grenz zone which is typical of superficial myofibroblastoma of the lower female genital tract is not a feature of fibroepithelial polyp, and the former is characterized by a more heterogeneous appearance with a variety of architectural patterns. Negative staining with S100 helps to exclude a neural lesion which may enter into the differential diagnosis since some of the morphological features, such as the presence of wavy nuclei, may raise this possibility.

Other Mesenchymal Neoplasms

Many other mesenchymal tumors have been reported as primary neoplasms in the cervix, but these are generally more common in the corpus. They include endometrial stromal neoplasms, uterine tumor resembling ovarian sex cord tumor (UTROSCT), alveolar soft part sarcoma (more common in the cervix than uterine corpus), inflammatory myofibroblastic tumor, epithelioid sarcoma, perivascular epithelioid cell

tumor (PEComa), malignant rhabdoid tumor, schwannoma, neurofibroma, hemangioma, rhabdomyoma, liposarcoma, angiosarcoma, tumors in the Ewing family, and malignant peripheral nerve sheath tumor (malignant schwannoma) ([18–25]; reviewed in [25]). The morphological and immunohistochemical features are identical to when these neoplasms occur at more usual sites, but the pathologist may not think of the diagnosis given the rarity of these neoplasms in the cervix. Three cases of an S100- and CD34-positive cervical sarcoma which the authors termed fibroblastic malignant peripheral nerve sheath tumor (neurofibrosarcoma) have been reported [26]. Rare cases of pseudoneoplastic myxoid change of the cervical stroma have been reported (Fig. 10.5) [27, 28]. Fibroepithelial polyps, similar to those occurring in the vulva or vagina, rarely arise within the cervix and may contain a population of atypical stromal fibroblasts.

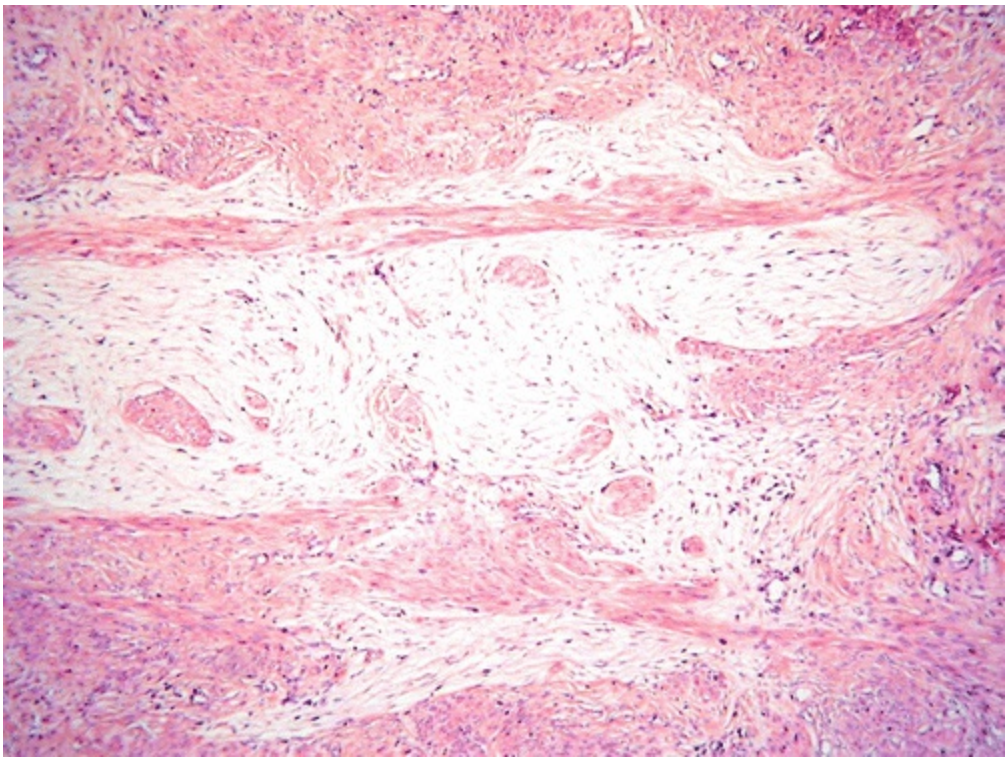

Fig. 10.5 Pseudoneoplastic myxoid change of cervical stroma

Mixed Epithelial and Mesenchymal Neoplasms

The same variety of mixed epithelial and mesenchymal neoplasms (mixed Mullerian tumors) that affect the uterine corpus, namely, carcinosarcoma, adenofibroma, and adenosarcoma, occurs more uncommonly in the cervix [29]. There is also a specific type of adenomyoma, termed an endocervical adenomyoma, which occurs within the cervix.

Carcinosarcoma

Carcinosarcomas of the cervix are much less common than their counterpart within the uterine corpus [30]. Morphologically they are characterized by malignant epithelial and mesenchymal components, both of which are typically high grade and sharply demarcated from one another. The epithelial component may be squamous, glandular of various types (including mesonephric), or undifferentiated. Compared to carcinosarcomas of the uterine corpus, the epithelial component is more likely to be squamous, adenoid cystic, adenoid cystic-like, or adenoid basal in type (Fig. 10.6). Occasional mesonephric adenocarcinomas of the cervix contain spindle cell elements, and this could be regarded as a mesonephric carcinosarcoma [31, 32]. The mesenchymal component in cervical carcinosarcomas may comprise undifferentiated sarcoma, fibrosarcoma and leiomyosarcoma, or heterologous elements such as chondrosarcoma or rhabdomyosarcoma may be present. Before making a diagnosis of a primary carcinosarcoma of the cervix, spread from a neoplasm in the uterine corpus should be excluded. Carcinosarcoma of the cervix with a squamous element should be distinguished from squamous carcinoma with a spindle cell component (spindle cell squamous carcinoma); positive staining with cytokeratins or p63 in the spindle cell elements may assist in diagnosing spindle cell squamous carcinoma, although expression of these markers is often absent or markedly reduced in the spindle cells. Some carcinosarcomas of the cervix have been found to contain high-risk human papillomavirus (HPV), especially HPV16 [33].

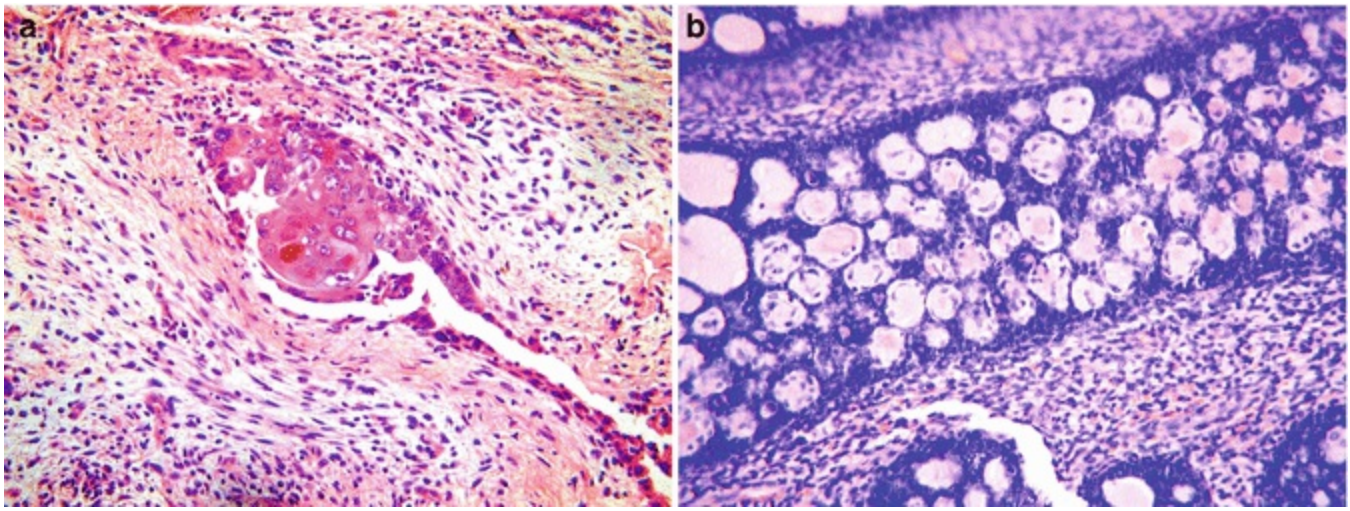

Fig. 10.6 Carcinosarcoma of the cervix composed of malignant epithelial and mesenchymal components. The epithelial element is squamous in type (**a**). In (**b**), the epithelial component is adenoid cystic-like

Adenofibroma and Adenosarcoma

Adenofibroma and adenosarcoma are rare primary cervical neoplasms and are much

less common than their counterparts in the uterine corpus; the latter may involve the cervix by direct extension [29, 34, 35]. In fact, adenofibroma is not included in the 2014 World Health Organization (WHO) Classification of cervical neoplasms, although it is included in the category of mixed epithelial and mesenchymal neoplasms of the uterine corpus [36]. Adenofibromas and adenosarcomas are composed of a benign epithelial component and a stromal component which is benign (adenofibroma) or malignant (adenosarcoma). Adenofibroma is much less common than adenosarcoma, and some doubt the existence of the former [34, 37].

Grossly adenofibromas and adenosarcomas are usually polypoid lesions, sometimes with a lobulated architecture or a "spongy" appearance on cut surface, which project into the cervical canal (Fig. 10.7a). Morphologically, the low-power architecture is "club-like," "leaflike," or "phyllodes-like" (Fig. 10.7b). The surface is covered by benign glandular epithelium of a variety of Mullerian types; there may be foci of the squamous epithelium. The stromal component is usually morphologically quite bland and nondescript fibrous or endometrial stroma-like. According to the prior 2003 WHO Classification, adenosarcoma is distinguished from adenofibroma by increased cellularity surrounding the epithelial elements (cambium layer), more than mild stromal atypia, and mitotic activity in excess of 2 per 10 high-power fields (Fig. 10.7c) [38]. However, in practice, a diagnosis of adenosarcoma is usually made in the absence of this degree of mitotic activity if the characteristic low-power architecture and cambium layer are present [39]. This is reflected in the 2014 WHO Classification where no mitotic cutoff is given to distinguish between adenosarcoma and adenofibroma [36].

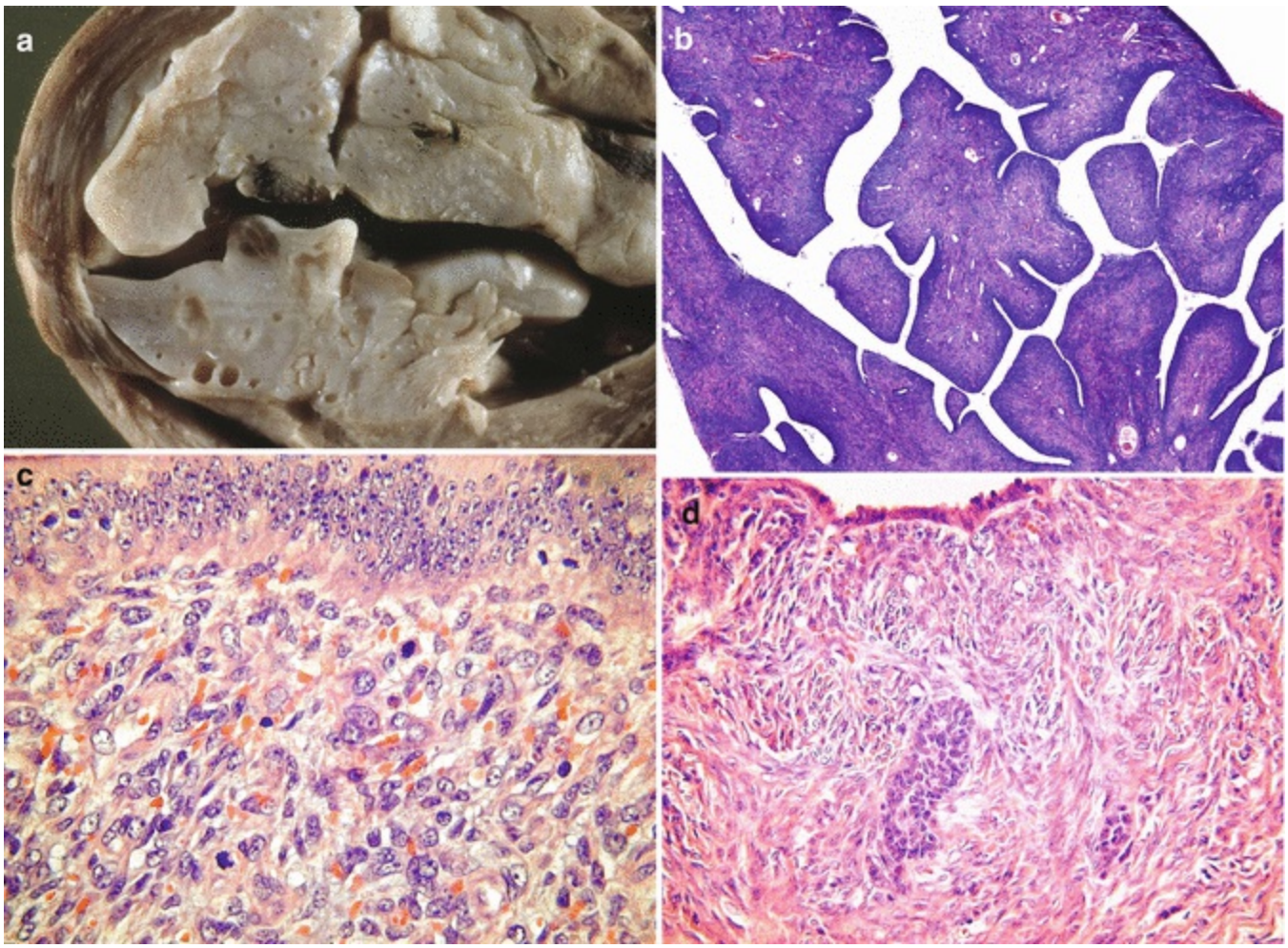

Fig. 10.7 Adenosarcoma of the cervix exhibiting a gross lobulated architecture (**a**). On low power there is a "phyllodes-like" architecture with increased cellularity around glands (cambium layer) (**b**). There is mitotic activity within the cambium layer (**c**). Adenosarcoma exhibiting sex cord-like foci within the stromal component (**d**)

Sometimes, there is focal "sex cord-like" differentiation within the stromal component where the stromal cells have an epithelioid appearance and are arranged in nests, cords, and trabeculae, resembling ovarian sex cord cells (Fig. 10.7d); sometimes, but not always, the sex cord-like foci are positive with markers of ovarian sex cord-stromal tumors such as inhibin and calretinin. Rarely there are heterologous elements in the form of rhabdomyoblasts or cartilage.

The treatment of choice of adenofibromas and adenosarcomas is hysterectomy given the risk of recurrence following polypectomy. Even adenofibromas may recur. The main adverse prognostic features in adenosarcoma are deep stromal invasion and sarcomatous overgrowth, both of which are uncommon [34]. Sarcomatous overgrowth is defined as areas of pure sarcoma without epithelium involving greater than 25% of the neoplasm. The areas of sarcomatous overgrowth, which often comprise much more than 25% of the neoplasm such that residual benign epithelium may be identified only focally and by extensive sampling, are usually composed of poorly differentiated sarcoma,

resembling undifferentiated sarcoma, with much more atypia and mitotic activity than in the sarcomatous element of the residual adenosarcoma. As such, this can be regarded as dedifferentiation of the low-grade stromal component. Heterologous elements, most commonly rhabdomyosarcoma, may be present in the areas of sarcomatous overgrowth.

Previously, there was no staging system for uterine adenosarcoma, but FIGO staging systems for uterine sarcomas were introduced in 2009 [40]. Adenosarcomas have a separate staging system to leiomyosarcomas and endometrial stromal sarcomas. Stage 1 adenosarcomas are confined to the uterus (corpus and cervix) with substages of IA, IB, and IC (tumor limited to endometrium/endocervix with no myometrial invasion, less than or equal to half myometrial invasion, more than half myometrial invasion, respectively) [40].

It has been proposed to combine adenofibroma and adenosarcoma into a single category of mixed epithelial and mesenchymal neoplasm. This approach recognizes that these are a spectrum of tumors composed of a benign epithelial component and a stromal element which is an integral part of the neoplasm and which is generally of low-grade malignancy [34, 37]. One reason for this approach is that it has been shown that occasional tumors which would be categorized as adenofibroma on the basis of a low mitotic count can recur or even metastasize [37]. Additionally, there are multiple problems in counting mitotic figures with significant interobserver variation among pathologists.

Occasional benign endocervical (or endometrial) polyps contain focal areas which raise the possibility of a lesion in the adenofibroma/adenosarcoma category. For example, focally there may be a "phyllodes-like" architecture and/or increased cellularity surrounding the glands. Such cases are best reported as benign endocervical (or endometrial) polyps with unusual features. A recent study has shown that follow-up in such cases is usually uneventful [41]. The differential diagnosis between adenosarcoma and embryonal rhabdomyosarcoma is discussed in the section on "Embryonal Rhabdomyosarcoma".

Endocervical Adenomyoma

Adenomyomas of endocervical type (endocervical adenomyomas) are uncommon lesions usually occurring in women of reproductive or postmenopausal age [39, 42]. They vary in size and are most commonly polypoid and project from the mucosal surface of the cervix. Rare examples are intramural or exophytic. They are grossly well circumscribed, usually gray-white to tan, and may contain small cysts. Histologically, they are composed of bland mucinous glands of endocervical type, often with a somewhat lobular arrangement, embedded in a stroma containing abundant smooth muscle. Some of the glands may be dilated. There is often a thin rim of fibrous tissue surrounding the glands which is in turn surrounded by smooth muscle (Fig. 10.8). There

may be focal mild nuclear atypia of the epithelial component and minor foci of tubal, endometrioid, or squamous differentiation, but there is no stromal desmoplasia. Other findings which are occasionally seen include gland rupture with mucin extravasation, small intraglandular papillary proliferations (adenofibroma-like), a component of adipose tissue, and symplastic change in the smooth muscle component [39, 42]. These are benign lesions but occasionally persist or recur following local excision.

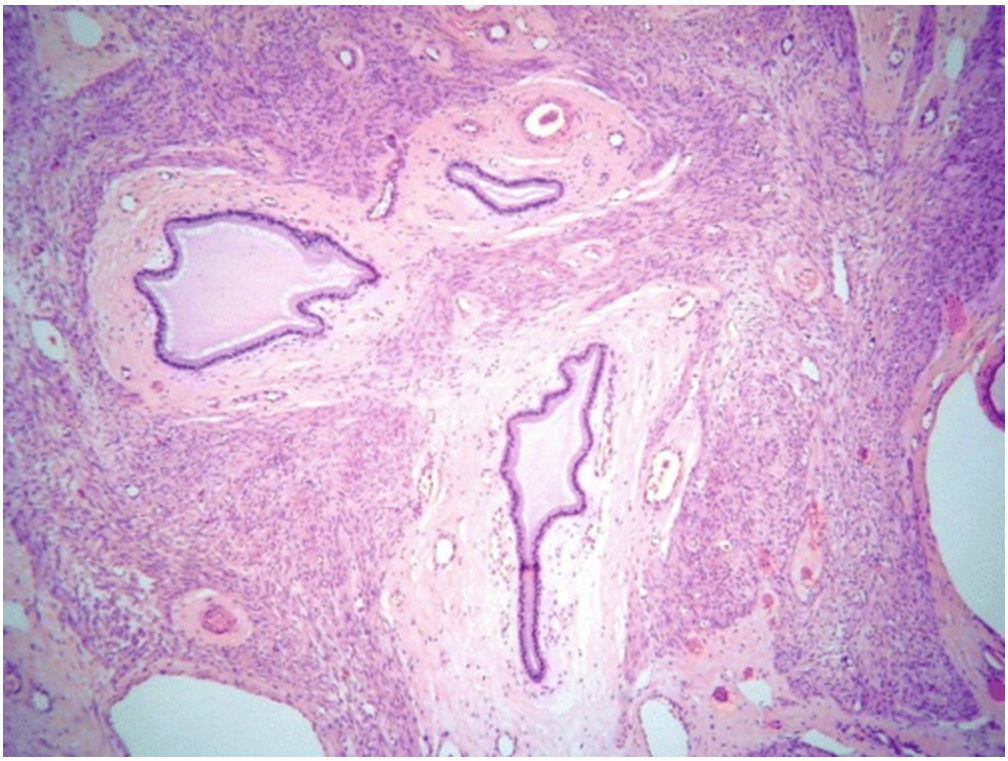

Fig. 10.8 Endocervical adenomyoma. Mucinous endocervical glands are surrounded by a thin rim of loose stroma which in turn is surrounded by mature smooth muscle

The main differential diagnoses are adenoma malignum (mucinous variant of minimal deviation adenocarcinoma) and lobular endocervical glandular hyperplasia (see Chap. 9). The circumscription of endocervical adenomyoma together with an absence of irregular stromal infiltration, a desmoplastic stromal response and focal significant nuclear atypia, and the presence of abundant smooth muscle assists in excluding adenoma malignum. Lobular endocervical glandular hyperplasia may be considered since in endocervical adenomyoma the glands can have a somewhat lobular arrangement. However, lobular endocervical glandular hyperplasia is not polypoid, is often an incidental microscopic finding, and lacks a smooth muscle component. ER staining may be useful in that the glands in endocervical adenomyoma are positive, while adenoma malignum and lobular endocervical glandular hyperplasia (part of the spectrum of "gastric-type" endocervical glandular lesions; see Chap. 9) are usually "flat" negative [43]. Benign endocervical polyps may contain a minor population of

smooth muscle fibers within the stroma, but this should not result in diagnostic confusion.

References

1. Lesh RE. Fibromyoma of the uterine cervix. J Int Coll Surg. 1950;14:122–3.
[PubMed]

2. Grayson W, Fourie J, Tiltman AJ. Xanthomatous leiomyosarcoma of the uterine cervix. Int J Gynecol Pathol. 1998;17:89–90.
[Crossref][PubMed]

3. Daya D, Scully RE. Sarcoma botryoides of the uterine cervix in young women: a clinicopathological analysis of 13 cases. Gynecol Oncol. 1998;29:290–304.
[Crossref]

4. McClean GE, Kurian S, Walter N, Kekre A, McCluggage WG. Cervical embryonal rhabdomyosarcoma and ovarian Sertoli-Leydig cell tumour: a more than coincidental association of two rare neoplasms? J Clin Pathol. 2007;60:326–8.
[Crossref][PubMed][PubMedCentral]

5. Golbang P, Khan A, Scurry J, Maclsaac I, Planner R. Cervical sarcoma botryoides and ovarian Sertoli-Leydig cell tumor. Gynecol Oncol. 1997;67:102–6.
[Crossref][PubMed]

6. Dehner LP, Jarzembowski JA, Hill DA. Embryonal rhabdomyosarcoma of the uterine cervix: a report of 14 cases and a discussion of its unusual clinicopathological associations. Mod Pathol. 2012;25:602–14.
[Crossref][PubMed]

7. Li FL, Gupta M, McCluggage WG, Ronnett BM. Embryonal rhabdomyosarcoma (Botryoid Type) of the uterine corpus and cervix in adult women: report of a case series and review of the literature. Am J Surg Pathol. 2013;37:344–55.
[Crossref][PubMed]

8. Foulkes WD, Bahubeshi A, Hamel N, et al. Expanding the phenotypes associated with DICER1 mutations. Hum Mutat. 2011;32:1381–4.
[Crossref][PubMed]

9. Houghton JP, McCluggage WG. Embryonal rhabdomyosarcoma of the cervix with focal pleomorphic areas. J Clin Pathol. 2007;60:88–9.
[Crossref][PubMed][PubMedCentral]

10. Priest JR, Watterson J, Strong L, et al. Pleuropulmonary blastoma: a marker for familial disease. J Pediatr. 1996;128:220–4.
[Crossref][PubMed]

11. Hill DA, Ivanovich J, Priest JR, et al. DICER1 mutations in familial pleuropulmonary blastoma. Science. 2009;325:965.
[Crossref][PubMed][PubMedCentral]

12.

Krisemen ML, Wang W-L, Sullinger J, et al. Rhabdomyosarcoma of the cervix in adult women and younger patients. Gynecol Oncol. 2012;126:351–6.
[Crossref]

13. de Kock L, Boshari T, Martinelli F, et al. Adult-onset cervical embryonal rhabdomyosarcoma and DICER1 mutations. J Low Genit Tract Dis. 2016;20(1):e8–e10.
[Crossref][PubMed]

14. Kirsch CH, Goodman M, Esiashvili N. Outcome of female pediatric patients diagnosed with genital tract rhabdomyosarcoma based on analysis of cases registered in SEER database between 1973 and 2006. Am J Clin Oncol. 2014;37:47–50.
[Crossref][PubMed]

15. Laskin WB, Fetsch JF, Tavassoli FA. Superficial cervicovaginal myofibroblastoma: fourteen cases of a distinctive mesenchymal tumor arising from the specialized subepithelial stroma of the lower female genital tract. Hum Pathol. 2001;32:715–25.
[Crossref][PubMed]

16. Ganesan R, McCluggage WG, Hirschowitz L, Rollason TP. Superficial myofibroblastoma of the lower female genital tract: report of a series including tumours with a vulval location. Histopathology. 2005;46:137–43.
[Crossref][PubMed]

17. Stewart CJ, Amanuel B, Brennan BA, et al. Superficial cervicovaginal myofibroblastoma: a report of five cases. Pathology. 2005;37:144–8.
[Crossref][PubMed]

18. Nielsen GP, Oliva E, Young RH, et al. Alveolar soft-part sarcoma of the female genital tract. Int J Gynecol Pathol. 1995;24:131–5.

19. Rabban JT, Zaloudek CJ, Shekitka KM, Tavassoli FA. Inflammatory myofibroblastic tumor of the uterus: a clinicopathologic study of 6 cases emphasizing distinction from aggressive mesenchymal tumors. Am J Surg Pathol. 2005;29:1348–55.
[Crossref][PubMed]

20. Kabbani W, Deavers MT, Malpica A, et al. Uterine tumor resembling ovarian sex-cord tumor: report of a case mimicking cervical adenocarcinoma. Int J Gynecol Pathol. 2003;22:297–302.
[Crossref][PubMed]

21. Wei EX, Albores-Saavedra J, Fowler MR. Plexiform neurofibroma of the uterine cervix: a case report and review of the literature. Arch Pathol Lab Med. 2005;129:783–6.
[PubMed]

22. Wang YC, Chen CH, Su HY, et al. Huge spindle cell hemangioma of the cervix mimicking a pelvic tumor. Gynecol Obstet Investig. 2005;60:98–101.
[Crossref]

23. Keel SB, Clement PB, Prat J, Young RH. Malignant schwannoma of the uterine cervix: a study of three cases. Int J Gynecol Pathol. 1998;17:223–30.
[Crossref][PubMed]

24. Cenacchi G, Pasquinelli G, Montanaro L, et al. Primary endocervical extraosseous Ewing's sarcoma/PNET. Int J Gynecol Pathol. 1998;17:83–8.
[Crossref][PubMed]

25. Fadare O. Uncommon sarcomas of the uterine cervix: a review of selected entities. Diagn Pathol. 2006;1:30. [Crossref][PubMed][PubMedCentral]

26. Mills AM, Karamchandani JR, Vogel H, Longacre TA. Endocervical fibroblastic malignant peripheral nerve sheath tumor (neurofibrosarcoma): report of a novel entity possibly related to endocervical CD34 fibrocytes. Am J Surg Pathol. 2011;35:404–12. [Crossref][PubMed]

27. McCluggage WG, Young RH. Myxoid change of the myometrium and cervical stroma: description of a hitherto unreported non-neoplastic phenomenon with discussion of myxoid uterine lesions. Int J Gynecol Pathol. 2010;29:351–7. [Crossref][PubMed]

28. Pugh A, McCluggage WG, Hirschowitz L. Multifocal uterine myxoid change: a newly recognized association with neurofibromatosis type 1. Int J Gynecol Pathol. 2012;31:580–3. [Crossref][PubMed]

29. McCluggage WG. A practical approach to the diagnosis of mixed epithelial and mesenchymal tumours of the uterus. Mod Pathol. 2016;29(Supp1):S78–91. [Crossref][PubMed]

30. Clement PB, Zubovits JT, Young RH, Scully RE. Malignant mullerian mixed tumors of the uterine cervix: a report of nine cases of a neoplasm with morphology often different from its counterpart in the corpus. Int J Gynecol Pathol. 1998;17:211–22. [Crossref][PubMed]

31. Clement PB, Young RH, Keh P, Ostor AG, Scully RE. Malignant mesonephric neoplasms of the uterine cervix. A report of eight cases, including four with a malignant spindle cell component. Am J Surg Pathol. 1995;19:1158–71. [Crossref][PubMed]

32. Bague S, Rodriquez IM, Prat J. Malignant mesonephric tumors of the female genital tract. A clinicopathologic study of 9 cases. Am J Surg Pathol. 2004;28:601–7. [Crossref][PubMed]

33. Grayson W, Taylor LF, Cooper K. Carcinosarcoma of the uterine cervix: a report of eight cases with immunohistochemical analysis and evaluation of human papillomavirus status. Am J Surg Pathol. 2001;25:338–47. [Crossref][PubMed]

34. McCluggage WG. Mullerian adenosarcoma of the female genital tract. Adv Anat Pathol. 2010;17:122–9. [Crossref][PubMed]

35. Clement PB, Scully RE. Mullerian adenosarcoma of the uterus: a clinicopathologic analysis of 100 cases with a review of the literature. Hum Pathol. 1990;21:363–81. [Crossref][PubMed]

36. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO classification of tumours of female reproductive organs. Lyon: International Agency for Research on Cancer; 2014.

37. Gallardo A, Prat J. Mullerian adenosarcoma: a clinicopathologic and immunohistochemical study of 55 cases challenging the existence of adenofibroma. Am J Surg Pathol. 2009;33:278–88. [Crossref][PubMed]

38. Tavassoli FA, Devilee P. World health organization classification of tumours. In:Pathology and genetics. Tumours

of the breast and female genital organs. Lyon: IARC Press; 2003.

39. Casey S, McCluggage WG. Adenomyomas of the uterine cervix: report of a cohort including endocervical and novel variants. Histopathology. 2015;66:420–9.
[Crossref][PubMed]

40. Prat J. FIGO staging for uterine sarcomas. Int J Gynecol Obstet. 2009;104:177–8.
[Crossref]

41. Howitt BE, Quade BJ, Nucci MR. Uterine polyps with features overlapping with those of Mullerian adenosarcoma: a clinicopathologic analysis of 29 cases emphasizing their likely benign nature. Am J Surg Pathol. 2015;39:116–26.
[Crossref][PubMed]

42. Gilks CB, Young RH, Clement PB, Hart WR, Scully RE. Adenomyomas of the uterine cervix of endocervical type: a report of ten cases of a benign cervical tumor that may be confused with adenoma malignum. Mod Pathol. 1996;9:220–4.
[PubMed]

43. Mikami Y, McCluggage WG. Endocervical glandular lesions exhibiting gastric differentiation: an emerging spectrum of benign, premalignant and malignant lesions. Adv Anat Pathol. 2013;20:227–37.
[Crossref][PubMed]

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11. Other Cervical Neoplasms

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Abstract
This chapter focuses on tumors not covered elsewhere in the book. These include neuroendocrine tumors, adenosquamous carcinoma, glassy cell carcinoma, basaloid tumors, melanocytic lesions, germ cell tumors, hematologic disorders and secondary tumors.

Keywords Small cell neuroendocrine carcinoma – Large cell neuroendocrine carcinoma – Adenosquamous carcinoma – Glassy cell carcinoma – Adenoid basal carcinoma – Cervical melanoma – Undifferentiated carcinoma – Lymphoma – Leukemia – Langerhans cell histiocytosis – Metastasis

Neuroendocrine Tumors and Carcinomas (NETs)

Clinical and Gross Features

Neuroendocrine tumors (NETs) of the cervix are rare tumors and comprise less than 2% of all invasive carcinomas [1]. The clinical presentation of NETs is very similar to other carcinomas of the cervix, that is, vaginal bleeding/discharge, detection of a cervical mass, or abnormal cytology [2]. The macroscopic appearance of NETs is also not distinctive. Importantly, a variety of peptides such as calcitonin, gastrin, serotonin, substance P, vasoactive intestinal peptide, pancreatic polypeptide, somatostatin, and adrenocorticotrophic hormone may be produced by NETs [3, 4], although patients rarely present with symptoms or biochemical evidence of ectopic hormone production.

Nevertheless, NET may rarely be associated with the syndrome of inappropriate antidiuretic hormone secretion [5]. Subsequent metastatic disease can be accompanied by the development of carcinoid syndrome [6]. Gynecological cytology may not detect NETs in many cases [7, 8].

Histopathology

Four types of NETs are recognized [9]. Cervical carcinoid tumor, also known as low-grade neuroendocrine tumor grade 1 (ICD-O Code 8240/3), is extremely rare and is primarily defined by the same organoid architecture and cytological features used at other sites. Immunohistochemistry for synaptophysin, chromogranin, CD56, and neuron-specific enolase can support the histological diagnosis.

Cervical atypical carcinoid tumors, also known as low-grade neuroendocrine tumor grade 2 (ICD-O 8249/3), are extremely rare and are distinguished from the usual carcinoid tumor by their greater degree of nuclear atypia and mitotic activity.

High-grade neuroendocrine carcinoma, small cell type, also known as small cell neuroendocrine carcinoma (SCNEC, ICD-O 8041/3), is the most common of the NETs and resembles its counterpart in the lung. SCNECs are primarily defined by their morphological appearance and have a monotonous population of small cells with ovoid hyperchromatic nuclei, often exhibiting molding, and scanty cytoplasm (Fig. 11.1). The amount of cytoplasm can vary from minimal ("oat cell") to moderate ("intermediate") in amount [10]. Abundant mitotic and apoptotic activity with extensive necrosis and lymphovascular and perineural invasion is usually present (Fig. 11.2). SCNEC may be accompanied by in situ or invasive squamous or glandular neoplasia [3, 11–14]. Recently, a case of mixed SCNEC and endocervical adenocarcinoma was reported in a woman with a family history of Muir-Torre syndrome [15].

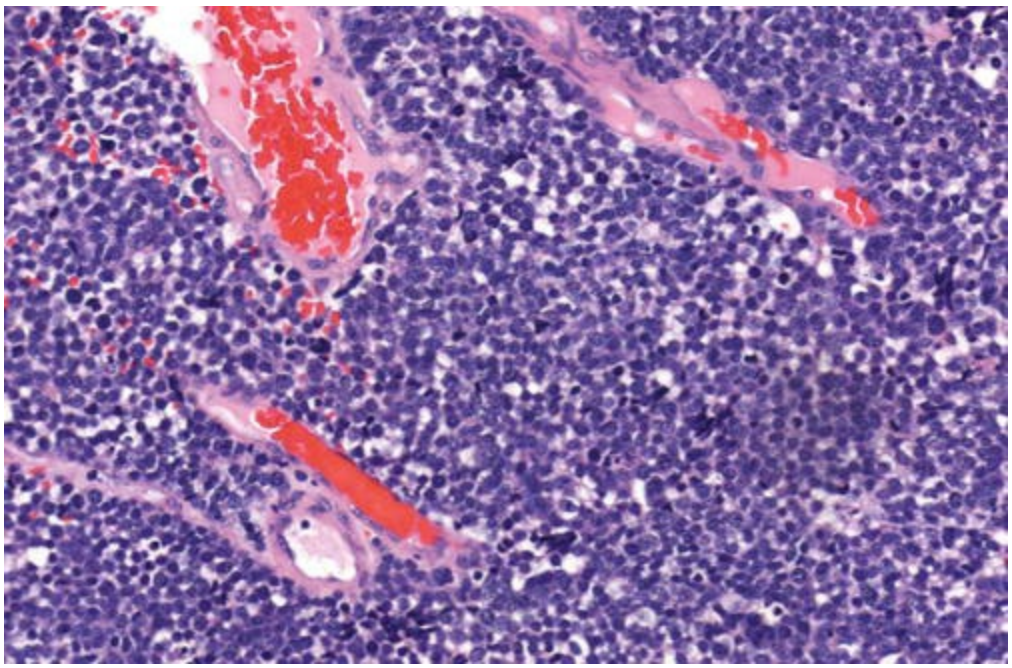


Fig. 11.1 Small cell neuroendocrine carcinoma consisting of sheets of malignant cells with minimal cytoplasm. The peripheral circumscription of these sheets suggests epithelial differentiation, that is, carcinoma. Immunohistochemistry could provide further supportive evidence

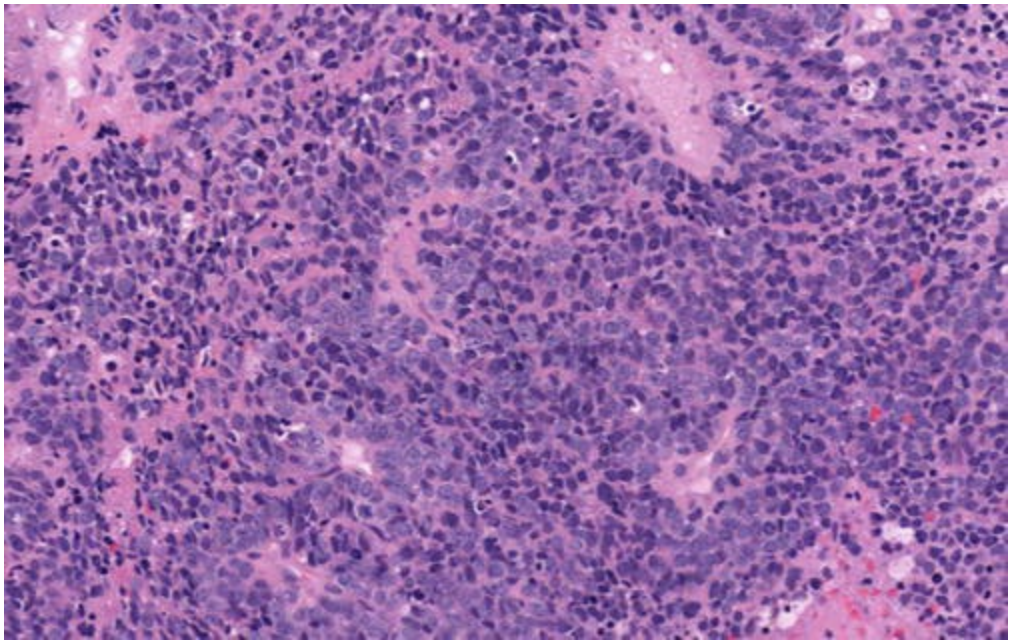


Fig. 11.2 Small cell neuroendocrine carcinoma with poorly delineated trabeculae of small malignant cells showing no architectural differentiation. Crush artifact is prominent and commonly seen

Previously, argyrophilia was used to detect neuroendocrine differentiation [16, 17]. Immunohistochemical staining for neuroendocrine markers (chromogranin, synaptophysin, CD56) may provide support for a diagnosis of SCNEC (Fig. 11.3), but some of these neoplasms may not express any neuroendocrine markers, and only

epithelial markers such as cytokeratin and epithelial membrane antigen may be found [1, 18]. Immunoreactivity for serotonin, somatostatin, gastrin, glucagon, and pancreatic polypeptide has been demonstrated [10]. TTF1 can be expressed in cervical SCNEC, so this antibody cannot be used to distinguish SCNEC from a pulmonary primary [18, 19]. The prime differential diagnostic considerations for SCNEC are lymphoma and the small cell variant of squamous cell carcinoma, although in some cases granulocytic sarcoma, stromal sarcoma, and rhabdomyosarcoma may need to be considered. Diffuse p63 nuclear immunoreactivity is typical of the small cell variant of squamous carcinoma. SCNEC may occasionally fail to express cytokeratins. CD56 and synaptophysin are the most sensitive markers for SCNEC; chromogranin and PGP9.5 are less so. CD56 staining can be present in non-neuroendocrine carcinomas [18].

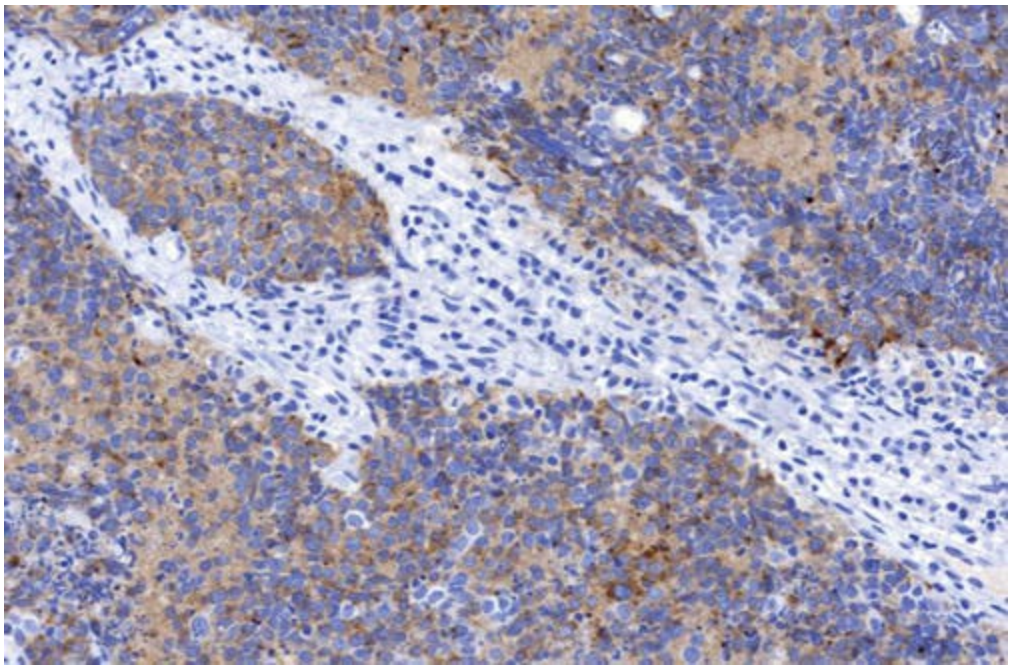


Fig. 11.3 Synaptophysin immunostain in small cell neuroendocrine carcinoma. Immunostains for neuroendocrine markers can provide support for the histopathologic diagnosis but are not essential

Large cell neuroendocrine carcinoma (LCNEC), also known as high-grade neuroendocrine carcinoma, large cell type (ICD-O 8013/3), is characterized by a diffuse, organoid, trabecular, or cord-like pattern (Fig. 11.4). The neoplastic cells have abundant cytoplasm, large nuclei, prominent nucleoli, and a high mitotic rate. Focal glandular differentiation may be present [20–22]. Strong and diffuse positive staining for neuroendocrine markers is essential for a definitive diagnosis (Fig. 11.5). Mixed LCNEC and SCNEC may occur [23, 24]. Non-neuroendocrine cervical carcinomas of both squamous and glandular types need to be distinguished from LCNEC. Neuroendocrine differentiation is not exclusively found in LCNEC, and a scanty number of cells with neuroendocrine features can be found in squamous carcinomas and

adenocarcinomas and should not lead to a diagnosis of LCNEC if the typical morphological features of LCNEC are absent. LCNECs frequently express TTF1 and may be p63 positive [18].

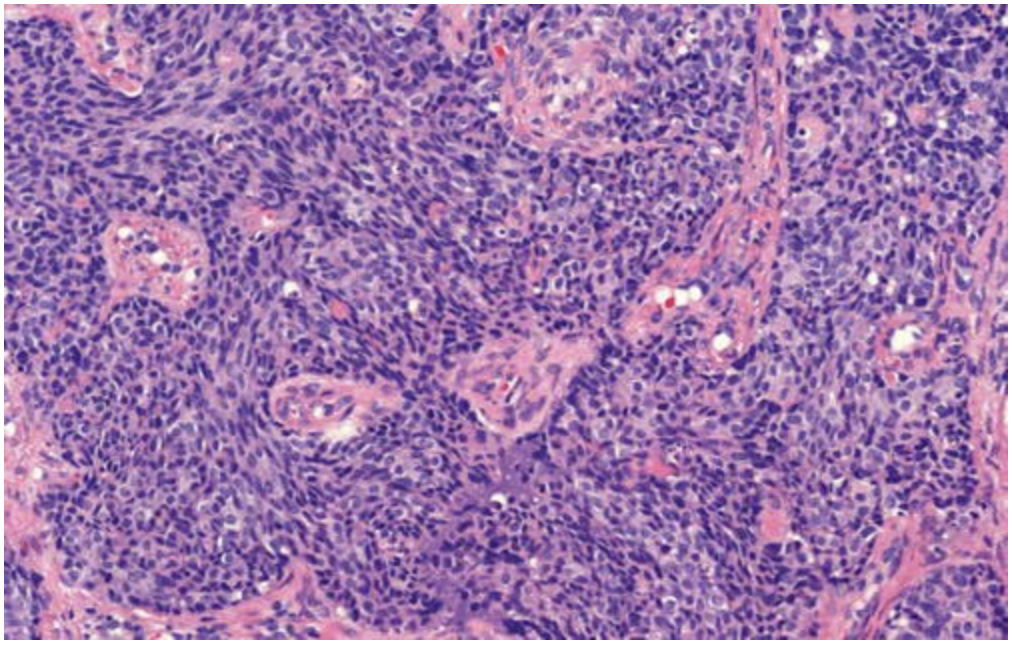


Fig. 11.4 This large cell neuroendocrine carcinoma shows the characteristic organoid trabecular composed of large cells

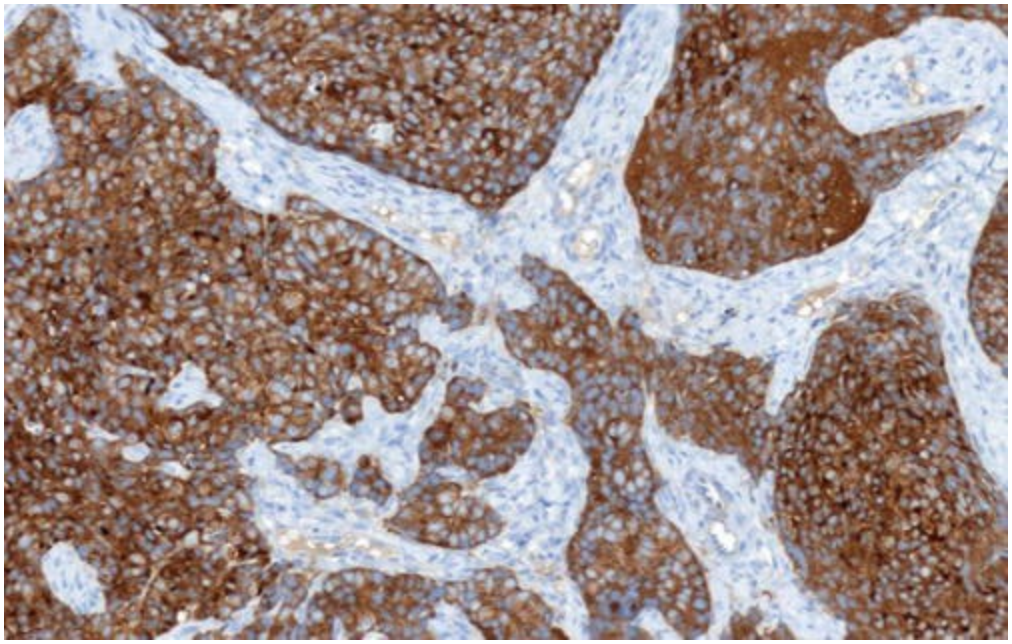


Fig. 11.5 This large cell neuroendocrine carcinoma (LCNEC) shows strong and diffuse cytoplasmic staining for synaptophysin. Positivity for neuroendocrine markers is essential for a diagnosis of LCNEC

Histogenesis

Neuroendocrine differentiation occurs within neoplasms arising from the cervical epithelium. Cells that express neuroendocrine markers are present in some cases of cervical adenocarcinoma in situ and could be the precursor of cervical neuroendocrine tumors [18, 25]. Both preinvasive and invasive squamous and glandular neoplasia may be found in association with cervical NETs, but glandular lesions are proportionately commoner (Fig. 11.6). High-risk human papillomavirus (HPV) can be identified in most cervical NETs [8, 14, 26, 27]. HPV 16 predominates in LCNEC, while, in most studies, HPV 18 has been found to be most common in SCNECs [3, 8, 10, 27, 28]. Strong nuclear staining for p16 is typically found in SCNECs as a result of Rb dysfunction [8]. The most frequent allelic loss in NETs is localized 3p deletion [14, 26]. Occasional 9p21 deletions have also been identified [26]. Amplification of chromosome 3q has been identified in LCNEC, similar to LCNEC of the lung [29].

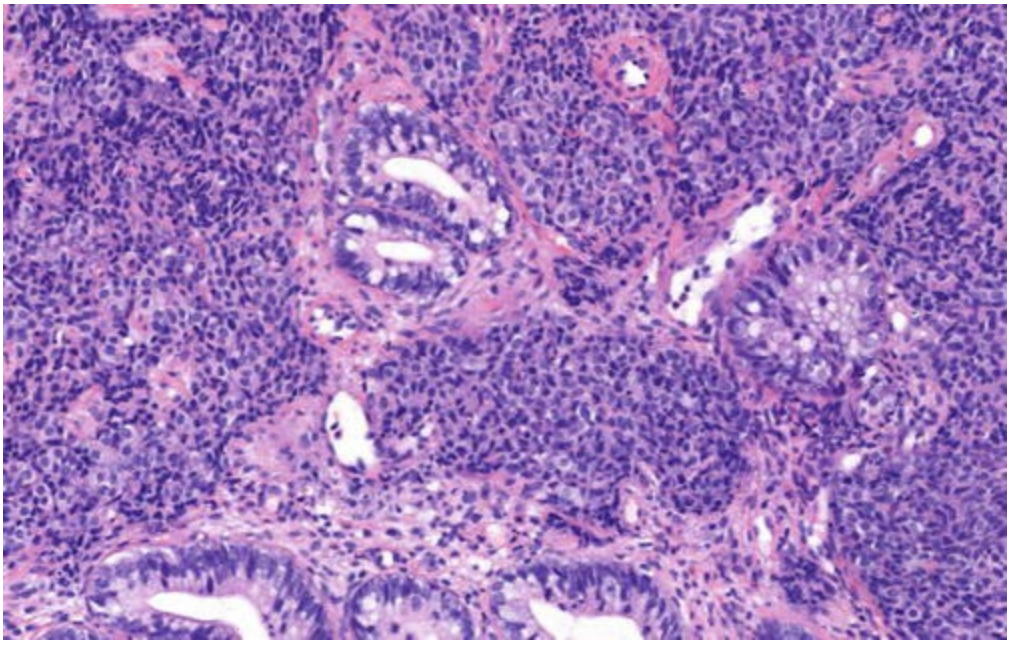


Fig. 11.6 This large cell neuroendocrine carcinoma is associated with adenocarcinoma in situ of intestinal type

Prognosis and Management

The prognosis of NETs is determined by histological type. Carcinoid tumors of the cervix generally follow a benign course. Atypical carcinoid tumors are aggressive neoplasms, although reports of cases with follow-up are few [30]. Their behavior may be similar to large cell neuroendocrine carcinomas [31]. SCNEC frequently presents at an advanced stage and is a highly aggressive carcinoma. Five-year survival for SCNEC of all stages is reported to be 14–39%, with poorer survival in higher stage disease [3, 11, 14, 32]. Similar to SCNEC, cervical LCNEC is an aggressive neoplasm with a poor prognosis [33].

The management of SCNEC may include surgery, chemoradiation, specific

neuroendocrine-based systemic chemotherapy, and axial radiation therapy [25, 34, 35]. A management algorithm using multiple modalities has been defined by the Society of Gynecologic Oncology [36].

Adenosquamous Carcinoma

Clinical and Gross Features

The clinical presentation and macroscopic appearance of adenosquamous carcinoma (ICD-O 8560/3) are not distinctive.

Histopathology

An admixture of malignant epithelial elements exhibiting both glandular and squamous architecture is the defining characteristic of adenosquamous carcinoma (Fig. 11.7). Scattered mucin-producing cells may occur in a squamous cell carcinoma [37], and this finding is not sufficient for a diagnosis of adenosquamous carcinomas. Routine staining for mucin in squamous carcinomas is not recommended since the identification of mucin has no clinical value [37]. Carcinomas having abundant mucin-producing cells without evidence of squamous differentiation (intercellular bridges, keratinization) should be diagnosed as poorly differentiated adenocarcinoma. A clear cell variant of adenosquamous carcinoma characterized by a clear appearance of the squamous component due to extensive glycogen has been described [38].

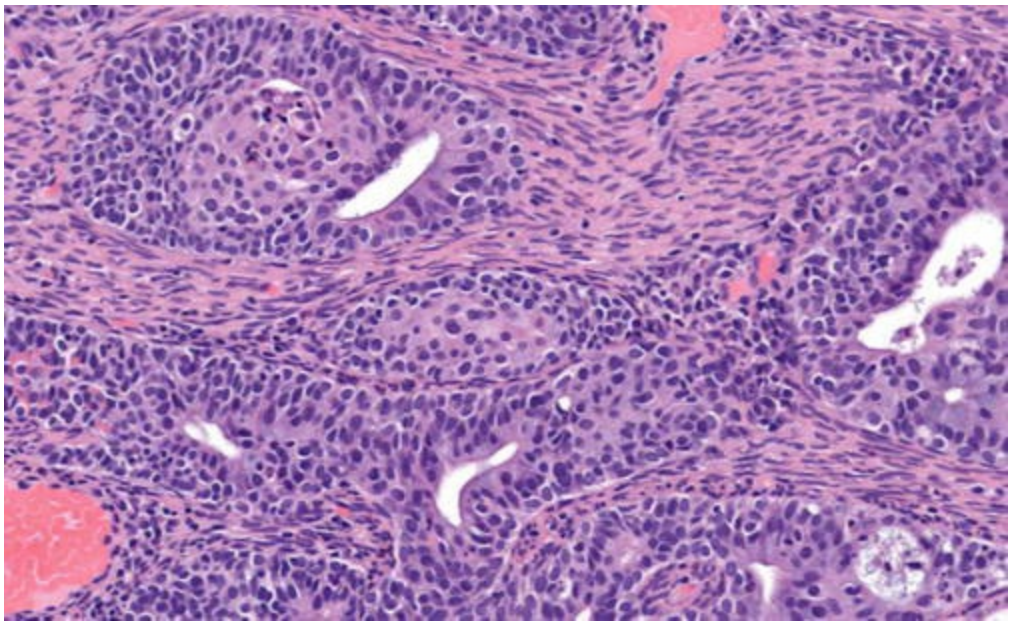


Fig. 11.7 This adenosquamous carcinoma exhibits both glandular and squamous architectural differentiation. Columnar epithelium lines the glandular lumina and serves to distinguish this adenosquamous carcinoma from squamous carcinoma with pseudo-acinar change. Mucin production alone within a squamous carcinoma does not

qualify as an adenosquamous carcinoma

Mucoepidermoid carcinomas are a distinctive variant of adenosquamous carcinoma and characterized by a three cell types (epidermoid, mucin-producing, and intermediate).

Histogenesis

Both cervical intraepithelial neoplasia (CIN)/squamous intraepithelial lesions (SILs) (see Chap. 6) and adenocarcinoma in situ (AIS) (see Chap. 8) are precursor lesions for adenosquamous carcinomas. Adenosquamous carcinomas likely are derived from epithelial reserve cells with subsequent bidirectional differentiation. Multiplex real-time polymerase chain reaction detects HPV in 80% of adenosquamous carcinomas, usually HPV 16/HPV 18; mixed HPV infection occurs in about a third of cases [39]. HPV 18 is the most prevalent HPV type in adenosquamous carcinoma, followed by HPV 16 [40]. The expression of adenine-thymine-rich interactive domain 1A (ARID1A) is downregulated in adenosquamous carcinoma as compared to squamous cell carcinoma, suggesting that this gene may have a role in the pathogenesis of adenosquamous carcinoma [41]. Consequently, the protein expression of ARID1A is frequently lost in adenosquamous carcinomas [42]. The t(11;19)-associated *CRTC1-MAML2* gene fusion is identified in cervical mucoepidermoid, but not adenosquamous, carcinomas [43].

Prognosis and Management

Whether adenosquamous differentiation is an independent predictor of poor outcome is unclear [44]. Some studies have indicated that adenosquamous carcinoma has a poorer outcome than adenocarcinoma and/or squamous carcinoma [44–47]. The balance of evidence suggests that adenosquamous carcinoma has a similar behavior and prognosis to squamous and adenocarcinomas [48–57]. Cervical carcinomas of an advanced-stage adenosquamous differentiation may be a predictor of poorer outcome [58]. HPV negativity in an adenosquamous carcinoma may be an indicator of poor prognosis [59].

Glassy Cell Carcinoma Variant of Adenosquamous Carcinoma

Clinical and Gross Features

Glassy cell carcinoma (ICD-O 8015/3) is a poorly differentiated variant of adenosquamous carcinoma and comprises no more than 2% of cervical carcinomas. Typically it occurs in young women, is characterized by a rapid course, and may have distant metastases. There are no distinctive gross features of glassy cell carcinoma.

Histopathology

The distinctive features of glassy cell carcinoma include sharp cytoplasmic margins, glassy eosinophilic cytoplasm, and large round to ovoid nuclei with prominent nucleoli [60]. A prominent eosinophilic infiltrate may be found in the adjacent stroma [61]. However, eosinophilic infiltrates may also be identified in invasive squamous carcinoma. The tumor cells lack estrogen and progesterone receptors [62].

Histogenesis

Glassy cell carcinomas are associated with HPV 18 and probably originate from multipotential stem or reserve cells [63].

Prognosis and Management

Some studies of glassy cell carcinomas have identified a poor prognosis and worse outcome than other cervical carcinomas [60]. Recent studies have not confirmed these prognostic findings [64, 65]. Surgery is the mainstay of treatment for early-stage glassy cell carcinoma; neoadjuvant radiochemotherapy has been recommended for more advanced disease [61, 66–68].

Undifferentiated carcinoma

Undifferentiated carcinoma (ICD-O 8020/3) is defined as a malignant epithelial neoplasm lacking evidence of specific differentiation (WHO) and is therefore a diagnosis of exclusion. In most cases of cervical carcinoma, squamous or glandular differentiation can be seen at least focally. Undifferentiated carcinomas represent 0.2–5% of cervical carcinomas, depending on the population [69–71]. In the vast majority of cases evaluated, undifferentiated carcinoma of the cervix is associated with high-risk HPV [71, 72]. It is aggressive and tends to present at later stages [69].

The classification of undifferentiated carcinoma is distinct from poorly differentiated large cell neuroendocrine carcinomas (see above). The differential diagnosis of undifferentiated carcinoma includes poorly differentiated squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma, and carcinosarcoma. Because of the lack of differentiation, undifferentiated carcinoma can also be difficult to distinguish from malignant melanoma, large cell lymphoma, and sarcoma with large epithelioid cells. Endometrial undifferentiated carcinoma may also involve the cervix [73].

Adenoid Basal Carcinoma

Clinical and Gross Features

Adenoid basal carcinoma (ABC), also known as adenoid basal epithelioma (ICD-O 8098/3), is a rare tumor and usually occurs in women older than 50. Patients are usually asymptomatic and without detectable clinical or gross abnormality of the cervix unless associated with another carcinoma type. The tumor is usually discovered as an incidental microscopic finding [74].

Histopathology

ABC is composed solely of small well-differentiated rounded nests and cords of basaloid cells with scanty cytoplasm and focal gland or squamous formation (Figs. 11.8, 11.9, and 11.10). These nests infiltrate the cervical stroma and are often associated with CIN [74, 75]. The small cells are p16 positive on immunohistochemistry [76]. Invasive squamous carcinoma and small cell neuroendocrine carcinoma may be seen in association with ABC. The presence of such an invasive carcinoma excludes a case from categorization as ABC [77] and such cases should be labeled as "mixed carcinoma." Consequently, a confident diagnosis of "pure" ABC cannot be rendered on a small biopsy, and definitive diagnosis requires the evaluation of the entire tumor [76, 77]. "Adenoid basal hyperplasia" has been described and is characterized by a proliferation of small basaloid HPV-negative nests extending less than 1 mm from the basement membrane [78]. Until additional descriptions are made, such lesions are better classified within adenoid basal carcinoma. The differential diagnosis of ABC includes adenoid cystic (ACC), squamous, and neuroendocrine carcinomas [79]. ABC and ACC share many immunohistochemical similarities [80]. ABC may be distinguished from ACC by its CD117 negativity [81]. A single report has suggested that p16 IHC can be used to distinguish low-grade, noninvasive ABC from invasive ABC [82].

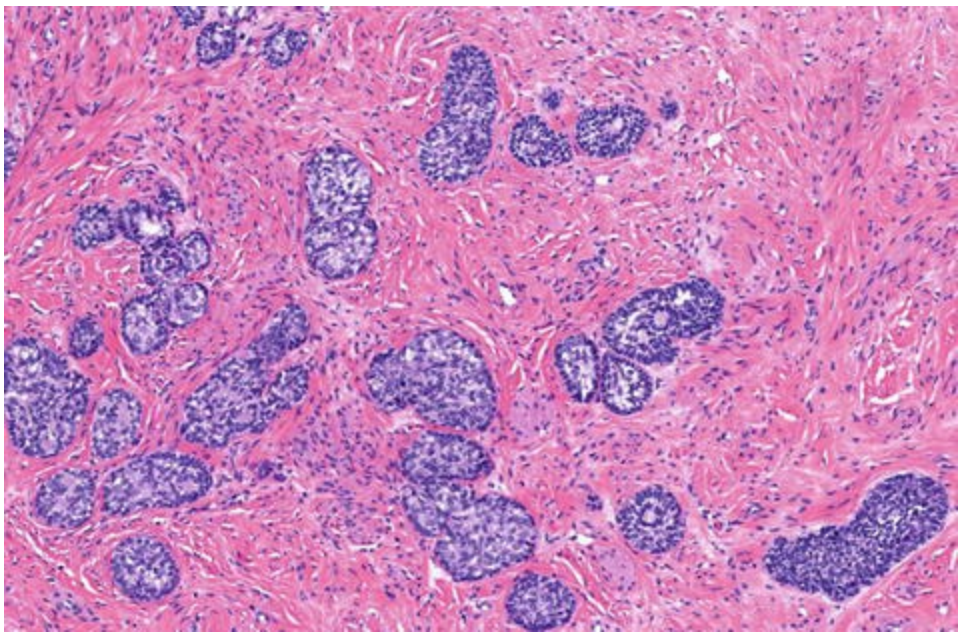


Fig. 11.8 Adenoid basal carcinoma is composed of nests and cords of basaloid cells coursing throughout the stroma. The cells have scanty cytoplasm that lack any nuclear atypia

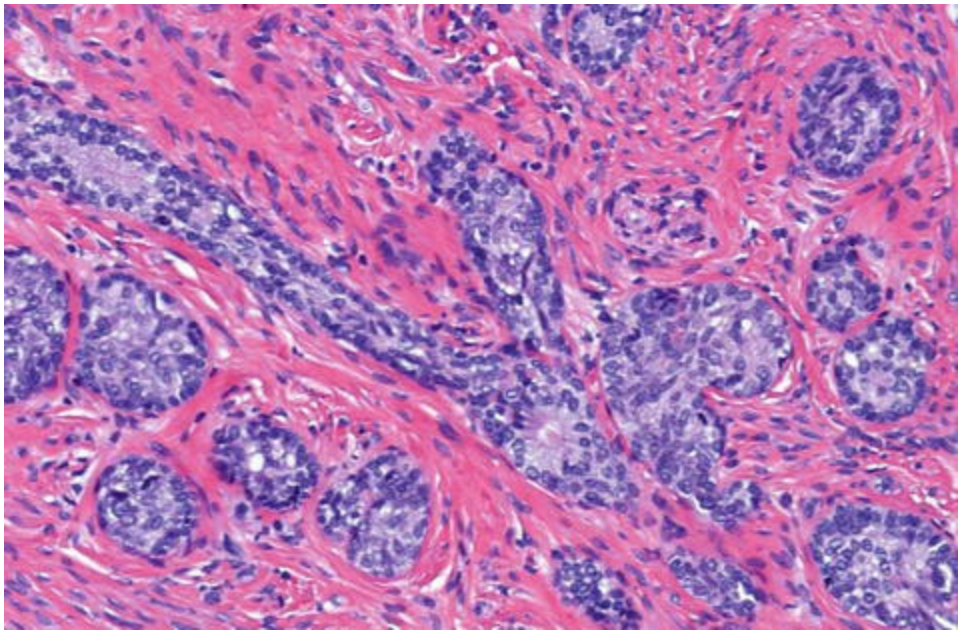


Fig. 11.9 Some nests of adenoid basal carcinoma show central lumina (center of field)

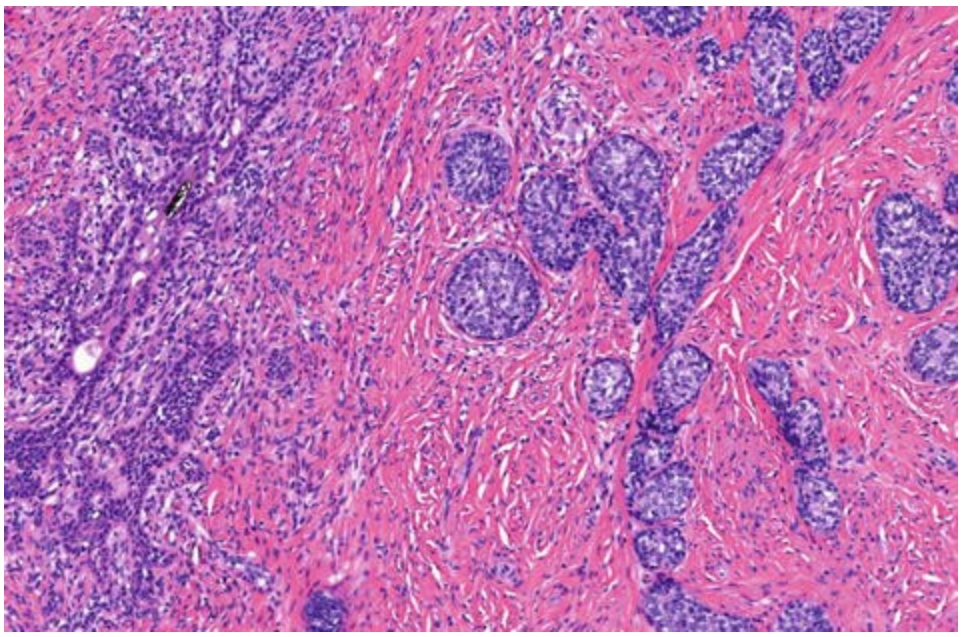


Fig. 11.10 Adenoid basal carcinoma may also exhibit squamous differentiation (left of field)

Histogenesis

It is postulated that ABC has its origin from a reserve cell [80, 83]. ACC is another tumor within this morphological spectrum. ABC is a high-risk HPV-related tumor with HPV types 16 and 33 being identified [76, 84, 85].

Prognosis and Management

Pure ABC is a low-grade tumor, has an excellent prognosis, and rarely metastasizes [74]. Although the term "adenoid basal epithelioma" has been suggested to reflect this benign behavior, ABC is a well-known and accepted entity. The outcome of mixed carcinomas and ABC is largely dependent upon prognostic features of the non-ABC component. The detection of an invasive component of usual type in an excisional biopsy showing ABC, that is a "mixed carcinoma," should direct appropriate management.

Adenoid Cystic Carcinoma

Clinical and Gross Features

Adenoid cystic carcinoma (ACC, ICD-O 8200/3) of the cervix is very rare, representing fewer than 1% of all cervical carcinomas [86]. Cervical ACC is clinically aggressive, often presenting as a prominent mass and showing signs of deep invasion including bloody, watery, or purulent discharge [87–90]. ACC most frequently occurs in postmenopausal black women [80, 91, 92], although cases in Hispanic, Asian, and

white patients have also been reported [81, 92].

Histopathology

ACC of the cervix is characterized by morphological features resembling ACC of the salivary gland [93, 94]. The tumors are poorly circumscribed; perineural and vascular invasion are frequent. Tumor cells grow in both large and small nests, within which cribriform, trabecular, solid, and cord-like patterns can be seen. In most cases, there is at least focal formation of round pseudoglandular spaces filled with globules of hyaline basement membrane material or mucin. The stromal areas between tumor nests have a predominantly hyaline appearance, although desmoplastic or myxoid changes can be seen. Tumor necrosis is common, and focal calcifications may also be present [87, 92].

Both at the periphery of tumor nests and surrounding the pseudoglandular spaces, the cells tend to palisade in a more compact formation. These palisading cells usually have very scant cytoplasm, dark compact nuclei, and a more bland basaloid appearance. By contrast, the cells found throughout the tumor can have a range of nuclear atypia, and mitotic figures may be abundant, although bizarre pleomorphic cells are generally not present. The palisading ("abluminal") cells represent a distinct cell population having a predominantly myoepithelial phenotype and can be highlighted with p63 immunohistochemistry [89]. The remaining ("adluminal") cells have a more heterogeneous epithelial phenotype, without clear squamous or glandular appearance, and are variably positive for CD117 [81]. The hyaline/mucinous globules are strongly highlighted by a PAS stain. The diagnosis of cervical ACC can also be made on a Papanicolaou smear, when basaloid cells surrounding hyaline globules are seen [95].

In the cervix, the presence of a solid undifferentiated component is not unusual in ACC and may be generally underappreciated [96]. Unlike ACC of the salivary gland, the prognostic significance of an undifferentiated component ("solid ACC") is unclear for cervical ACC, given that the latter is already treated as an aggressive clinical entity [88]. Cervical ACC can also be seen as a component of a mixed carcinoma having conventional squamous or other divergent epithelial differentiation [79, 89]. These variant patterns may be more closely associated with high-risk HPV compared to pure ACC [97].

The most important differential diagnosis is adenoid basal carcinoma (ABC, previous section), which in its pure form carries a favorable prognosis. Unlike ACC, ABC is a tumor with a single cell population that is characteristically CD117 negative. ABC also lacks the stromal changes and nuclear variability seen in ACC [79].

Histogenesis

ACC is part of a spectrum of cervical carcinomas with basaloid features, postulated to arise from the reserve cells [79]. Unlike other basaloid tumors, ACC arising in

numerous sites is associated with a distinct t(6:9) resulting in a *MYB-NFIB* fusion gene [98]. Overexpression of the MYB protein has been reported in three cases of cervical ACC with mixed squamous differentiation—in all three cases, high-risk HPV DNA was also detected [89]. However, another study of both mixed-type ACC and pure ACC showed that these two patterns are distinct with respect to HPV status. Whereas cervical carcinomas of mixed differentiation have high-risk HPV in its ACC component, pure cervical ACC is not associated with high-risk HPV and does not demonstrate p16 overexpression [97].

Prognosis and Management

ACC shows a high propensity for perineural and vascular invasion and for distant metastasis in the long term, especially to the lung [99, 100]. Even when presenting at Stage I, the 5-year survival is as low as 56% based on a review of early cases [91]. ACC is considered radiosensitive, and some authors recommend a low threshold for radiation therapy following hysterectomy [88, 101].

Melanocytic Lesions: Melanoma and Blue Nevus

Blue Nevus

Clinical and Gross Features

Blue nevi (ICD-O 8780/0) are rare benign melanocytic lesions of the cervix. The mean age of affected women is about 50 [102]. Blue nevi are usually an incidental pathological finding in a hysterectomy specimen as detected by either by the presence of a blue-black nodule, often in the posterior endocervix, or histopathological examination [103, 104]. Occasionally, cervical blue nevi may be clinically or colposcopically apparent as a dark or blue macule. One to three blue nevi up to 2 cm. in size may be present [102].

Histopathology

Blue nevi are characterized by the presence of clusters of pigmented, dendritic spindle cells in the superficial stroma beneath the epithelium [102]. These spindle cells appear cytologically benign and mitoses are absent (Fig. 11.11). In the cellular blue nevus, variant epithelioid cells with round to ovoid nuclei and clear cytoplasm predominate and form a circumscribed nodule [104]. Blue nevi with features intermediate between spindle cell and cellular variants occur [102]. The cells are immunoreactive for S100, although HMB45 and Melan-A may be negative in some cases [105]. Cytological appearance must be used to distinguish blue nevi from melanoma. Blue nevi should be distinguished from melanotic macules [102].

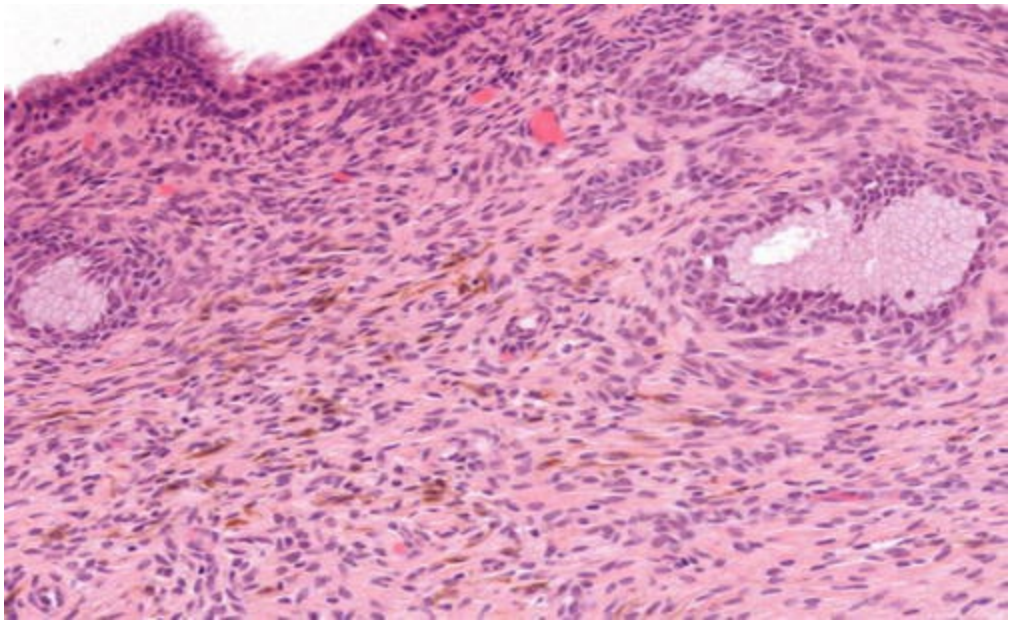


Fig. 11.11 Blue nevus of the endocervix. The endocervical stroma contains numerous pigmented spindle cells. The spindle cells are oriented parallel to the overlying mucosal surface and lack any cytologic atypia

Histogenesis

The cervix does not usually contain melanocytes. Blue nevi are considered to arise from Schwann cells or nerves or from melanocytic precursors which have arrived through aberrant migration from the neural crest into stroma [105].

Prognosis and Management

Blue nevi follow a benign course. However, cases of melanoma associated with malignant melanoma have been described [106].

Melanoma

Clinical and Gross Features

Melanoma of the cervix (ICD-O 8720/3) is a very rare cervical malignancy. The usual presentation is the onset of vaginal bleeding in a woman in her sixth decade [107]. On examination an exophytic ulcerating lesion is often seen on the cervix. Blackish hue or discoloration may serve to distinguish melanoma from cervical carcinoma.

Histopathology

Similar to other body sites, cervical melanoma has a variable histopathological appearance (Figs. 11.12 and 11.13). Immunoreactivities for S100, HMB45, and Melan-A are all useful markers for melanocytic differentiation, although no single marker, or

combination thereof, establishes an unequivocal diagnosis of melanoma (Fig. 11.14) [108]. Amelanotic melanoma can be mistaken for carcinoma and sarcomas, and their diagnosis can be particularly challenging; immunohistochemistry is especially important in reaching the correct diagnosis [109, 110]. Melanoma metastatic to the cervix should be excluded, particularly in those cases which lack a junctional component. An intraepithelial component, however, is often absent in primary cervical melanoma. Clear cell and malignant peripheral nerve sheath tumor-like variants of cervical melanoma have been described [111, 112].

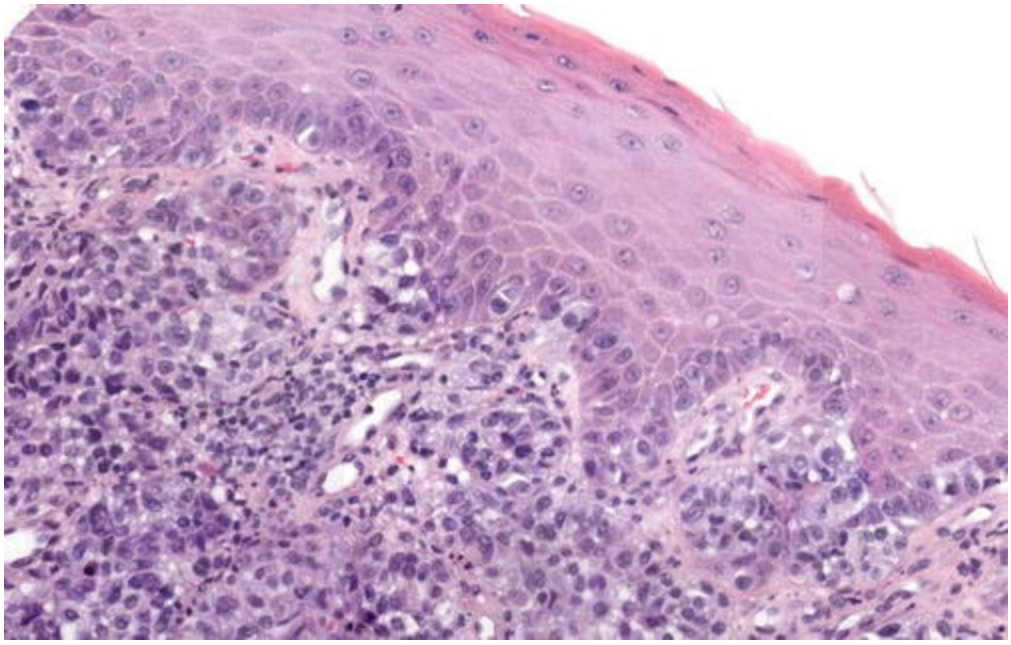


Fig. 11.12 This invasive malignant melanoma of the cervix shows sheets of malignant cells within the submucosa. The overlying squamous epithelium exhibits an intraepithelial component

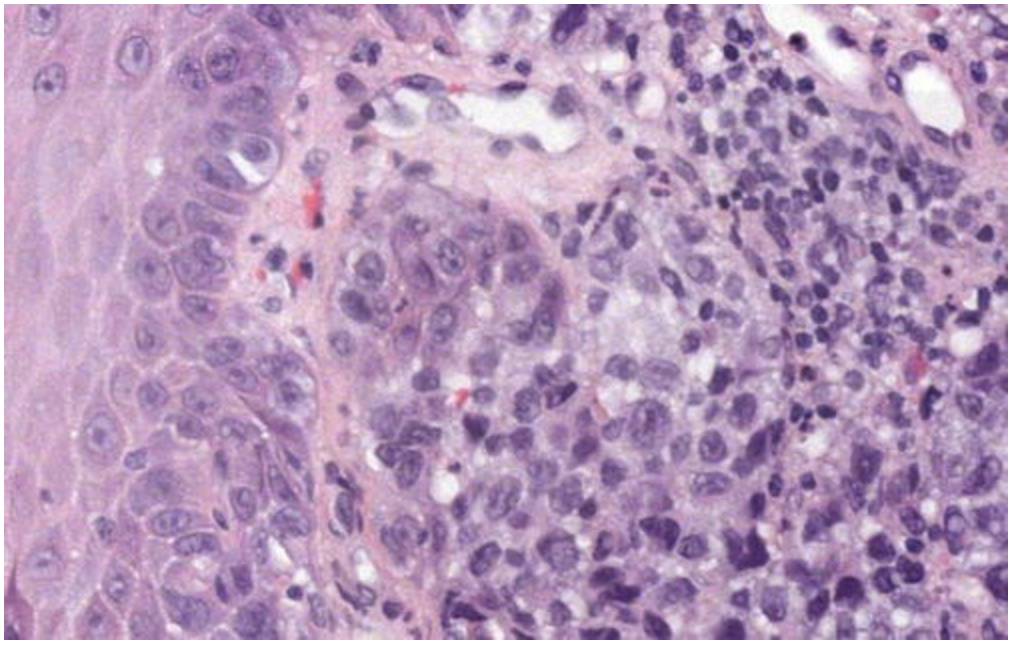


Fig. 11.13 The infiltrating cells of this invasive malignant melanoma are epithelioid in type and have ovoid nuclei often with prominent nucleoli

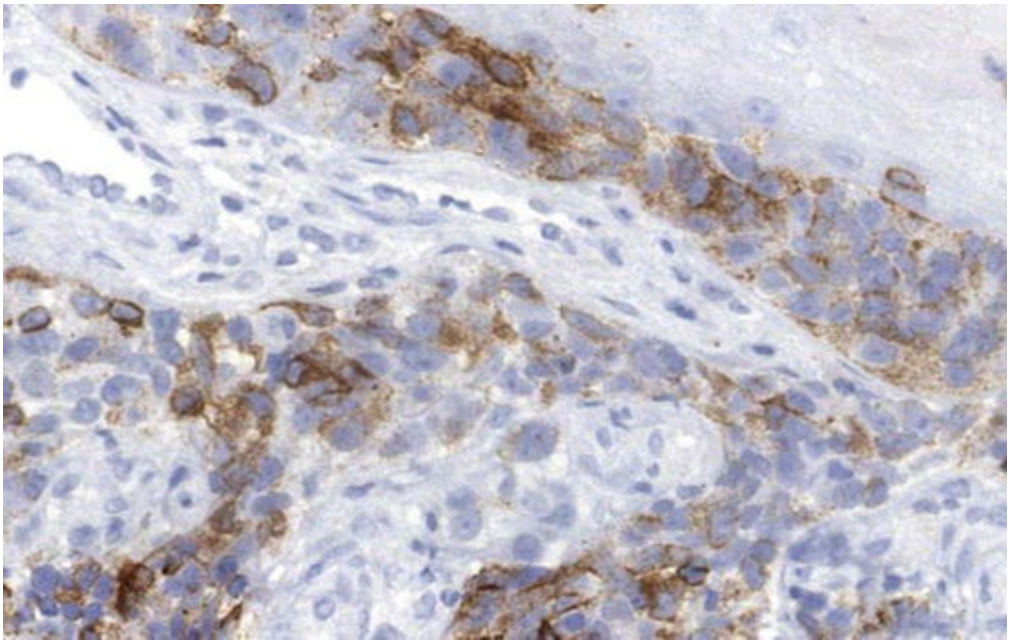


Fig. 11.14 An HMB45 immunostain of this malignant melanoma shows strong and diffuse staining in both the invasive and intraepithelial components

Histogenesis

Melanoma is considered to arise from cervical melanocytes which can be identified in a very small proportion of cervices.

Prognosis and Management

Although the course of melanoma is unpredictable, most cervical melanomas present with advanced local and/or regional disease and have a poor outcome. Patients with thinner and smaller melanomas which are amenable to surgical resections have a better prognosis [113]. In the past cytotoxic chemotherapy has been used in advanced disease. In the past few years, however, treatment for advanced disease using targeted anticancer agents and immunotherapy has advanced considerably.

Germ Cell Tumors of the Cervix

Extragonal germ cell tumors of various types are uncommon and are usually found in midline structures. These germ cell tumors are considered to be parthenogenetic in origin from oocytes after completion of the first division. Cervical teratoma appears to be the most common uterine germ cell tumor and may present as a mass, polyp, or ulcerating lesion [114–116]. Immature teratomas with the presence of immature

neuroepithelium are even less common [117]. Immature teratomas may contain other malignant germ cell elements and merit designation as a malignant mixed germ cell tumor (Figs. 11.15, 11.16, and 11.17). Primary yolk sac tumors may also arise in the uterus and are usually associated with somatic tumors, such as endometrioid adenocarcinoma, and should be distinguished from primary germ cell tumors [118]. Immature and malignant teratomas may recur after resection, with aggressive disease [116, 119].

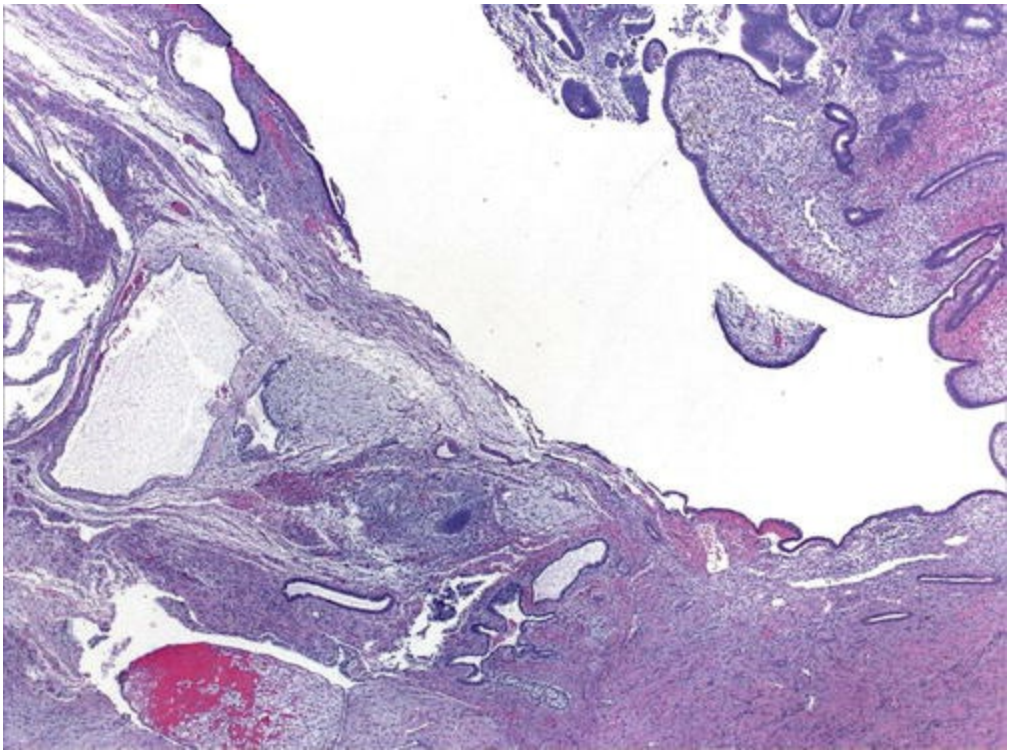


Fig. 11.15 This malignant mixed germ cell tumor of the cervix exhibits a mixture of epithelial and mesenchymal teratomatous elements (*left*) and abuts the body of the uterus with proliferative-type endometrium (*right*)

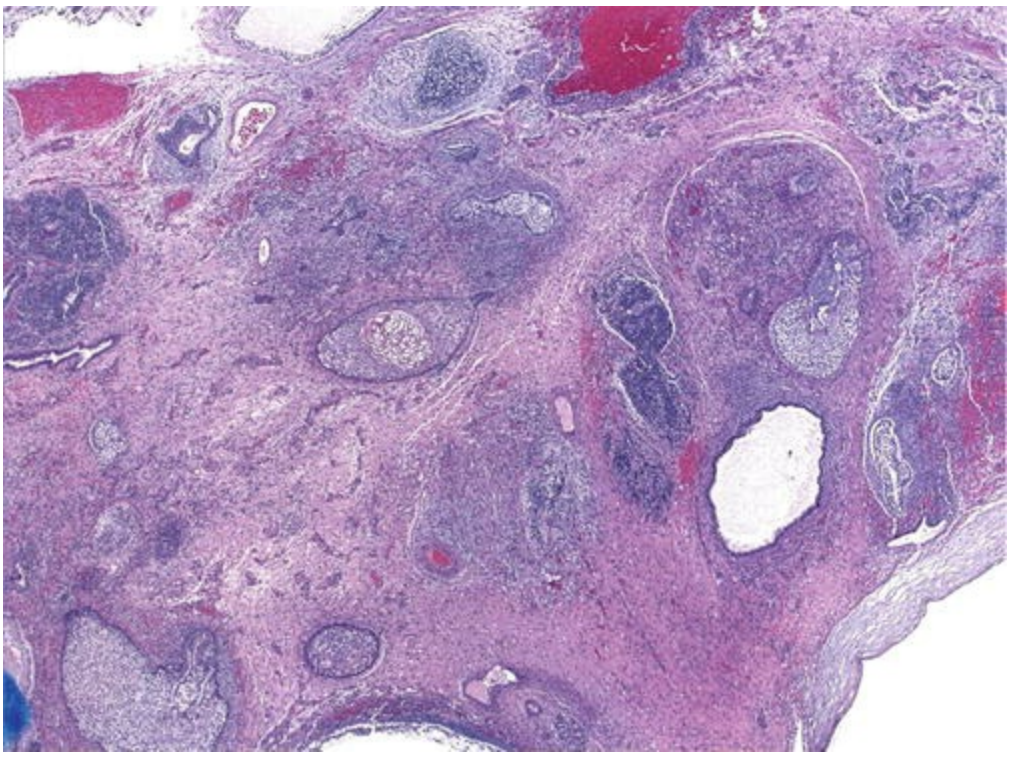


Fig. 11.16 The teratomatous component of this malignant mixed germ cell tumor has a variety of teratomatous elements, including immature neuroepithelial elements with tubules (*upper left*)

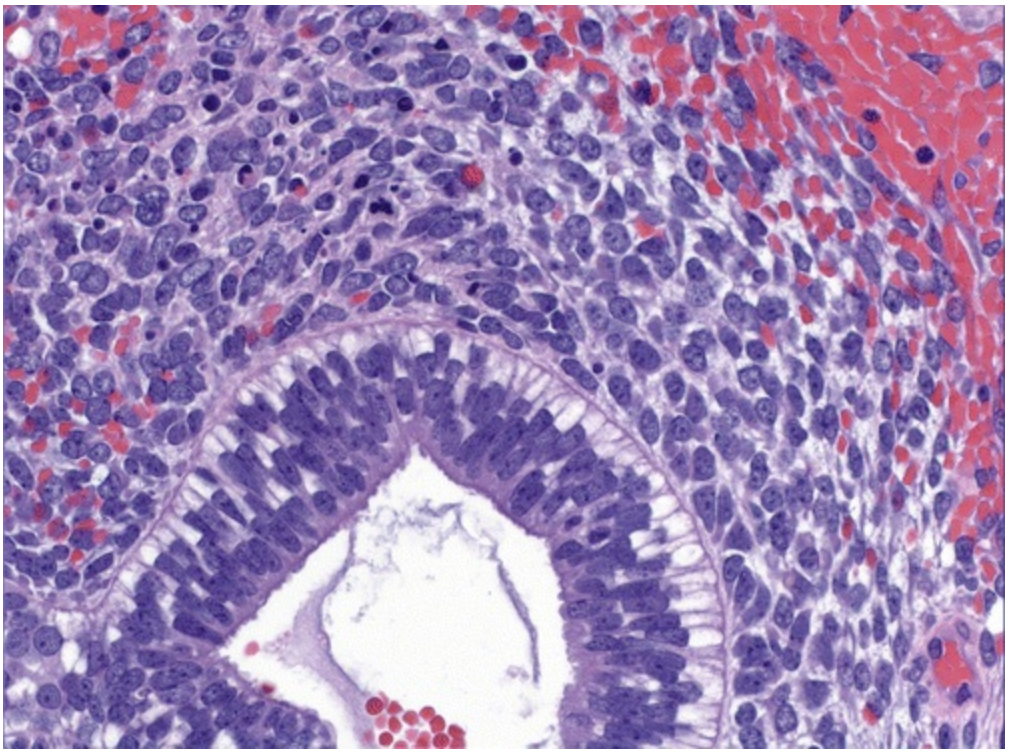


Fig. 11.17 Foci of this malignant mixed germ cell tumor showed endodermal type tubules embedded in cellular primitive stroma indicating a minor yolk sac component

Lymphoid and Myeloid Tumors

Lymphoma can involve the uterine cervix as a primary malignancy but is more frequently a manifestation of multisite disease [120]. Among all lymphomas, fewer than 0.07% involve the cervix without evidence of other systemic disease [121, 122]. The most common lymphomas of the cervix are diffuse large B-cell lymphoma (Figs. 11.18 and 11.19), followed by follicular lymphoma (of any grade) and Burkitt lymphoma. By contrast, marginal zone lymphoma occurs exclusively in the endometrial mucosa, and lymphoplasmacytic lymphoma is restricted to the vulvovaginal area [123]. Lymphomas of the uterine cervix have an unpredictable prognosis, even when disease appears extensive. Chemotherapy and radiation are considered more appropriate than surgical debulking [124], underscoring the importance of distinguishing lymphoma from more common cervical malignancies. Cervical lymphoma has also been reported as a local manifestation of both B-cell and T-cell acute lymphoblastic leukemias [125, 126] and of chronic lymphocytic leukemia [127].

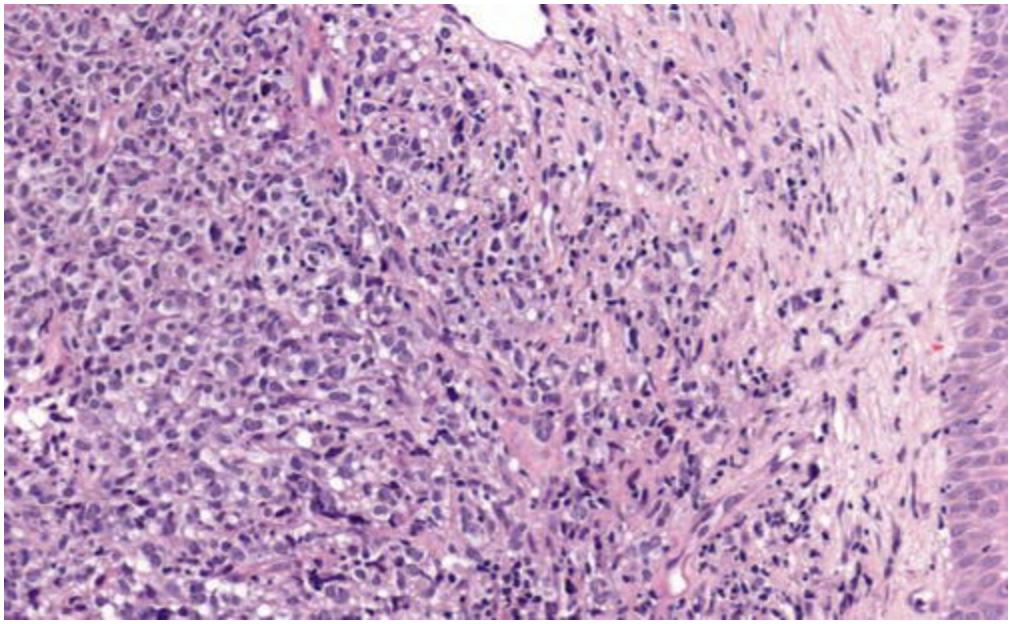


Fig. 11.18 This diffuse large B-cell lymphoma presented as a cervical mass. This lower-power view shows the mass to be submucosal in this case and composed of a discohesive infiltrate of large mononuclear cells. Fine bands of fibrous sclerosis are also seen

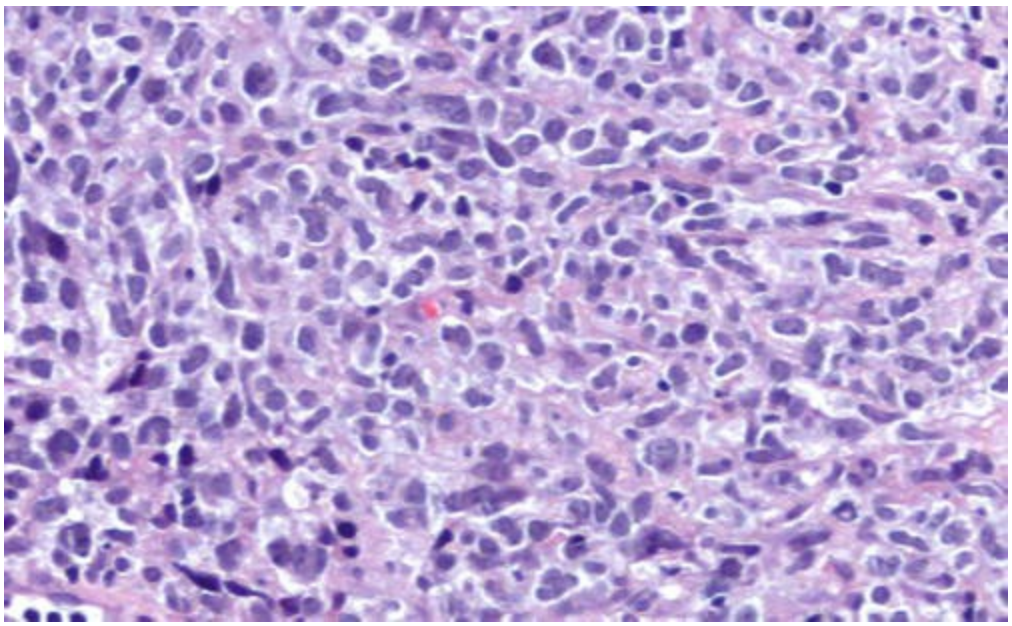


Fig. 11.19 A higher power view of this diffuse large B-cell lymphoma shows that many of the cells have prominent nucleoli. The diagnosis of cervical lymphoma is confirmed by an immunohistochemical panel and flow cytometric analysis if available

Myeloid or granulocytic sarcoma is a tumor mass formed by immature granulocytic cells at an extramedullary site (Figs. 11.20 and 11.21). (The older descriptive term "chloroma" also refers to this entity.) It is most commonly seen as a manifestation of acute myeloid leukemia (AML) or transformation of myelodysplastic syndrome to AML [128]. It is less frequently the first manifestation of AML and may present at any body site including the uterine cervix [129, 130]. It can also present as relapsing disease in a patient with previously diagnosed AML [131] or less commonly as a manifestation of chronic myeloid leukemia [132]. When detected, it is considered equivalent to a diagnosis of myeloid leukemia with respect to prognosis and treatment [133, 134]. Any variant of myeloid leukemia can present as myeloid sarcoma, and the diagnosis is made according to the same pathology criteria [135].

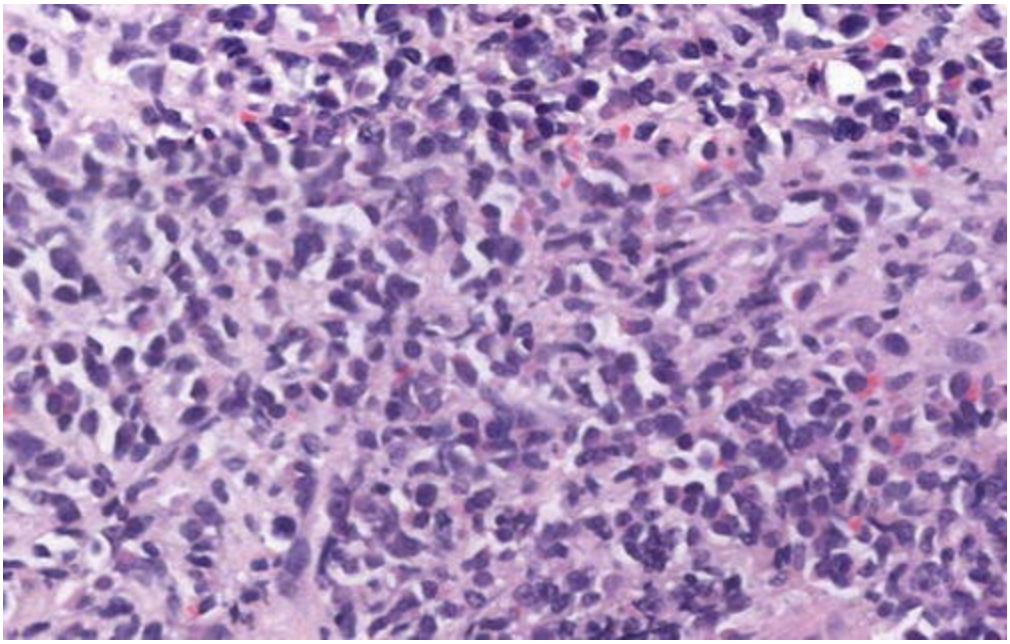


Fig. 11.20 This cervical myeloid sarcoma is composed predominantly of immature myeloblasts although scattered promyelocytes having more prominent eosinophilic cytoplasm can also be seen

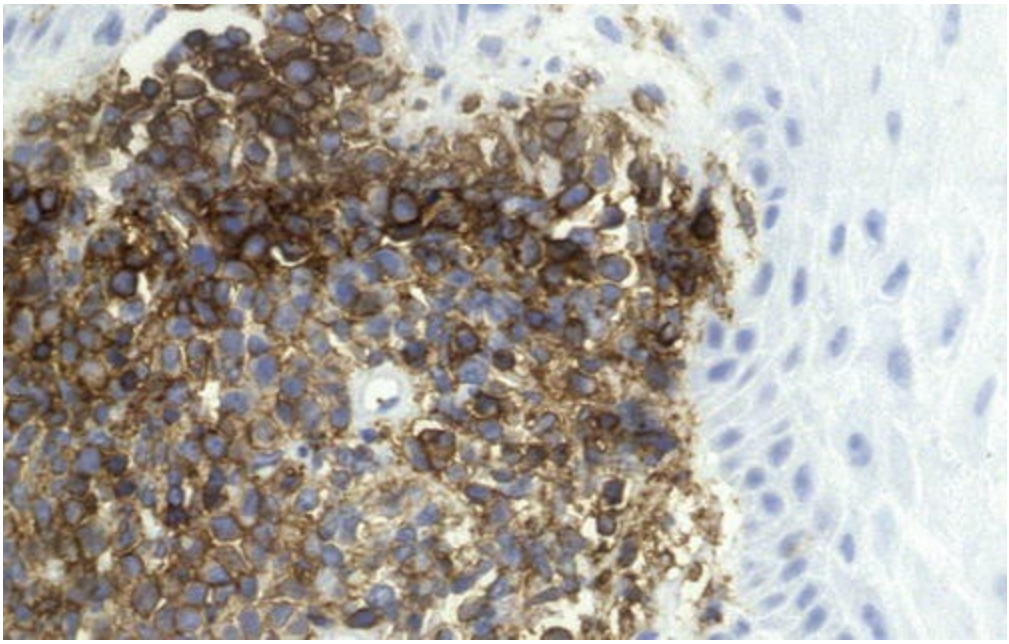


Fig. 11.21 Most myeloid sarcomas will express CD43, as shown here, or myeloperoxidase, lysozyme, and chloroacetate esterase. The classification of the myeloid cells is based on the same criteria as the underlying leukemia

Langerhans Cell Histiocytosis

Langerhans cell histiocytosis (LCH) is a rare neoplastic proliferation of Langerhans cells and has a wide range of clinical manifestations, from limited lesions to widespread disease. Involvement of the female genital tract is rare and usually

concomitant with disease at other sites [136, 137]. In a subset of cases, a syndrome in which diabetes insipidus precedes the genital lesions has been identified [138]. LCH presenting as a primary or originating genital lesion is exceptionally rare [136, 139]. The clinical course appears related to the extent of disease [140].

Langerhans cells are marrow-derived antigen-presenting cells in the dendritic cell family and are present mainly at mucocutaneous sites. They are characterized by ovoid nuclei with a grooved or folded appearance and abundant pale eosinophilic cytoplasm (Fig. 11.22). The cells express S100 [141], CD1a [142], and langerin (CD207) proteins [143] (Fig. 11.23). On electron microscopy characteristic rod-shaped Birbeck granules are seen. The mass lesions of LCH include a mixed inflammatory infiltrate, often with a predominance of eosinophils [135]. The differential diagnosis includes lymphoproliferative or myeloproliferative disorders, Rosai-Dorfman disease, and reactive histiocytosis.

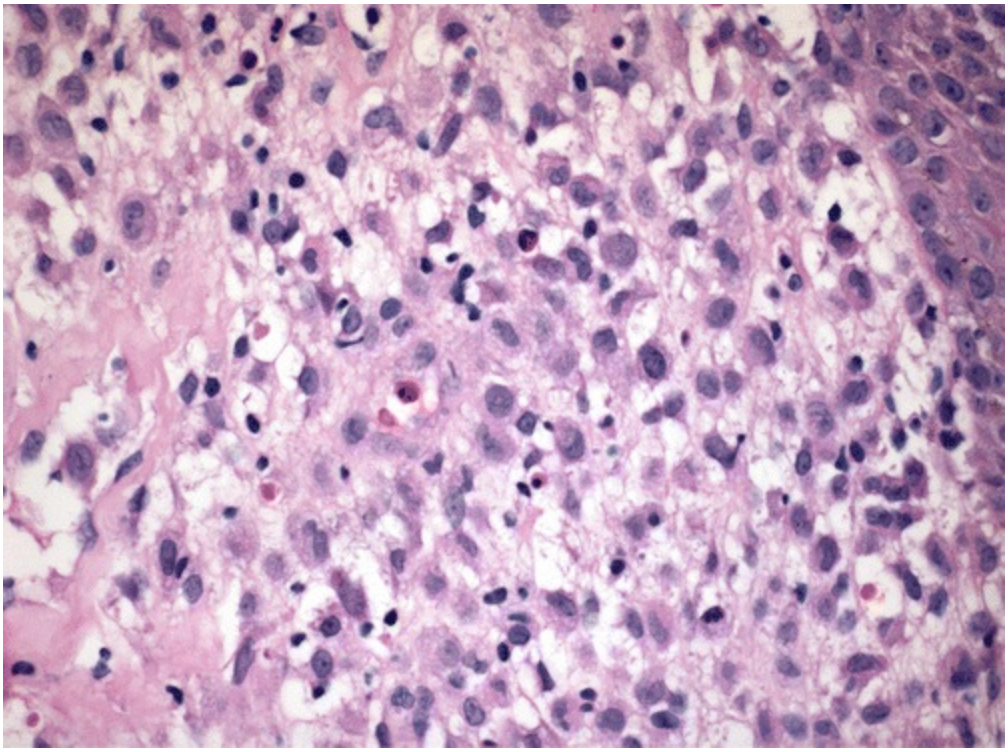


Fig. 11.22 This cervical Langerhans cell histiocytosis is composed of cells with folded, reniform-to-lobated nuclear morphology and abundant eosinophilic cytoplasm. Scattered inflammatory cells, including eosinophils, can be seen

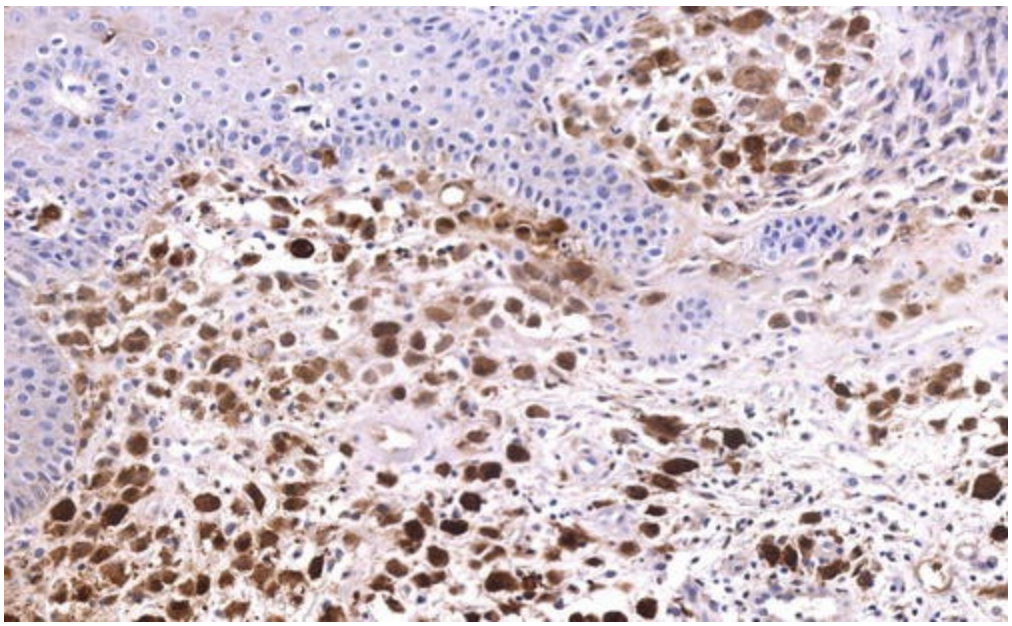


Fig. 11.23 Langerhans cell histiocytosis is immunoreactive to S100, as seen here, CD1a, and langerin

Secondary Malignancies

Involvement of the cervix by endometrial carcinoma (usually by direct surface or stromal extension) is relatively common. The main differential diagnosis in these cases is primary cervical adenocarcinoma (see Chap. 8). Vaginal squamous cell carcinoma can also extend directly into the cervix as part of its natural course. By contrast, metastasis from an ovarian/fallopian tube primary carcinoma is uncommon (in one series less common than metastases from the breast and gastrointestinal tract) [144]. The presence of ovarian carcinoma in the cervix, with little to no stromal or lymphovascular invasion, raises the possibility of transtubal/intrauterine spread in at least a subset of cases [145].

Overall, the cervix is an uncommon site for metastatic cancer. Breast, gastric, and colorectal carcinomas are the three most common non-gynecological primary malignancies that present as cervical metastasis (Fig. 11.24) [144]. Spread from pancreatic, appendiceal, renal cell carcinomas and malignant melanoma has also been reported [145–148]. The diagnosis can usually be made by morphological and immunohistochemical means. However, the relative rarity of secondary tumors at this site results in the mimicking of primary cervical carcinoma. Papanicolaou smear cytology has detected metastatic carcinoma in several reported cases [149, 150].

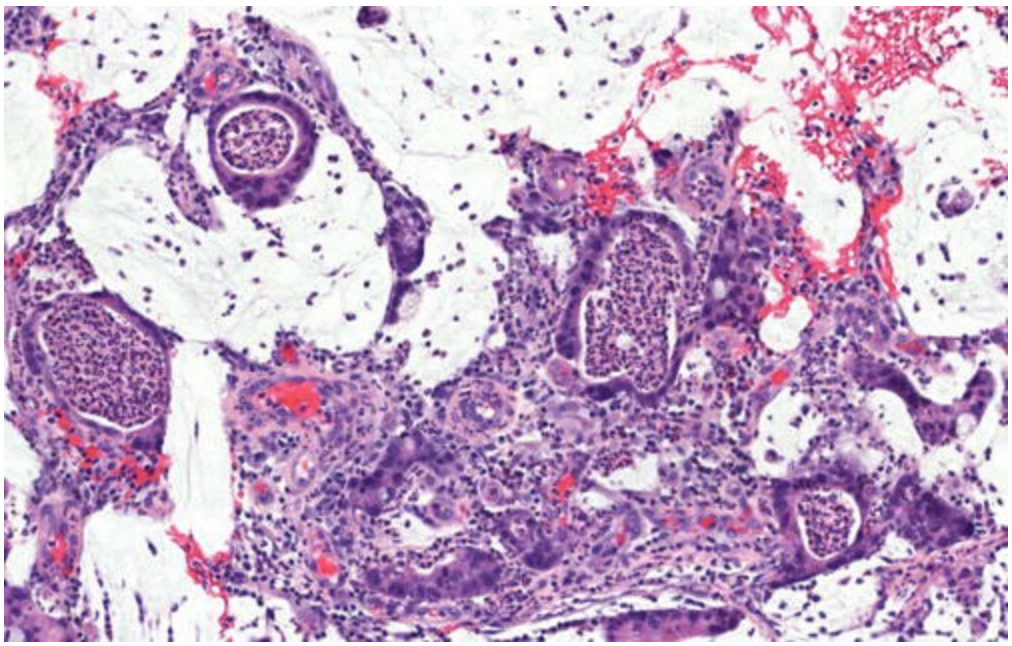


Fig. 11.24 This metastatic colonic adenocarcinoma presented as a cervical mass. The findings are of an infiltrative glandular malignancy with abundant mucin, and hyperchromatic columnar cells are arranged around inflammatory or necrotic debris

References

1. Van Nagell JR, Powell DE, Gallion HH, et al. Small cell carcinoma of the uterine cervix. Cancer. 1988;62:1586–93.
[PubMed]

2. Park HJ, Choi YM, Chung CK, et al. Pap smear screening for small cell carcinoma of the uterine cervix: a case series and review of the literature. J Gynecol Oncol. 2011;22:39–43.
[PubMed][PubMedCentral]

3. Abeler VM, Vergote IB, Kjorstad KE. Small cell carcinoma of the cervix: a clinicopathologic study of 26 patients. Cancer. 1994;73:98–105.

4. Seckl MJ, Mulholland PJ, Bishop AE, et al. Hypoglycemia due to an insulin-secreting small-cell carcinoma of the cervix. N Engl J Med. 1999;341:733–6.
[PubMed]

5. Kim Do Y, Yun HJ, Lee YS, Lee HN, Kim CJ. Small cell neuroendocrine carcinoma of the uterine cervix presenting with syndrome of inappropriate antidiuretic hormone secretion. Obstet Gynecol. 2013;56:420–5.

6. Koch CA, Azumi N, Furlong MA, Jha RC, Kehoe TE, Trowbridge CH, et al. Carcinoid syndrome caused by an atypical carcinoid of the uterine cervix. J Clin Endocrinol Metab. 1999;84:4209–13.
[PubMed]

7. Moss EL, Pearmain P, Askew S, et al. Neuroendocrine carcinoma of the cervix: a review of clinical management and survival. Int J Gynecol Cancer. 2011;21((S)3):248.

8. Wang PH, Liu YC, Lai C, Chao HT, Yuan CC, Yu KJ. Small cell carcinoma of the cervix:analysis of clinical and pathological findings. Eur J Gynaecol Oncol. 1998;19:189–92.
[PubMed]

9. Albores-Saavedra J, Manvivel C, Mora A, Henson DE, Lindberg G, Santiago H, et al. Terminology of endocrine tumors of the uterine cervix: results of a workshop sponsored by the College of American Pathologists and the National Cancer Institute. Arch Pathol Lab Med. 1997;121:34–9.
[PubMed]

10. Ishida GM, Kato N, Hayasaka T, Saito M, Kobayashi H, Katayama Y, et al. Small cell neuroendocrine carcinomas of the uterine cervix: a histological, immunohistochemical, and molecular genetic study. Int J Gynecol Pathol. 2004;23:366–72.
[PubMed]

11. Bermudez A, Vighi S, Garcia A, Sardi J. Neuroendocrine cervical carcinoma: a diagnostic and therapeutic challenge. Gynecol Oncol. 2001;82:32–9.
[PubMed]

12. Faul C, Kounelis S, Karasek K, et al. Small cell carcinoma of the uterine cervix: overexpression of p53, BcL2, and CD 44. Int J Gynecol Cancer. 1996;6:369–75.

13. Perrin L, Ward B. Small cell carcinoma of the cervix. Int J Gynecol Cancer. 1995;5:200–3.
[PubMed]

14. Mannion C, Park WS, Man YG, Zhuang Z, Albores-Saavedra J, Tavassoli FA. Endocrine tumors of the cervix: morphologic assessment, expression of human papillomavirus, and evaluation for loss of heterozygosity on 1p, 3p, 11q, and 17 p. Cancer. 1998;83:1391–400.
[PubMed]

15. Donati P, Paolino G, Donati M, Panetta C. Adenocarcinoma of the cervix associated with a neuroendocrine small cell carcinoma of the cervix in the spectrum of Muir-Torre syndrome. Eur J Gynaecol Oncol. 2015;36:213–5.
[PubMed]

16. Ueda G, Shimizu C, Shimizu H, et al. An immunohistochemical study of small-cell and poorly differentiated carcinomas of the cervix using neuroendocrine markers. Gynecol Oncol. 1989;34:164–9.
[PubMed]

17. Barrett RJ, Davos I, Leuchter RS, Lagasse LD. Neuroendocrine features in poorly differentiated and undifferentiated carcinomas of the cervix. Cancer. 1987;60:2325–30.
[PubMed]

18. McCluggage WG, Kennedy K, Busam KJ. An immunohistochemical study of cervical neuroendocrine carcinomas: neoplasms that are commonly TTF1 positive and which may express CK20 an\d p63. Am J Surg Pathol. 2010;34:525–32.
[PubMed]

19. Li JD, Zhuang Y, Li YF, et al. A clinicopathological aspect of primary small-cell carcinoma of the uterine cervix: a single-centre study of 25 cases. J Clin Pathol. 2011;64:1102–7.
[PubMed]

20. Cui S, Lespinasse P, Cracchiolo B, et al. Large cell neuroendocrine carcinoma of the cervix associated with adenocarcinoma in situ: evidence of a common origin. Int J Gynecol Pathol. 2001;20:311–2.
[PubMed]

21. Krivak TC, McBroom JW, Sundborg MJ, et al. Large cell neuroendocrine cervical carcinoma: a report of two cases and review of the literature. Gynecol Oncol. 2001;82:187–91.
[PubMed]

22. Rhemtula H, Grayson W, van Iddekinge B, et al. Large cell neuroendocrine carcinoma of the uterine cervix- a clinicopathologic study of five cases. S Afr Med J. 2001;91:525–8.
[PubMed]

23. Rekhi B, Patil B, Deodhar KK, et al. Spectrum of neuroendocrine carcinomas of the uterine cervix, including histopathologic features, terminology, immunohistochemical profile, and clinical outcomes in a series of 50 cases from a single institution in India. Ann Diagn Pathol. 2003;17:1–9.

24. Tsou M, Tan T, Cheng SK, Chiou Y-K. Small cell carcinoma of the uterine cervix with large cell neuroendocrine carcinoma component. Gynecol Oncol. 1998;68:69–72.
[PubMed]

25. Lee SJ, Rollason TP. Argyrophilic cells in cervical intraepithelial glandular neoplasia. Int J Gynecol Pathol. 1994;13:131–2.
[PubMed]

26. Wistuba I, Thomas B, Behrens C, et al. Molecular abnormalities associated with endocrine tumors of the uterine cervix. Gynecol Oncol. 1999;72:3–9.
[PubMed]

27. Stoler MH, Stacey EM, Gerseell DJ, et al. Small cell neuroendocrine carcinoma of the cervix: a HPV type-18 associated cancer. Am J Surg Pathol. 1991;15:28–32.
[PubMed]

28. Grayson W, Rhemtula HA, Taylor LF, Allard U, Tiltman AJ. Detection of human papillomavirus in large cell neuroendocrine carcinoma of the uterine cervix: a study of 12 cases. J Clin Pathol. 2002;55:108–14.
[PubMed][PubMedCentral]

29. Kawauchi S, Okuda S-I, Morioka H, et al. Large cell neuroendocrine carcinoma of the uterine cervix with cytogenetic analysis by comparative genomic hybridization: a case study. Hum Pathol. 2005;36:1096–100.
[PubMed]

30. Yoshida Y, Sato K, Yamaguchi A, et al. Atypical metastatic carcinoid of the uterine cervix and review of the literature. J Obstet Gynaecol Res. 2011;37:636–40.
[PubMed]

31. Soga J, Osaka M, Yakuwa Y. Gut-endocrinomas (carcinoids and related endocrine variants) of the uterine cervix: an analysis of 205 reported cases. J Exp Clin Cancer Res. 2001;20:327–34.
[PubMed]

32. Straughn Jr JM, Richter HE, Conner MG, et al. Predictors of outcome in small cell carcinoma of the cervix: a case series. Gynecol Oncol. 2001;83:216–20.
[PubMed]

33. Gilks CB, Young RH, Gersell DJ, et al. Large cell neuroendocrine carcinoma of the uterine cervix: a clinicopathologic study of12 cases. Am J Surg Pathol. 1997;21:905–14.
[PubMed]

34. Cohen JG, Kapp DS, Shin JY, et al. Small cell carcinoma of the cervix: treatment and survival outcomes of 188

patients. Am J Obstet Gynecol. 2010;203:347 e1.

35. Nagao S, Miwa M, Maeda N, et al. Clinical features of neuroendocrine carcinoma of the uterine cervix: a single-institution retrospective review. Int J Gynecol Cancer. 2015;25:1300–5.
[PubMed]

36. Gardner GJ, Reidy-Lagunes D, Gehrig PA. Neuroendocrine tumors of the gynecologic tract: A Society of Gynecologic Oncology (SGO) clinical document. Gynecol Oncol. 2011;122:190–8.
[PubMed]

37. Samlal RA, Ten Kate FJ, Hart AA, et al. Do mucin-secreting squamous cell carcinomas of the uterine cervix metastasize more frequently to pelvic lymph nodes? A case–control study. Int J Gynecol Pathol. 1988;17:201–4.

38. Garg MM, Arora VK. Clear cell adenosquamous carcinoma of the cervix: a case report with discussion of the differential diagnosis. Int J Gynecol Pathol. 2012;31:294–6.
[PubMed]

39. Quddus MR, Manna P, Sung CJ, et al. Prevalence, distribution, and viral burden of all 15 high-risk human papillomavirus types in adenosquamous carcinoma of the uterine cervix: a multiplex real-time polymerase chain reaction-based study. Hum Pathol. 2014;45:303–9.

40. Yoshida T, Sano T, Oyama T, Kanuma T, Fukuda T. Prevalence, viral load, and physical status of HPV 16 and 18 in cervical adenosquamous carcinoma. Virchows Arch. 2009;455:253–9.
[PubMed]

41. Solakoglu Kahraman D, Diniz G, Sayhan S, et al. Differences in the ARID-1 alpha expressions in squamous and adenosquamous carcinomas of uterine cervix. APMIS. 2015;123:847–50.
[PubMed]

42. Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Ishikawa M, Ishibashi T, Iida K, Otsuki Y, Nakayama S, Miyazaki K. Frequent loss of tumor suppressor ARID1A protein expression in adenocarcinomas/adenosquamous carcinomas of the uterine cervix. Int J Gynecol Cancer. 2012;22:208–12.
[PubMed]

43. Lennerz JK, Perry A, Mills JC, et al. Mucoepidermoid carcinoma of the cervix: another tumor with the t(11;19)-associated CRTC1-MAML2 gene fusion. Am J Surg Pathol. 2009;33:835–43.
[PubMed]

44. Huang YT, Wang CC, Tsai CS, Lai CH, Chang TC, Chou HH, et al. Clinical behaviors and outcomes for adenocarcinoma or adenosquamous carcinoma of cervix treated by radical hysterectomy and adjuvant radiotherapy or chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2012;84:420–7.
[PubMed]

45. Lee JY, Lee C, Hahn S, et al. Prognosis of adenosquamous carcinoma compared with adenocarcinoma in uterine cervical cancer: a systematic review and meta-analysis of observational studies. Int J Gynecol Cancer. 2014;24:289–94.
[PubMed]

46. Chen JL, Huang CY, Huang YS, et al. Differential clinical characteristics, treatment response and prognosis of locally advanced adenocarcinoma/adenosquamous carcinoma and squamous cell carcinoma of cervix treated with definitive radiotherapy. Acta Obstet Gynecol Scand. 2014;93:661–8.
[PubMed]

47.

Donnelly ED, Refaat T, Gentile M, et al. Evaluation of outcomes in patients with carcinoma of the cervix treated with concurrent radiation and cisplatin versus cisplatin/5-FU compared with radiation alone. Am J Clin Oncol. 2015;38:437–41.
[PubMed]

48. Alfsen GC, Kristensen GB, Skovlund E, Pettersen EO, Abeler VM. Histologic subtype has minor importance for overall survival in patients with adenocarcinoma of the uterine cervix: a population-based study of prognostic factors in 505 patients with nonsquamous cell carcinomas of the cervix. Cancer. 2001;92:2471–83.
[PubMed]

49. Lee JY, Lee C, Hahn SK, et al. A comparison of adenosquamous carcinoma and adenocarcinoma of the cervix after radical hysterectomy. Gynecol Obstet Invest. 2015;80:15–20.
[PubMed]

50. Baek MH, Park JY, Kim D, et al. Comparison of adenocarcinoma and adenosquamous carcinoma in patients with early-stage cervical cancer after radical surgery. Gynecol Oncol. 2014;135:462–7.
[PubMed]

51. Rose PG, Java JJ, Whitney CW, et al. Locally advanced adenocarcinoma and adenosquamous carcinomas of the cervix compared to squamous cell carcinomas of the cervix in gynecologic oncology group trials of cisplatin-based chemoradiation. Gynecol Oncol. 2014;135:208–12.
[PubMed][PubMedCentral]

52. Mabuchi S, Okazawa M, Kinose Y, et al. Comparison of the prognoses of FIGO 1 to stage 2 adenosquamous carcinoma and adenocarcinoma of the uterine cervix treated with radical hysterectomy. Int J Gynecol Cancer. 2012;22:1389–97.
[PubMed]

53. Grigsby PW, Perez CA, Kuske RR, et al. Adenocarcinoma of the uterine cervix: lack of evidence for a poor prognosis. Radiother Oncol. 1988;12:289–96.
[PubMed]

54. Noh JM, Park W, Kim YS, et al. Comparison of clinical outcomes of adenocarcinoma and adenosquamous carcinoma in uterine cervical cancer patients receiving surgical resection followed by radiotherapy: a multicentre retrospective study (KROG 13-10). Gynecol Oncol. 2014;132:618–23.
[PubMed]

55. Look KY, Brunetto VL, Clarke-Pearson DL, et al. An analysis of cell type in patients with surgically staged IB carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1996;63:304–11.
[PubMed]

56. Lea JS, Coleman RL, Garner EO, et al. Adenosquamous histology predicts poor outcome in low risk stage IB1 cervical adenocarcinoma. Gynecol Oncol. 2003;91:558–62.
[PubMed]

57. Shingleton HM, Bell MC, Fremgen A, et al. Is there really a difference in survival of women with squamous cell carcinoma, adenocarcinoma and adenosquamous cell carcinoma of the cervix? Cancer. 1995;76:1948–55.
[PubMed]

58. Farley JH, Hickey KW, Carlson JW, et al. Adenosquamous histology predicts a poor outcome for patients with advanced-stage, but not early-stage, cervical carcinoma. Cancer. 2003;97:2196–202.
[PubMed]

59. Lai CH, Chou HH, Chang CJ, et al. Clinical implications of human papillomavirus genotype in cervical adeno-adenosquamous carcinoma. Eur J Cancer. 2013;49:633–41.
[PubMed]

60. Littman P, Clement PB, Henriksen B, et al. Glassy cell carcinoma of the cervix. Cancer. 1976;37:2238–346.
[PubMed]

61. Lotocki RJ, Krepart GV, Paraskevas M, Vadas G, Heywood M, Fung FK. Glassy cell carcinoma of the cervix: a bimodal treatment strategy. Gynecol Oncol. 1992;44:254–9.
[PubMed]

62. Atlas I, Gajewski W, Falkenberry S, Granai CO, Steinhoff MM. Absence of estrogen and progesterone receptors in glassy cell carcinoma of the cervix. Obstet Gynecol. 1998;91:136–8.
[PubMed]

63. Kato N, Katayama Y, Kaimori M, Motoyama T. Glassy cell carcinoma of the uterine cervix: histochemical, immunohistochemical, and molecular genetic observations. Int J Gynecol Pathol. 2002;21:134–40.
[PubMed]

64. Hopkins MP, Morley GW. Glassy cell adenocarcinoma of the uterine cervix. Am J Obstet Gynecol. 2004;190:67–70.
[PubMed]

65. Gray HJ, Garcia R, Tamimi HK, et al. Glassy cell carcinoma of the cervix revisited. Gynecol Oncol. 2002;85:274–7.
[PubMed]

66. Mikami M, Ezawa S, Sakaiya N, Komuro Y, Tei C, Fukuchi T, et al. Response of glassy-cell carcinoma of the cervix to cisplatin, epirubicin, and mitomycin C. Lancet. 2000;355:1159–60.
[PubMed]

67. Matsuura Y, Murakami N, Nagashio E, Toki N, Kashimura M. Glassy cell carcinoma of the uterine cervix: combination chemotherapy with paclitaxel and carboplatin in recurrent tumor. J Obstet Gynaecol Res. 2001;27:129–32.
[PubMed]

68. Zolciak-Siwinska A, Jonska-Gmyrek J. Glassy cell carcinoma of the cervix: a literature review. Eur J Obstet Gynecol Reprod Biol. 2014;179:232–5.
[PubMed]

69. Geirsson G, Jóhannesson G, Tulinius H. Tumours in Iceland. 5. Malignant tumours of the cervix uteri. Histological types, clinical stages and the effect of mass screening. Acta Pathol Microbiol Immunol Scand A. 1982;90:139–43.
[PubMed]

70. Olu-Eddo AN, Ekanem VJ, Umannah I, Onakevhor J. A 20 year histopathological study of cancer of the cervix in Nigerians. Nig Q J Hosp Med. 2011;21:149–53.
[PubMed]

71. Lizano M, De la Cruz-Hernández E, Carrillo-García A, García-Carrancá A, Ponce de Leon-Rosales S, Dueñas-González A, et al. Distribution of HPV16 and 18 intratypic variants in normal cytology, intraepithelial lesions, and cervical cancer in a Mexican population. Gynecol Oncol. 2006;102:230–5.
[PubMed]

72. Fukushima M, Okagaki T, Twiggs LB, Clark BA, Zachow KR, Ostrow RS, et al. Histological types of carcinoma of the uterine cervix and the detectability of human papillomavirus DNA. Cancer Res. 1985;45:3252–5.
[PubMed]

73. Saad RS, Mashhour M, Noftech-Mozes S, Ismiil N, Dubé V, Ghorab Z, et al. P16INK4a expression in undifferentiated carcinoma of the uterus does not exclude its endometrial origin. Int J Gynecol Pathol. 2012;31:57–65.
[PubMed]

74. Brainard JA, Hart WR. Adenoid basal epitheliomas of the uterine cervix: a re-evaluation of distinctive cervical basaloid lesions currently classified as adenoid basal carcinoma and adenoid basal hyperplasia. Am J Surg Pathol. 1998;22:965–75.
[PubMed]

75. Ferry JA. Adenoid basal carcinoma of the uterine cervix: evolution of a distinctive clinicopathologic entity. Int J Gynecol Pathol. 1997;16:299–300.
[PubMed]

76. Parwani AV, Smith Sehdev AE, Kurman RJ, Ronnett BM. Cervical adenoid basal tumors comprised of adenoid basal epithelioma associated with various types of invasive carcinoma: clinicopathologic features, human papillomavirus DNA detection, and P16 expression. Hum Pathol. 2005;36:82–90.
[PubMed]

77. Russell MJ, Fadare O. Adenoid basal lesions of the uterine cervix: evolving terminology and clinicopathological concepts. Diagn Pathol. 2006;1:18.
[PubMed][PubMedCentral]

78. Kerdraon O, Cornelius A, Farine M-O, et al. Adenoid basal hyperplasia of the uterine cervix: a lesion of reserve cell type, distinct from adenoid basal carcinoma. Hum Pathol. 2012;43:2255–65.
[PubMed]

79. Grayson W, Cooper K. A reappraisal of “basaloid carcinoma” of the cervix, and the differential diagnosis of basaloid cervical neoplasms. Adv Anat Pathol. 2002;9:290–300.
[PubMed]

80. Grayson W, Taylor LF, Cooper K. Adenoid cystic and adenoid basal carcinoma of the uterine cervix: comparative morphologic, mucin, and immunohistochemical profile of two rare neoplasms of putative 'reserve cell' origin. Am J Surg Pathol. 1999;23:448–58.
[PubMed]

81. Chen TD, Chuang HC, Lee LY. Adenoid basal carcinoma of the uterine cervix: clinicopathologic features of 12 cases with reference to CD117 expression. Int J Gynecol Pathol. 2012;31:25–32.
[PubMed]

82. Goyal A, Wang Z, Przybycin CG, Yang B. Application of p16 immunohistochemistry and RNA in situ hybridization in the classification of adenoid basal tumors of the cervix. Int J Gynecol Pathol. 2016;35:82–91.
[PubMed]

83. Senzaki H, Osaki T, Uemura Y, Kiyozuka Y, Ogura E, Okamura A, et al. Adenoid basal carcinoma of the uterine cervix: immunohistochemical study and literature review. Jpn J Clin Oncol. 1997;27:437–41.
[PubMed]

84. Grayson W, Taylor LF, Cooper K. Adenoid basal carcinoma of the uterine cervix: detection of integrated human papillomavirus in a rare tumour of putative 'reserve cell' origin. Int J Gynecol Pathol. 1997;16:299–300.

85. Jones MW, Kounelis S, Papadaki H, Bakker A, Swalsky PA, Finkelstein SD. The origin and molecular characterization of adenoid basal carcinoma of the uterine cervix. Int J Gynecol Pathol. 1997;16:301–6. [PubMed]

86. Phillips Jr GL, Frye LP. Adenoid cystic carcinoma of the cervix: a case report with implications for chemotherapeutic treatment. Gynecol Oncol. 1985;22:260–2. [PubMed]

87. Young RH, Clement PB. Endocervical adenocarcinoma and its variants: their morphology and differential diagnosis. Histopathology. 2002;41:185–207. [PubMed]

88. Kaur P, Khurana A, Chauhan AK, Singh G, Kataria SP, Singh S. Adenoid cystic carcinoma of cervix: treatment strategy. J Clin Diagn Res. 2013;7:2596–7. [PubMed][PubMedCentral]

89. Shi X, Wu S, Huo Z, Ling Q, Luo Y, Liang Z. Co-existing of adenoid cystic carcinoma and invasive squamous cell carcinoma of the uterine cervix: a report of 3 cases with immunohistochemical study and evaluation of human papillomavirus status. Diagn Pathol. 2015;10:145. [PubMed][PubMedCentral]

90. Nishida M, Nasu K, Takai N, Miyakawa I, Kashima K. Adenoid cystic carcinoma ofthe uterine cervix. Int J Clin Oncol. 2005;10:198–200. [PubMed]

91. Prempree T, Villasanta U, Tang CK. Management of adenoid cystic carcinoma of the uterine cervix (cylindroma): report of six cases and reappraisal of all cases reported in the medical literature. Cancer. 1980;46:1631–5. [PubMed]

92. Gallager HS, Simpson CB, Ayala AG. Adenoid cystic carcinoma of the uterine cervix. Report of 4 cases. Cancer. 1971;27:1398–402. [PubMed]

93. Colgan TJ, Kim K-R, Hirschowitz L, McCluggage WG. Chapter 7: Other epithelial tumours. In:WHO classification of tumours of female reproductive organs. 4th ed. Lyon: International Agency for Research on Cancer; 2014. p. 194–6.

94. Paalman RJ, Counseller VS. Cylindroma of the cervix with procidentia. Am J Obstet Gynecol. 1949;58:184–7. [PubMed]

95. Vuong PN, Neveux Y, Schoonaert MF, Guettier C, Houissa-Vuong S. Adenoid cystic (cylindromatous) carcinoma associated with squamous cell carcinoma of the cervix uteri: cytologic presentation of a case with histologic and ultrastructural correlations. Acta Cytol. 1996;40:289–94. [PubMed]

96. Albores-Saavedra J, Manivel C, Mora A, Vuitch F, Milchgrub S, Gould E. The solid variant of adenoid cystic carcinoma of the cervix. Int J Gynecol Pathol. 1992;11:2–10. [PubMed]

97. Xing D, Schoolmeester JK, Ren Z, Isacson C, Ronnett BM. Lower female genital tract tumors with adenoid cystic differentiation: P16 expression and high-risk HPV detection. Am J Surg Pathol. 2016;40(4):529–36.
[PubMed]

98. Brill LB, Kenner WA, Fehr A, Andren Y, Moskaluk CA, Loning T, Stenman G, Frierson HF. Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms. Mod Pathol. 2011;24:1169–76.
[PubMed]

99. Fowler Jr WC, Miles PA, Surwit EA, Edelman DA, Walton LA, Photopulos GJ. Adenoid cystic carcinoma of the Cervix. Report of 9 cases and a reappraisal. Obstet Gynecol. 1978;52:337–42.
[PubMed]

100. Musa AG, Hughes RR, Coleman SA. Adenoid cystic carcinoma of the cervix: a report of 17 cases. Gynecol Oncol. 1985;22:167–73.
[PubMed]

101. Koyfman SA, Abidi A, Ravichandran P, Higgins SA, Azodi M. Adenoid cystic carcinoma of the cervix. Gynecol Oncol. 2005;99:477–80.
[PubMed]

102. Ta T, Niu G, Tomasello CA, et al. The spectrum of grossly visible pigmented lesions in the uterine cervix: a prospective study. Int J Gynecol Pathol. 2014;33:89–99.

103. Patel DS, Bhagavan BS. Blue nevus of the uterine cervix. Hum Pathol. 1985;16:79–85.
[PubMed]

104. Eskue K, Prieto VG, Malpica A. Cellular blue nevus of the uterus: a case report and review of the literature. Int J Gynecol Pathol. 2010;29:583–6.
[PubMed]

105. Craddock KJ, Bandarchi B, Khalifa MA. Blue nevi of the Mullerian tract: case series and review of the literature. J Low Genit Tract Dis. 2007;11:284–9.
[PubMed]

106. Parada D, Pena KB, Riu F. Coexisting malignant melanoma and blue nevus of the uterine cervix: an unusual combination. Case Rep Pathol. 2012;2012:986542.
[PubMed][PubMedCentral]

107. Pusceddu S, Bajetta E, Carcangiu ML, et al. A literature overview of primary cervical malignant melanoma: an exceedingly rare cancer. Crit Rev Oncol Hematol. 2012;81:185–95.
[PubMed]

108. Prieto VG, Shea CR. Immunohistochemistry of melanocytic proliferations. Arch Pathol Lab Med. 2011;135:853–9.
[PubMed]

109. Mardato VD, Kobal B, Di Stefano A, et al. Amelanotic malignant melanoma of the uterine cervix with ten-year follow-up. Eur J Gynaecol Oncol. 2009;30:106–9.

110. Duggal R, Srinivasan R. Primary amelanotic melanoma of the cervix: case report with review of literature. J Gynecol Oncol. 2010;21:199–202.
[PubMed][PubMedCentral]

111. Furuya M, Shimizu M, Nishihara H, et al. Clear cell variant of malignant melanoma of the uterine cervix: a case report and review of the literature. Gynecol Oncol. 2001;80:409–12.
[PubMed]

112. Pusceddu S, Bajetta E, Buzzoni R, et al. Primary uterine cervix melanoma resembling malignant peripheral nerve sheath tumor: a case report. Int J Gynecol Pathol. 2008;27:596–600.
[PubMed]

113. Tcheung WJ, Selim MA, Herndon 2nd JE, Abernethy AP, Nelson KC. Clinicopathologic study of 85 cases of melanoma of the female genitalia. J Am Acad Dermatol. 2012;67:598–605.
[PubMed]

114. Khoor A, Fleming MV, Purcell CA, et al. Mature teratoma of the uterine cervix with pulmonary differentiation. Arch Pathol Lab Med. 1995;119:848–50.
[PubMed]

115. Lim SC, Kim YS, Lee YH, Lee MS, Lim JY. Mature teratoma of the uterine cervix with lymphoid hyperplasia. Pathol Int. 2003;53:327–31.
[PubMed]

116. Newsom-Davis T, Poulter D, Gray R, et al. Case report: malignant teratoma of the uterine corpus. BMC Cancer. 2009;9:195.
[PubMed][PubMedCentral]

117. Panesar NK, Sidhu JS. Uterine cervical teratoma with divergent neuroepithelial differentiation and development of an oligodendroglioma: report of a case and review of the literature. Ann Diagn Pathol. 2007;11:293–6.
[PubMed]

118. Damato S, Haldar K, McCluggage WG. Primary endometrial yolk sac tumor with endodermal-intestinal differentiation masquerading as metastatic colorectal adenocarcinoma. Int J Gynecol Pathol. 2015;35(4):316–20.. Nov 23 e-pub.

119. Ben Ameur El Youbi M, Mohtaram A, Kharmoum J, et al. Primary immature teratoma of the uterus relapsing as malignant neuroepithelioma: a case report and review of the literature. Case Rep Oncol Med. 2013;2013:971803.
[PubMed][PubMedCentral]

120. Harris NL, Scully RE. Malignant lymphoma and granulocytic sarcoma of the uterus and vagina. A clinicopathologic analysis of 27 cases. Cancer. 1984;53:2530–45.
[PubMed]

121. Chorlton I, Karnei Jr RF, King FM, Norris HJ. Primary malignant reticuloendothelial disease involving the vagina, cervix, and corpus uteri. Obstet Gynecol. 1974;44:735–48.
[PubMed]

122. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. Cancer. 1972;29:252–60.
[PubMed]

123. Kosari F, Daneshbod Y, Parwaresch R, Krams M, Wacker HH. Lymphomas of the female gential tract: a study of 186 cases and review of the literature. Am J Surg Pathol. 2005;29:1512–20.
[PubMed]

124. Perren TJ, Selby PJ, Milan S, Meldrum M, McElwain TJ. Etoposide and Adriamycin containing combination chemotherapy (HOPE-Bleo) for relapsed Hodgkin's disease. Br J Cancer. 1990;61:919–23.

[PubMed][PubMedCentral]

125. Kazi S, Szporn AH, Strauchen JA, Chen H, Kalir T. Recurrent precursor-B acute lymphoblastic leukemia presenting as a cervical malignancy. Int J Gynecol Pathol. 2013;32:234–7.
[PubMed]

126. Lyman MD, Neuhauser TS. Precursor T-cell acute lymphoblastic leukemia/lymphoma involving the uterine cervix, myometrium, endometrium, and appendix. Ann Diagn Pathol. 2002;6:125–8.
[PubMed]

127. Mainiero A, Schnatz PF. Chronic lymphocytic leukemia presenting with localized gynecologic symptoms. J Low Genit Tract Dis. 2010;14:63–4.
[PubMed]

128. Sears HF, Reid J. Granulocytic sarcoma: local presentation of a systemic disease. Cancer. 1976;37:1808–13.
[PubMed]

129. Oliva E, Ferry JA, Young RH, Prat J, Srigley JR, Scully RE. Granulocytic sarcoma of the female genital tract: a clinicopathologic study of 11 cases. Am J Surg Pathol. 1997;21:1156–65.
[PubMed]

130. Pathak B, Bruchim I, Brisson ML, Hammouda W, Bloom C, Gotlieb WH. Granulocytic sarcoma presenting as tumors of the cervix. Gynecol Oncol. 2005;98:493–7.
[PubMed]

131. Delaflor-Weiss E, Zauber NP, Kintiroglou M, Berman EL, DeWitt R, Malczynski D. Acute myelogenous leukemia relapsing as granulocytic sarcoma of the cervix. A case report. Acta Cytol. 1999;43:1124–30.
[PubMed]

132. Chen Z, Wang W, Rich A, Tang G, Hu S. Myeloid sarcoma as the initial presentation of chronic myelogenous leukemia, medullary chronic phase in era of tyrosine kinase inhibitors: a report of 11 cases. Am J Hematol. 2015;90:E146–8.
[PubMed]

133. Avni B, Rund D, Levin M, Grisariu S, Ben-Yehuda D, Bar-Cohen S, et al. Clinical implications of acute myeloid leukemia presenting as myeloid sarcoma. Hematol Oncol. 2012;30:34–40.
[PubMed]

134. Pileri SA, Ascani S, Cox MC, et al. Myeloid sarcoma: clinic-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. Leukemia. 2007;21:340–50.
[PubMed]

135. Pileri SA, Orazi A, Falini B. Myeloid sarcoma. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of Tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008.

136. Axiotis CA, Merino MJ, Duray PH. Langerhans cell histiocytosis of the female genital tract. Cancer. 1991;67:1650–60.
[PubMed]

137. Jiang W, Li L, He YM, Yang KX. Langerhans cell histiocytosis of the female genital tract: a literature review with additional three case studies in China. Arch Gynecol Obstet. 2012;285:99–103.
[PubMed]

138. Epstein JG, Kierland RB, Weber WE. Eosinophilic granuloma of skin and mucous membrane; association with

diabetes insipidus. AMA Arch Derm. 1957;75:45–54.
[PubMed]

139. Montero AJ, Díaz-Montero CM, Malpica A, Ramirez PT, Kavanagh JJ. Langerhans cell histiocytosis of the female genital tract: a literature review. Int J Gynecol Cancer. 2003;13:381–8.
[PubMed]

140. Greenberger JS, Crocker AC, Vawter G, Jaffe N, Cassady JR. Results of treatment of 127 patients with systemic histiocytosis. Medicine (Baltimore). 1981;60:311–38.

141. Nakajima T, Watanabe S, Sato Y, Shimosato Y, Motoi M, et al. S-100 protein in Langerhans cells, interdigitating reticulum cells and histiocytosis X cells. Gan. 1982;73:429–32.
[PubMed]

142. Ray A, Schmitt D, Dezutter-Dambuyant C, Hanau D, Thivolet J. Comparative study of in vitro CD1a and HLA-class I antigens endocytosis by human thymocytes and Langerhans cells. Thymus. 1988-1989;12:239–52.
[PubMed]

143. Valladeau J, Duvert-Frances V, Pin JJ, Dezutter-Dambuyant C, Vincent C, Massacrier C, et al. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. Eur J Immunol. 1999;29:2695–704.
[PubMed]

144. Pérez-Montiel D, Serrano-Olvera A, Salazar LC, Cetina-Pérez L, Candelaria M, Coronel J, et al. Adenocarcinoma metastatic to the uterine cervix: a case series. J Obstet Gynaecol Res. 2012;38:541–9.
[PubMed]

145. McCluggage WG, Hurrell DP, Kennedy K. Metastatic carcinomas in the cervix mimicking primary cervical adenocarcinoma and adenocarcinoma in situ: report of a series of cases. Am J Surg Pathol. 2010;34:735–41.
[PubMed]

146. Pan Z, Repertinger S, Leonard R, Bewtra C, Gatalica Z, et al. Cervical and endometrial metastases of appendiceal goblet cell carcinoid. Arch Pathol Lab Med. 2010;134:776–80.
[PubMed]

147. Zafrakas M, Papanikolaou AN, Venizelos ID, Kellartzis D, Agorastos T, et al. A rare case of renal cell carcinoma metastasizing to the uterine cervix. Eur J Gynaecol Oncol. 2009;30:239–40.
[PubMed]

148. Bokun R, Perković M, Bakotin J, Milasinović D, Mojsović D. Cytology and histopathology of metastatic malignant melanoma involving a polyp on the uterine cervix. A case report. Acta Cytol. 1985;29:612–5.
[PubMed]

149. Giordano G, Gnetti L, Pilato FP, Viviano L, Silini EM. The role of cervical smear in the diagnosis and management of extrauterine malignancies metastatic to the cervix: three case reports. Diagn Cytopathol. 2010;38:41–6.
[PubMed]

150. Gupta N, Bhar V, Dey P, Rajwanshi A, Suri V. Direct sampling of metastatic ovarian carcinoma masquerading as endocervical adenocarcinoma in liquid-based cytology cervical sample. J Cytol. 2014;31:165–7.
[PubMed][PubMedCentral]

Appendix 1: Surgical Cut Up of Cervical Specimens

Introduction

A variety of specimens are received as part of diagnosis and surgical treatment of precancerous lesions and malignant cervical pathology. Specimen handling is crucial for accurate diagnosis, staging, and reporting, to enable optimal patient management. Handling, sampling, and processing of different specimen types are outlined below [1 – 4].

Cervical Punch/Wedge Biopsies

Cervical biopsies are usually colposcopically directed and carried out as a diagnostic procedure, generally after an abnormal smear. These are usually received in formalin and are 4–7 mm in their greatest dimension and 2–4 mm thick. Wedge biopsies are larger than punch biopsies but smaller than excision specimens and are carried out colposcopically as an alternative diagnostic procedure to a punch biopsy to confirm neoplasia before definitive treatment.

Macroscopic Description

The following should be recorded:

- Number of fragments.
- Color and consistency of the biopsies.
- Size of each fragment in three dimensions, measured in millimeters, or the range of the largest and smallest dimensions: for mucoid samples and/or those with immeasurably small tissue fragments, an aggregate measurement may be given in three dimensions or a volume dimension in milliliters.

Specimen Dissection and Block Selection

- Process all tissue, including mucoid material.
- If fragments are very small, wrap in tissue or process in mesh bags/wire cages to prevent loss during processing.
- For easier visibility and handling during embedding and cutting, the tissue may be inked using eosin before transfer to cassettes.

- Specimens greater than 5 × 5 mm may be bisected along the mucosal surface or sliced perpendicular to this surface; if sectioned, this should be recorded.
- For wedge biopsies, identify the squamocolumnar junction where possible, and slice perpendicularly to this; if sectioned, this should be recorded.

Processing/Staining

- Use standard H&E.
- A minimum of three levels should be examined in each case.
- Further levels, with a minimum of three additional levels, should be examined if there is a discrepancy between cytology and histology or to visualize the epithelium if this is not seen.

Excision Specimens: Cervical Cone Biopsy and Large Loop Excision of the Transformation Zone (LLETZ; Also Known as Loop Electrosurgical Excision Procedure, LEEP)

Cone/LLETZ biopsies are carried out on women with abnormal cytology samples as a "see and treat" procedure, or following a positive punch biopsy. The biopsy can be diagnostic or therapeutic. Large loop diathermy is most commonly used and favored because of reduced levels of bleeding, improved healing and preservation of cervical anatomy, and ability to be performed as an outpatient procedure, without general anesthetic. Electrothermal artefact can, however, impair histological diagnosis and render the assessment of excision margins difficult, especially in cases of glandular neoplasia. Cone biopsies are performed using a scalpel ("cold knife"). This is carried out as an in-patient procedure under general anesthetic. A "cold knife" cone biopsy is traditionally a preferred procedure for assessing glandular lesions of the cervix, especially after a diagnostic biopsy, to enable excision of a greater length of the endocervical canal ensuring complete excision and to avoid difficulties in diagnostic interpretation due to diathermy artefact.

Intact cone or loop biopsies are roughly conical in shape. The specimen may arrive free in the specimen pot, or it may be orientated and/or pinned to a corkboard. It may be open at one end (giving a U-shape) or opened and drawn out into a flattened, curved specimen. Alternatively, it may be received as multiple loop fragments, e.g., superficial, deeper/"top-hat" or marginal fragments. Marking the specimen with a suture by the clinician (e.g., at 12 o'clock position) may be helpful for orientation of the specimen during cutting.

Macroscopic Description

The following should be recorded:

- It is customary to record the measurements of the intact central loop/cone biopsy in three dimensions (anteroposterior, side to side, and thickness).
- Measurements of flat/opened loop biopsy in three dimensions (noting which dimension is being measured).
- In practice there tends to be variation in what is considered "depth"; to be relevant clinically [5] and to allow for standard measurements to be recorded, the description should clearly indicate the following three dimensions:
  - Length of the mucosal surface, i.e., a radial measure of distance from endocervical to ectocervical (EE) edges
  - Maximum thickness of tissue
  - Circumference or perpendicular diameters of tissue, for open and intact specimens, respectively.
- For multiple loop biopsies, the number of pieces, with the smallest and largest measured in the maximum dimension where the sample is small or in three dimensions where it is larger.
- The color, consistency, and presence of any surface lesions.

Specimen Dissection and Block Selection

- For all loop/cone biopsies, all slices must be blocked sequentially and not in random order.
- All of the tissue should be processed.
- Consider the use of ink where the identification of margins is difficult. For example, inking the ectocervical rim can be useful when orientating individual slices in the presence of a large ectropion. However, this is not always necessary.
- Note that opening or probing an intact loop/cone biopsy may damage the surface epithelium.
- When sectioning intact central loop/cone biopsies, two possible methods can be employed:
  - Slicing serially parallel to the sagittal plane at 2–3 mm intervals, from one edge to the other (beginning at the 3 or 9 o'clock edge), perpendicular to the transverse axis of the external os. The curved lateral ends may be

processed with the flat or curved side downward; a useful alternative is to cut these slices perpendicular to the longest axis of the slice so that the entire curved edge can be examined at 2–3 mm intervals.

  - Sampling radially, in wedge-shaped slices.

- Opened loop biopsies should be processed in sequential transverse slices, with the same surface facing down so that successive blocks allow examination of tissue at 2–3 mm intervals rather than of contiguous planes or of opposite surfaces which would be 4–6 mm apart.
- Fragments (e.g., superficial, deep/"top-hat," or marginal) should be processed in designated sequential cassettes.
- Only one slice should be processed in one cassette; this may contain more than one piece of tissue as when transverse slices are made parallel to the sagittal plane or when the curved lateral edges are submitted in multiple slices parallel to the longest axis of the slices.

Processing/Staining

- Use standard H&E. A single full-face section is sufficient from each block.
- Examine further levels if the full epithelial surface is not evident, if there is cytohistological discrepancy, or if invasive disease is suspected on the basis of the cytological, colposcopic, or histological features.

Simple Hysterectomy/Trachelectomy

This may be performed in cases where persistent abnormal cytology has been reported, after therapeutic conization or loop excision of an earlier cervical lesion, or for persistent cytological changes where the transformation zone cannot be visualized colposcopically (because of cervical stenosis or scarring from previous cervical loop biopsies or conization). This may also be carried out in women as an option instead of conization when the family is complete. In some instances, CIN may be an incidental finding when simple hysterectomy has been performed for other clinical reasons.

Macroscopic Description

The following should be recorded:

- Size of the specimen
- Size of the cervix (in hysterectomy specimens)

- Presence or absence of attached adnexal structures
- Presence or absence of parametrial tissues and vagina

Specimen Dissection and Block Selection

- When the uterus has already been opened and sampled before the cervical lesion was detected, two standard cervical blocks may already have been taken.
- In all cases, block all cervical tissue to examine the entire transformation zone. This will ensure that the whole lesion has been processed, exclude an invasive component, and allow assessment of the inferior (vaginal) excision margin.

Processing/Staining

- Use standard H&E.
- Cut a full-face single section from each block.
- If the surface epithelium is missing, sections are incomplete, or invasion is suspected consider cutting further levels.

Radical Trachelectomy

This procedure is performed for low-stage cervical cancers, where surgical treatment with preservation of fertility is desired. The following structures are included in radical trachelectomy specimens:

- Cervix
- Parametrium
- Vaginal cuff
- Pelvic lymphadenectomy (this may be carried out as a separate surgical procedure)

Macroscopic Description

The following should be recorded:

- Structures included and whether the specimen can be orientated. In most cases, the surgeon will place a suture at the 12 o'clock position to assist orientation; this should be encouraged. The peritoneum is present posteriorly, often taking a triangular shape with the apex pointing downward. There is usually no peritoneum over the anterior surface of vaginal trachelectomies, but a small amount may be present in specimens excised abdominally. Where the anterior and posterior

aspects of a specimen are difficult to identify, this should be clearly recorded.

- Height/length, lateral, and anteroposterior dimensions in millimeters.
- Length of vaginal cuff as a range (with maximum and minimum dimensions, as this usually varies around the circumference); also, the positions of the maximum and minimum lengths.
- Dimensions of resected parametrial/mesometrial tissues in three dimensions recorded separately for anterior, posterior, left, and right.
- Presence of any macroscopic abnormality: residual tumor, biopsy defect/scar or other lesions.
- Measurement of residual tumor, biopsy defect, or other lesion(s)
- Residual tumor should be measured in three dimensions, and the number of quadrants involved should be recorded.
- Position of residual tumor as clock face position and distance from the proximal, distal, and radial resection margins.

Specimen Dissection and Block Selection

- Parametrial margins should be inked. Using a standard protocol of right (Red or gReen) and left (bLue or yeLlow) helps to maintain orientation after slicing, as does use of a different color to mark the anterior and posterior aspects.
- The specimen should be blocked in its entirety. Block taking will vary according to local preferences and the nature of the individual specimen (see below).
- There is often a large central circumferential biopsy defect and no macroscopically visible residual tumor, while in some instances, there is macroscopic evidence of residual tumor:
    - Where residual tumor is clearly visible, the blocks should be taken in such a way that it is possible to measure tumor position and distance relative to margins (proximal, vaginal, cervical stromal (tumor-free stromal rim), and parametrial).
    - Blocks should be taken in a standard way for all other cases (i.e., when there is no residual visible tumor) and should also be taken to sample the remaining tissue after the tumor has been assessed.
- Recommended method:
    - Slice in parallel, horizontal slices of 2–3 mm thickness, including cervix

and attached parametrial tissues in continuity, starting from the upper end and stopping 10–15 mm above the external os, taking care not to slice through the vaginal fornices.

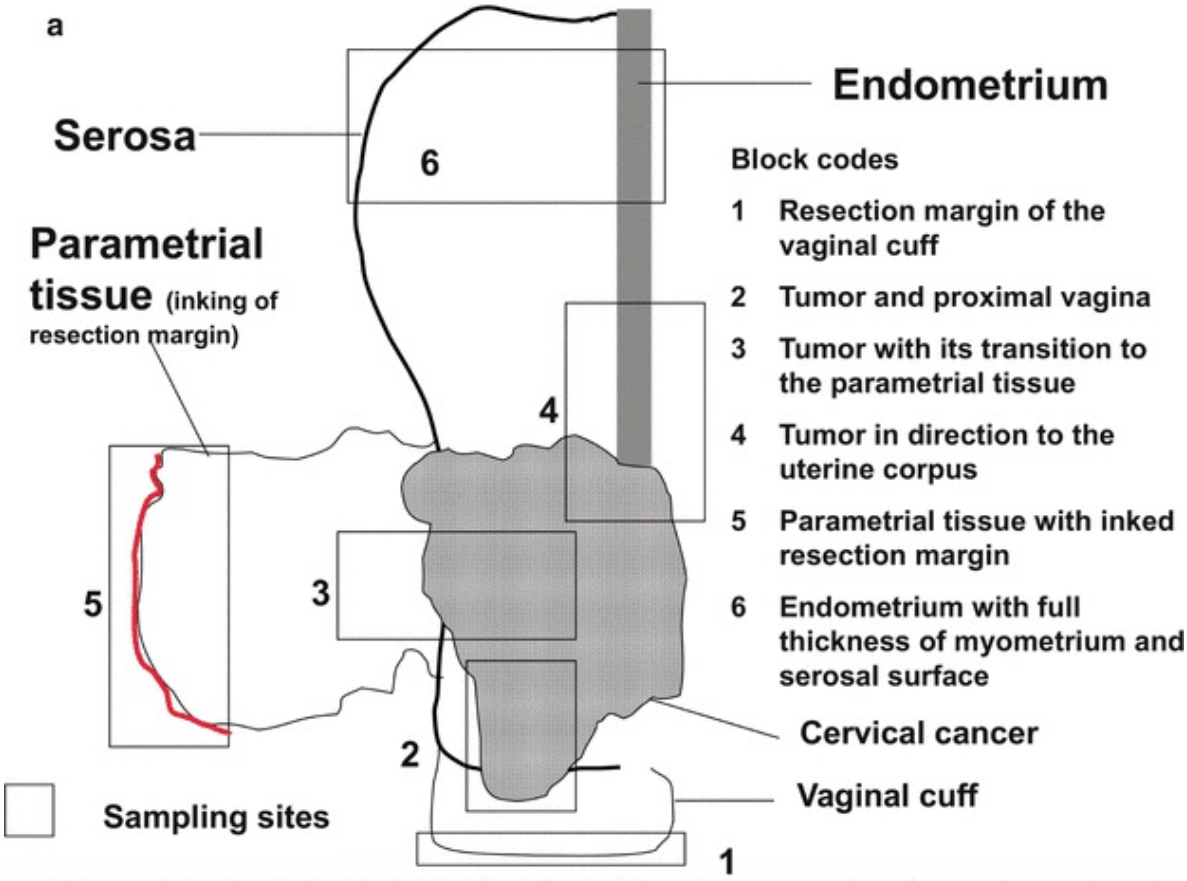
– Slice the lower part of the specimen, comprising the ectocervix and attached vaginal cuff, radially.

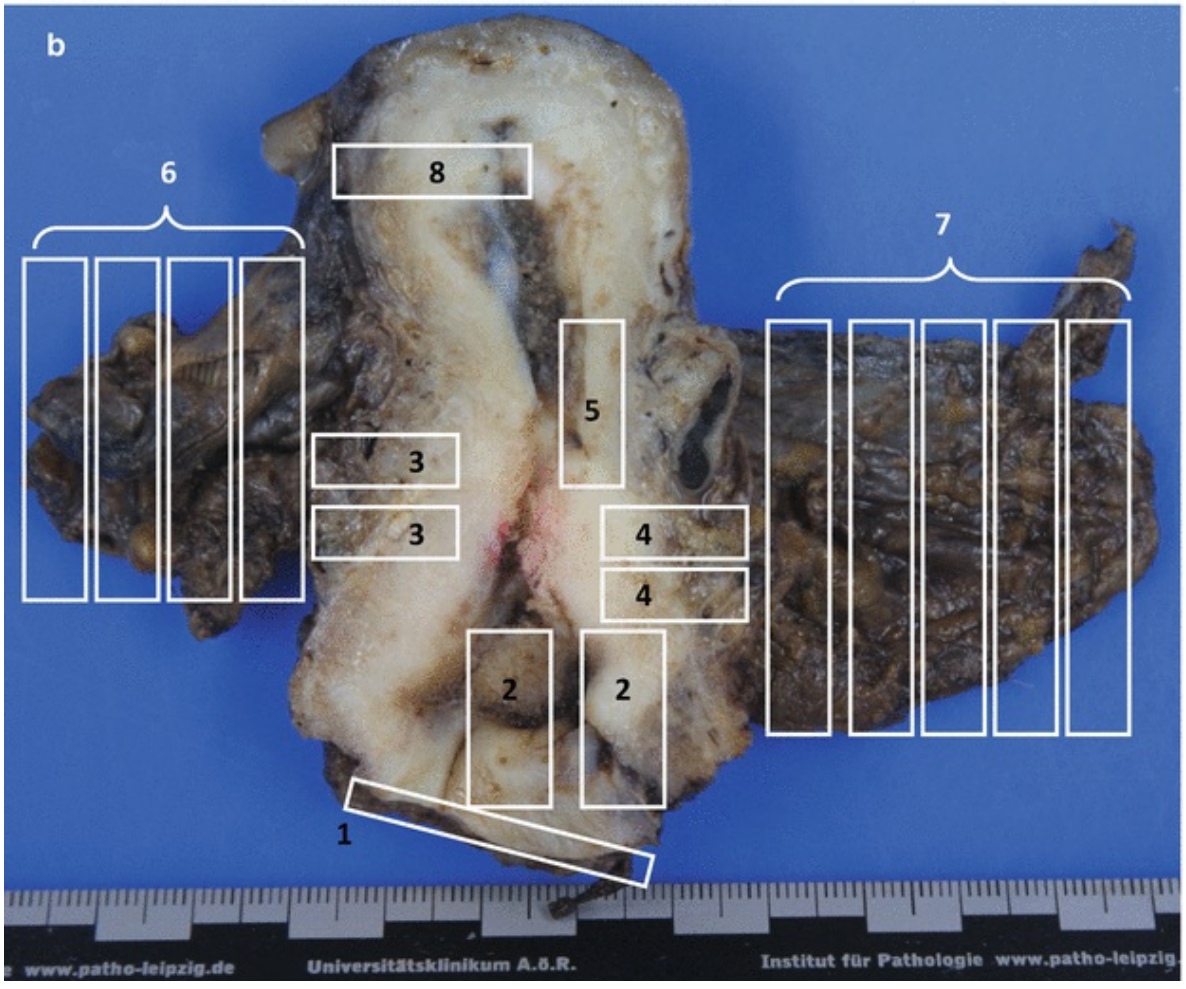
– Each slice is processed in a separate cassette.

– The proximal/upper margin (first slice) is embedded to allow examination of the superior surface.

– All transverse slices are embedded similarly, with the superior surface forming the cutting face of the block.

– Each slice may have to be bisected, or cut into three or four pieces, to fit into a cassette in a way that preserves all surgical margins. The cassettes must not be >80% filled with tissue. Large blocks can be used if preferred. No tissue should be trimmed or discarded.


a
Endometrium
Serosa
6
Parametrial tissue (inking of resection margin)
Block codes
1 Resection margin of the vaginal cuff
2 Tumor and proximal vagina
3 Tumor with its transition to the parametrial tissue
4 Tumor in direction to the uterine corpus
5 Parametrial tissue with inked resection margin
6 Endometrium with full thickness of myometrium and serosal surface
4
5
3
2
1
Cervical cancer
Vaginal cuff
Sampling sites


b
6
8
7
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2
2
1
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Universitätsklinikum A.ö.R.
Institut für Pathologie www.patho-leipzig.

Fig. A1.1 Figure A1.1: Embedding sites for a radical hysterectomy in a case of cervical carcinoma (**a**) Schematic description of embedding sites with block codes. (**b**) Radical hysterectomy after appropriate fixation and coronal sectioning of the specimen, showing embedding sites. Notes: Block codes 3 and 4 contain the transition between the tumor and the most proximal parametrial tissue (paracervix) for the examination for tiny foci of extrauterine disease. Block codes 6 and 7 - the parametrial tissue should be embedded completely

- Alternative method:
  - Take one sagittal slice through the length of the trachelectomy, leaving right and left hemicervices with parametrial and vaginal tissues attached. The vertical slice is processed as anterior and posterior portions of cervix and vagina
  - The remaining specimen is then handled as above, in horizontal slices for the upper portion and radial slices for the lower with vaginal cuff.
- For specimens that are smaller than 10–15 mm in their vertical dimension, processing as a cone or LLETZ may be preferable.

Processing/Staining

- Use standard H&E.
- Cut a full-face single section from each block.
- If the surface epithelium is missing or sections are incomplete, consider cutting further levels.

Radical Hysterectomy

Traditionally this has been the standard treatment for cases of cervical carcinoma at stage IA2, IB, and IIA. With the greater use and equivalent survival results using radical chemoradiotherapy, this procedure is becoming more infrequent, particularly in countries with a successful cervical screening program. This is carried out in cases where there is a very low chance of adjuvant therapy being indicated. The surgical specimen includes the parametria, vaginal cuff, and pelvic+/- para-aortic lymph node dissection. The adnexa are usually included but, in younger women, the ovaries may be conserved to prevent premature menopause.

Macroscopic Description

The following should be recorded:

- Structures included

- Height/length, lateral, and anteroposterior dimensions in millimeters
- Length of vaginal cuff as a range (with maximum and minimum dimensions, as this usually varies around the circumference); also, the positions of the maximum and minimum lengths
- Dimensions of resected parametrial/mesometrial tissues in three dimensions recorded separately for anterior, posterior, left, and right
- Presence of any macroscopic abnormality: residual tumor, biopsy defect/scar, or other lesions
- Dimensions of residual tumor, biopsy defect, or other lesion(s)
- Position of residual tumor as clock face position and distance from the proximal, distal, and radial resection margins

Specimen Dissection and Block Selection (Fig. A1.1a and b)

- Parametrial margins should be inked. Using a standard protocol of right (Red or gReen) and left (bLue or yeLlow) helps to maintain orientation after slicing, as does use of a different color to mark the anterior and posterior aspects.
- The cervix should be separated with the vaginal cuff and parametrial tissues attached.
- There may be a large central circumferential biopsy defect and no macroscopically visible residual tumor, while in some instances, there is macroscopically evidence residual tumor seen:
    - Where residual tumor is clearly visible, the blocks should be taken in such a way that it is possible to measure tumor position and distance relative to margins (proximal, vaginal, cervical stromal (tumor-free stromal rim), and parametrial).
    - Blocks should be taken in a standard way for all other cases (i.e., when there is no residual visible tumor) and should also be taken to sample the remaining tissue after the tumor has been assessed.
- Recommended method:
    - Slice in parallel, horizontal slices of 2–3 mm thickness, including cervix and attached parametrial tissues in continuity, starting from the upper end and stopping 10–15 mm above the external os, taking care not to slice through the vaginal fornices, as above for trachelectomy specimens.
    - The lower part of the specimen, comprising the ectocervix and attached

vaginal cuff, is radially sliced, as above for trachelectomy specimens.

- Examine each slice carefully for residual tumor.
- Where there is no macroscopically evidence residual tumor, sample all the tissue in the same way as described for radical trachelectomy.
- In the presence of residual tumor, at least one block of tumor per centimeter of its maximum dimension should be taken.
- Blocks should enable measurement of the deepest portion of the tumor to the external rim of cervix and to the nearest parametrial margin.
- For tumors positioned low in the cervix, blocks should enable measurement to the vaginal margin.
- All vaginal tissue should be sampled to detect/exclude microscopic vaginal involvement.
- All parametrial tissue should be sampled to exclude microscopic parametrial invasion.
- Representative sections should be taken of remaining structures, i.e., uterine corpus and adnexa.

Processing/Staining

- Use standard H&E.
- Cut a full-face single section from each block.
- If the surface epithelium is missing or sections are incomplete, consider cutting further levels.

Pelvic Exenteration (Figs. A1.2 and A1.3)

Pelvic exenteration may be performed for advanced cervical carcinoma or central recurrent disease, sometimes after treatment with chemoradiation. Because of the highly individual surgical approach in a given patient, the following recommendations must be carefully adapted to the specimen. The hysterectomy specimen will be accompanied by adjacent or adherent organs, e.g., bladder, large bowel, and (in rare cases) pelvic sidewall and/or bone. Prior chemoradiation may obscure the primary tumor and also the extent of macroscopic tumor spread. Appropriate fixation is mandatory in these generally large specimens to ensure adequate handling and microscopic examination. It may be necessary to fix the specimen for 24–48 h. Tamponade of the bladder, large bowel, and vagina with cellulose may be helpful to improve fixation and keep the

organs in shape before cutting.

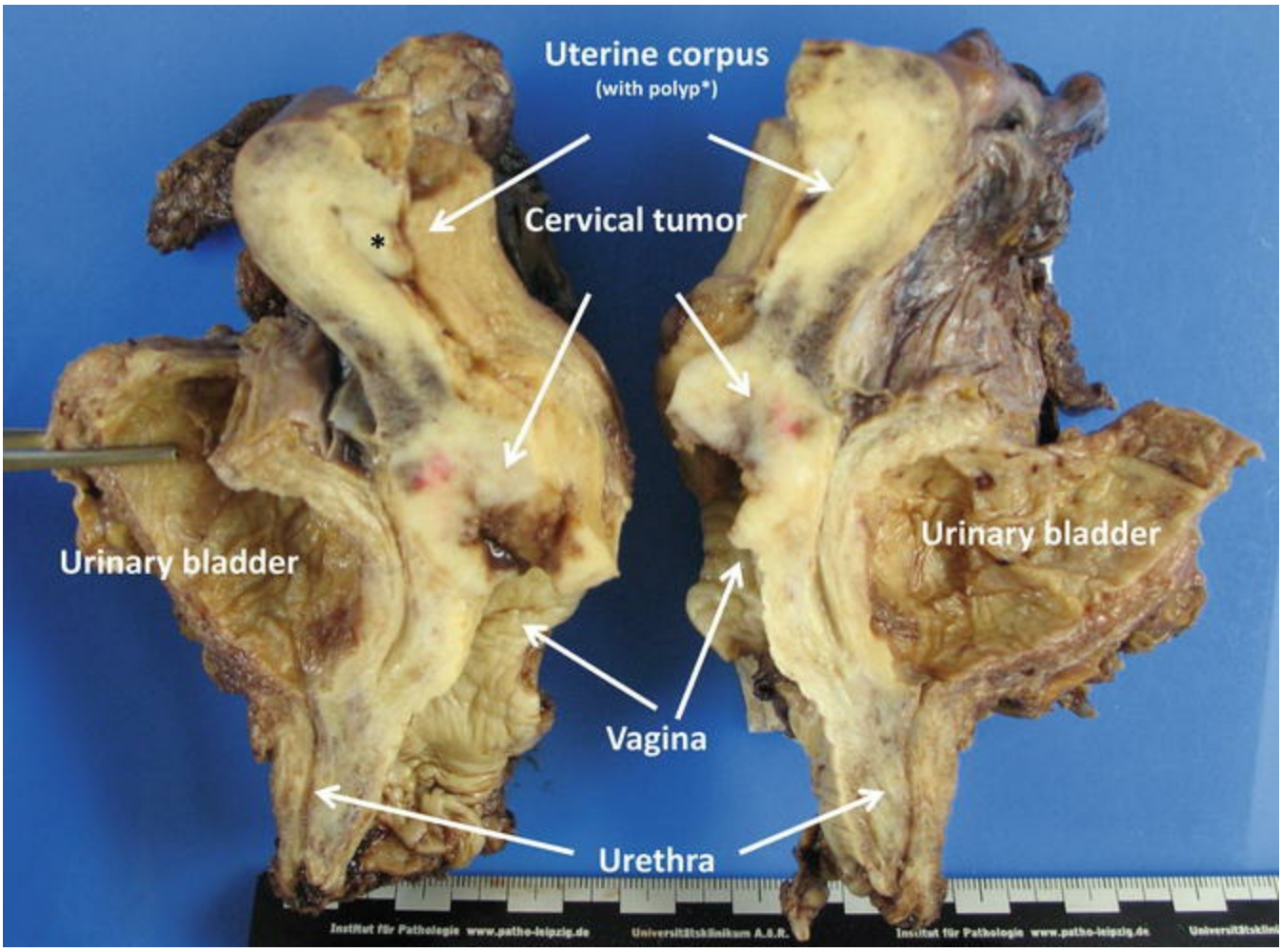

Fig. A1.2 Exenteration specimen from locally advanced cervical carcinoma containing urinary bladder and the uterus with sagittal cutting. Note the well-preserved shape of the urinary bladder after previous tamponade and fixation (see text). The uterus was previously opened at the 6 o'clock position during frozen section examination, and then the specimen was fixed for 48 h in buffered formalin

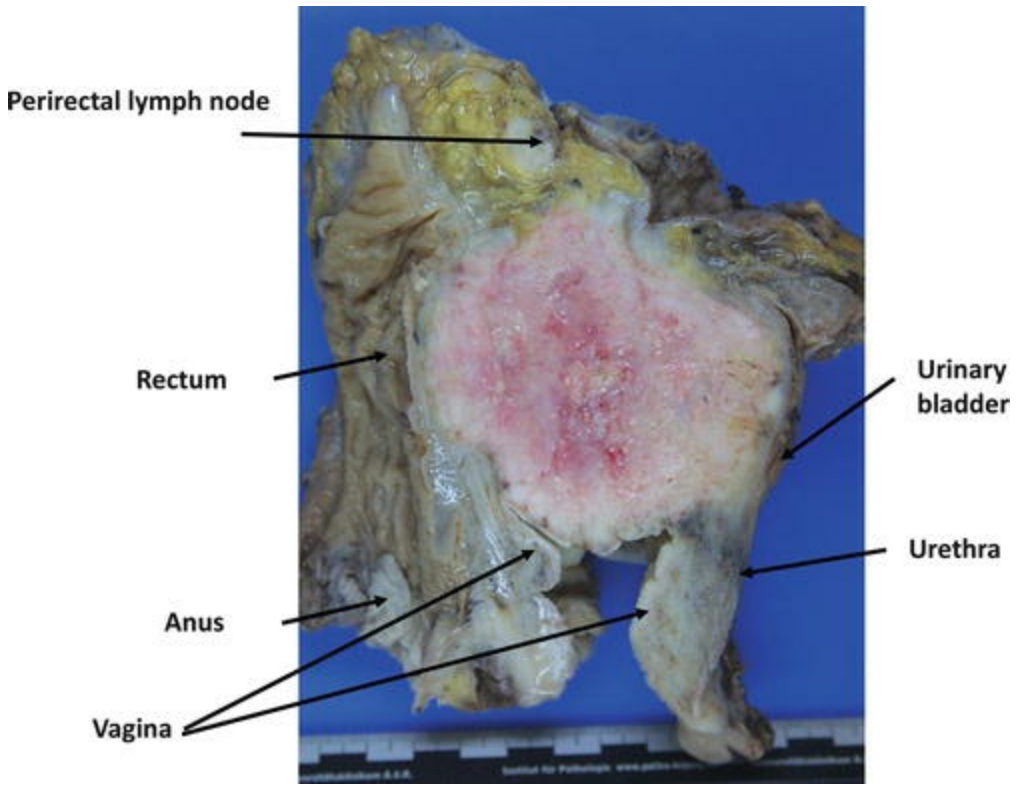

Fig. A1.3 Exenteration specimen from a patient with central pelvic recurrence of a squamous cell carcinoma of the uterine cervix, cut in the sagittal plane

Dissection of adherent or adjacent organs should be carried out in a way that does not compromise assessment of resection margins; a neat sagittal slice through all structures is helpful to assist fixation, demonstrate relationship of tumor to different structures and surgical margins and allow for block selection (Figs. A1.2 and A1.3). A photographic record of the specimen may be useful. Consider painting resection margins with different colors of ink/dye and inflating the urinary bladder with formalin prior to specimen opening. Open adherent or adjacent organs to allow fixation without compromising resection margins. Block selection will vary according to the position of the tumor, but, broadly speaking, perpendicular sections are favored over tangential sections for evaluating the resection margins and enabling measurement of the distance between the tumor and the given margin.

Macroscopic Description

The following should be recorded:

- Height/length, lateral, and anteroposterior dimensions in millimeters.
- Record and measure the specimen components, their gross appearances, and any macroscopic lesions, capturing relevant information on the relationship of the tumor to the bowel (usually the rectum) and urinary bladder.

- Describe the presence, and the extent of involvement, of any tumor in the vaginal fornices, parametria, urinary bladder, and rectum.
- Measure the distance from the tumor to the resection margins.
- Record the number and site of lymph nodes recovered from the specimen; note macroscopic involvement and dimensions of involved nodes.

Specimen Dissection and Block Selection

- Hemisect the entire specimen in the sagittal plane through the uterus and neoplasm. This allows detailed evaluation of the relationship of the tumor to adjacent anatomical structures and facilitates block selection.
- Consider taking blocks of the vaginal resection margin, in continuity with the tumor, where the vaginal cuff is short.
- Take separate blocks of the trimmed circumferential vaginal resection margin.
- Block the parametrial and paracervical tissues in their entirety, recording laterality.
- To assess infiltration of the rectum and bladder, sample the rectum and bladder perpendicular to the mucosa directly overlying the cervical tumor.
- Sample the closest circumferential resection margins. Inking may be helpful in determining the status and distance of the resection margins.
- Consider using oversized tissue blocks when examining cervical tumors in exenteration specimens, in order to retain anatomical relationships and assess resection margins. Process additional standard-sized blocks of tumor to allow immunohistochemistry or other special stains to be undertaken if necessary.

Processing/Staining

- Use standard H&E.
- Cut a full-face single section from each block.
- If sections are incomplete, consider cutting further levels.

Lymph Node Specimens

Lymph nodes are usually sent in separate pots, labeled according to the site of origin. In exenteration specimens, process nodes that are recovered from the mesocolon/mesorectum and parametria separately. The earliest site of nodal metastasis

is the subcapsular sinus, and sectioning of lymph nodes should be such that examination of this space is maximized. Sentinel lymph nodes should be processed the same way as non-sentinel nodes with additional procedures for ultra staging according to local protocols.

Macroscopic Description

The following should be recorded for each specimen site:

- Total amount of tissue
- Number of macroscopically identifiable nodes
- Range of sizes in three dimensions
- Macroscopic evidence of metastasis
- Macroscopic evidence of extranodal spread

Specimen Dissection and Block Selection

- Large lymph nodes should be sampled in separate cassettes.
- Small nodes, which are being processed without cutting, can be placed as multiple in one cassette.
- Block details should be carefully recorded, as it may not be possible to distinguish between a single node processed in several slices and multiple separate nodes in a single cassette.
- Only one block is necessary from any grossly involved node. It is recommended to leave a small rim of surrounding fatty tissue surrounding such nodes to determine the presence of extracapsular extension.
- Nodes that are not macroscopically involved should be processed entirely:
    - Nodes >5 mm in largest diameter should be bisected or serially sliced at 2 mm intervals perpendicular to the longest axis. Large lymph nodes may require processing in more than one block.
    - Nodes <5 mm should be processed whole.
- Ideally all remaining adipose tissue should be processed. If this amounts to an unusually large number of cassettes (>4 additional blocks), and the node yield is high, only representative sections may be taken at the discretion of the pathologist.

Processing/Staining

- Use standard H&E.
- Cut a full-face single section from each block.
- If sections are incomplete, consider cutting further levels.
- Procedures for sentinel node processing should be followed according to local protocols.

Appendix 2: Dataset for Reporting Cervical Neoplasia

The dataset presented here is based on the recommendations of the International Collaboration on Cancer Reporting (ICCR, http://www.iccr-cancer.org/datasets) [6]. The ICCR is an alliance between the Royal College of Pathologists of Australasia, the Royal College of Pathologists of the United Kingdom, the College of American Pathologists, and the Canadian Partnership Against Cancer. This was formed with a view to standardizing cancer reporting worldwide by developing evidence-based datasets for each cancer site and reducing the effort involved in cancer dataset development by different international institutions.

The following elements may be recorded in pathology reports on cervical neoplasia; in each it is indicated whether these are required or recommended.

Element name: Prior treatment
RECOMMENDED
Response type: Value list (single and multi-select)/ *text* :

Previous procedure performed :

- Loop
- Cone
- Trachelectomy
- No prior procedure
- Information not provided

Previous therapy :

- Administered
  - – Chemotherapy
  - – Radiation
  - – Chemoradiation
- No prior therapy

- Information not provided

Element name: Specimen(s) submitted
REQUIRED
Response type: Value list (multi-select, i.e., more than one option can be chosen)/ *text* :

- Not specified
- Loop excision*
- Cone biopsy
- Trachelectomy
- Hysterectomy:
    - – Simple
    - – Radical
    - – Part of exenteration
    - – Type not specified
- Left tube
- Right tube
- Left ovary
- Right ovary
- Left parametrium
- Right parametrium
- Vaginal cuff
- Pelvic exenteration:
    - – Urinary bladder
    - – Rectum
    - – Vagina
    - – Sigmoid colon
    - – Others (*specify*)
- Lymphadenectomy specimen(s)
    - – Left:

- Sentinel node(s)
- Regional nodes: pelvic
- Non-regional nodes: inguinal

– Right:

- Sentinel node(s)
- Regional nodes: pelvic
- Non-regional nodes: inguinal

– Non-regional: para-aortic

– Other node groups (*specify*)

- Others (*specify*)

** Loop excision includes loop electrosurgical excision procedure (LEEP) and large loop excision of the transformation zone (LLETZ)*

Element name: Specimen dimensions
REQUIRED
Response type: Numeric in mm/value list:

Number of tissue pieces: *___

Tissue piece dimensions: *___×___×___mm (*record for each piece*)

Cervix: ** Diameter of ectocervix ___×___mm

Depth of specimen ___mm

Vaginal cuff***: Minimum length ___mm

Maximum length ___mm

Not applicable

Left parametrium: Length ___mm or not applicable (RECOMMENDED)

Right parametrium: Length ___mm or not applicable (RECOMMENDED)

**Applicable to loop/cone biopsies only*
***Applicable to loop/cone biopsies and trachelectomy specimens only*
****Applicable to loop/cone biopsies, trachelectomy, and hysterectomy specimens*

Element name: Macroscopic tumor site(s)
RECOMMENDED
Response type: Value list (multi-select, i.e., more than one option can be chosen)/ *text* :

- No macroscopically visible tumor
- Indeterminate
- Anterior cervix
- Posterior cervix
- Left lateral cervix
- Right lateral cervix
- Circumference of cervix
- Extension to the vaginal cuff
- Extension to the isthmus of the uterus or the uterine body
- Left parametrium
- Right parametrium
- Other organs or tissues (*if additional tissue was resected,* e.g. *the urinary bladder mesothelium, the rectum or the bladder wall*)

Element name: Macroscopic appearance of tumor(s)
RECOMMENDED
Response type: Value list (multi-select, i.e., more than one option can be chosen)/ *text* :

- No macroscopically visible tumor
- Exophytic/polypoid
- Flat
- Ulcerated
- Circumferential/barrel shaped cervix
- Others (*specify*)

Element name: Block identification key
RECOMMENDED
Response type: *Text*
Element name: Tumor dimensions*
REQUIRED
Response type: Value list/numeric mm:

- Horizontal extent ___×___mm

- Depth of invasion ___mm OR not assessable
    - If not assessable record Thickness ___mm

**If separate tumors specify the dimensions for each tumor*

Element name: Histological tumor type
REQUIRED
Response type: Value list (multi-select, i.e., more than one option can be chosen)/ *text* :

- WHO 2014 listed tumors

Element name: Histological tumor grade
RECOMMENDED NO
Response type: Value list:

- G1: Well differentiated
- G2: Moderately differentiated
- G3: Poorly differentiated
- GX: Cannot be graded
- Not graded

Element name: Lymphovascular invasion
REQUIRED
Response type: Value list/ *text* :

- Not identified
- Indeterminate
- Present

Element name: Perineural involvement
RECOMMENDED
Response type: Value list (multi-select, i.e., more than one option can be chosen)/ *text*

Coexistent Pathology

ELEMENTS 1, 2 AND 3 REQUIRED FOR LOOP/CONE EXCISIONS/TRACHELECTOMIES ONLY; RECOMMENDED FOR OTHER SPECIMENSElement name 1: Squamous intraepithelial lesion (SIL) (CIN)
Response type: Value list:

- Not identified
- Present
    - Grade:
        - Low-grade SIL (LSIL) (CIN 1)
        - High-grade SIL (HSIL) (CIN 2/3)

Element name 2: Adenocarcinoma in situ (AIS)/high-grade cervical glandular intraepithelial neoplasia (HG CGIN)
Response type: Value list:

- Not identified
- Present

Element name 3: Stratified mucin-producing intraepithelial lesion (SMILE)
Response type: Value list:

- Not identified
- Present

Element name 4: Other possible precursor lesions
RECOMMENDED
Response type: Value list:

- Not identified
- Present:
    - Lobular endocervical glandular hyperplasia
    - Adenocarcinoma in situ of gastric type
    - Others (specify)

Element name: Extent of invasion
REQUIRED
Response type: Value list/ *text* :x

- Not applicable
- Vagina:
    - Involved:
        - Upper two thirds
        - Lower third

  - Not involved
- Endometrium:
  - Involved
  - Not involved
- Myometrium:
  - Involved
  - Not involved
- Parametrium:
  - Involved:
    - Left
    - Right
  - Not involved
- Fallopian tube:
  - Involved:
    - Left
    - Right
  - Not involved
- Ovary:
  - Involved:
    - Left
    - Right
  - Not involved
- Bladder:
  - Involved:
    - S *pecify compartment*
  - Not involved
- Rectum:
  - Involved:

- S *pecify compartment*

– Not involved

- Other organs or tissues:

– Involved:

- S *pecify*

– Not involved

Element name: Margin status
REQUIRED
Response type: Value list/numeric in mm/ *text*

- Margins cannot be assessed

OR complete the following:

For Carcinoma Hysterectomy/Trachelectomy Specimen

Margin	Involved	Not involved	Distance from tumor (mm) #
Radial/stromal margin			
Ectocervical/vaginal cuff margin			
Closest lateral margin	Right Left		
Endocervical margin *			

Loop/Cone

Margin	Involved	Not involved	Distance from tumor (mm) #
Ectocervical margin			
Endocervical margin			
Radial/stromal margin			
Unspecified margin **			

For Preinvasive Disease

Margin	HSIL	AIS/SMILE	Margin is not

							applicable to specimen
	Involved	Not involved	Distance of margin (mm) #	Involved	Not involved	Distance of margin (mm) #	
Ectocervical/vaginal cuff margin							
Endocervical margin							
Radial/stromal margin							
Unspecified margin							

Complete only if not involved and if less than 10 mm

* These measurements are required only for trachelectomy specimens

** Use for loop/cone biopsies where it is not possible to say whether the margin is ectocervical or endocervical

Element name: Lymph node status
REQUIRED
Response type: Value list / numeric */text*

- Not submitted
- Not involved
- Involved
- Left:
  - – Sentinel node(s):
    - Number of lymph nodes examined** __
    - Number of positive lymph nodes** __
  - – Regional nodes – pelvic:
    - Number of lymph nodes examined** __
    - Number of positive lymph nodes** __
  - – Non-regional nodes – inguinal:
    - Number of lymph nodes examined** __
    - Number of positive lymph nodes** __
- Right:

– Sentinel node(s):

- Number of lymph nodes examined** __
- Number of positive lymph nodes** __

– Regional nodes – pelvic:

- Number of lymph nodes examined** __
- Number of positive lymph nodes** __

– Non-regional nodes – inguinal:

- Number of lymph nodes examined** __
- Number of positive lymph nodes** __

- Non-regional – para-aortic:
  - Number of lymph nodes examined** __
  - Number of positive lymph nodes** __
- Other node group (*specify*):
  - Number of lymph nodes examined** __
  - Number of positive lymph nodes** __

*** In some cases, it may not be possible to record the actual number of nodes due to fragmentation of the specimen*

Element name: Ancillary studies
REQUIRED
Response type: Value list:

- Not performed
- Performed:

  – Human papillomavirus (HPV) testing (*specify details*)

  – Immunohistochemistry (*specify details*)

  – Others (*specify details*)

Element name: Pathologically confirmed distant metastases
REQUIRED
Response type: Value list (single select)/ *text*

- Not identified

- Present (*specify site(s)*)

Provisional Pathological Staging Pre-Multidisciplinary Team Meeting (MDTM)

Element name 1: FIGO 2009 EDITION
REQUIRED
Response type: FIGO list of values
Element name 2: TNM descriptors (UICC 8th Edition, 2016)
RECOMMENDED
Response type:

- m (multiple primary tumors)
- r (recurrent)
- y (post treatment)

Element name 3: Primary tumor T category (UICC 8th Edition, 2016)
REQUIRED
Response type: TNM pT value list
Element name 4: Regional lymph nodes N category (UICC 8th Edition, 2016)
REQUIRED
Response type:

- No nodes submitted or found
- TNM pN value list

Appendix 3: TNM and FIGO Staging of Cervical Carcinoma (ICD-O C53)

The definitions of the T and M categories correspond to the FIGO stages [7, 8]. FIGO staging does not take nodal involvement into account. Both systems are presented below for comparison.

TNM category	FIGO stage	Definition
T – primary tumor		
TX	–	Primary tumor cannot be assessed
T0	–	No evidence of primary tumor
Tis	–	Carcinoma in situ (preinvasive carcinoma)

T1	**I**	**Cervical carcinoma confined to the uterus (extension to corpus should be disregarded)**
T1a	IA	Invasive carcinoma, diagnosed only by microscopy, with deepest invasion ≤5.0 mm and largest extension ≤7.0 mm
T1a1	IA1	Measured stromal invasion ≤3.0 mm in depth and ≤7.0 mm in horizontal spread
T1a2	IA2	Measured stromal invasion of >3.0 mm and not >5.0 mm, and ≤7.0 mm in horizontal spread
T1b	IB	Clinically visible lesion limited to the cervix uteri or preclinical cancers greater than stage T1a/IA
T1b1	IB1	Clinically visible lesion ≤4.0 cm in greatest dimension
T1b2	IB2	Clinically visible lesion >4.0 cm in greatest dimension
T2	**II**	**Tumor invades beyond the uterus but not to pelvic wall or to lower third of vagina**
T2a	IIA	Without parametrial invasion
T2a1	IIA1	Clinically visible lesion ≤4.0 cm in greatest dimension
T2a2	IIA2	Clinically visible lesion >4.0 cm in greatest dimension
T2b	IIB	Tumor with parametrial invasion
T3	**III**	**Tumor extends to pelvic wall and/or involves lower third of vagina and/or causes hydronephrosis or nonfunctioning kidney** *
T3a	IIIA	Tumor involves lower third of vagina, with no extension to pelvic wall
T3b	IIIB	Extension to pelvic wall and/or hydronephrosis or nonfunctioning kidney
T4	**IV**	**Tumor has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. Bullous edema does not permit a case to be allocated to stage IV**
T4	IVA	Spread to adjacent organs
N – regional lymph nodes		
NX	–	Regional lymph nodes cannot be assessed
N0	–	No regional lymph node metastasis **
N1	–	Regional lymph node metastasis ***
M – distant metastasis		
M0	–	No distant metastasis
M1	IVB	Spread to distant organs****

Notes:

Depth of invasion should be taken from the base of the epithelium, either surface or glandular, from which it originates. The depth of invasion is defined as the measurement of the tumor from the epithelial-stromal junction of the adjacent most superficial papillae to the deepest point of invasion

Vascular space involvement, venous or lymphatic, does not affect stage classification

FIGO no longer includes stage 0 (Tis)

All macroscopically visible lesions, even with FIGO stage Ia dimensions, are T1b/IB

Bullous edema is not sufficient to classify a tumor as T4

* On rectal examination, there is no cancer-free space between the tumor and the pelvic wall. All cases with hydronephrosis or nonfunctioning kidney are included, unless they are known to be due to another cause

** Histological examination of a pelvic lymphadenectomy specimen will ordinarily include six or more lymph nodes. If the lymph nodes are negative, but the number ordinarily examined is not met, classify as pN0

*** Regional lymph nodes include paracervical, parametrial, and hypogastric (internal iliac, obturator); common and external iliac; and presacral and lateral sacral nodes. Para-aortic nodes are not regional

**** Distant metastasis includes inguinal lymph nodes and intraperitoneal disease except metastasis to pelvic serosa. It excludes metastasis to vagina, pelvic serosa, and adnexa

---

TNM Stage Grouping

TNM stage	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IA	T1a	N0	M0
Stage IA1	T1a1	N0	M0
Stage IA2	T1a2	N0	M0
Stage IB	T1b	N0	M0
Stage IB1	T1b1	N0	M0
Stage IB2	T1b2	N0	M0
Stage II	T2	N0	M0
Stage IIA	T2a	N0	M0
Stage IIA1	T2a1	N0	M0
Stage IIA2	T2a2	N0	M0
Stage IIB	T2b	N0	M0
Stage III	T3	N0	M0
Stage IIIA	T3a	N0	M0
Stage IIIB	T3b	Any N	M0
Stage IIIB	T1, T2, T3	N1	M0

Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

Additional/Optional Descriptors in the TNM Classification

Sentinel Lymph Node

The following designations are applicable for sentinel lymph node assessment:

pNX(sn) Sentinel lymph node could not be assessed.

pN0(sn) No sentinel lymph node metastasis.

pN0(sn) Sentinel lymph node metastasis.

Isolated Tumor Cells

Isolated tumor cells (ITCs) are single tumor cells or small clusters of cells no more than 0.2 mm in greatest dimension detected by routine H&E stains or immunohistochemistry. Cases with ITC in lymph nodes or distant metastatic sites should be classified as N0 or M0, respectively. This also applies to findings suggestive of tumor cells or tumor cell components detected by non-morphological techniques such as flow cytometry or DNA analysis.

The following designations are applicable to ITC in regional lymph nodes:

pN0 No regional lymph node metastasis histologically; no examination for ITCs

pN0(i-) No regional lymph node metastasis histologically; negative morphological findings for ITCs

pN0(i+) No regional lymph node metastasis histologically; positive morphological findings for ITCs

pN0(mol-) No regional lymph node metastasis histologically; negative non-morphological findings for ITCs

pN0(mol+) No regional lymph node metastasis histologically; positive non-morphological findings for ITCs

The following designations are applicable to ITCs in sentinel lymph nodes:

pN0(i-)(sn) No sentinel lymph node metastasis histologically; negative morphological findings for ITCs

pN0(i+)(sn) No sentinel lymph node metastasis histologically; positive morphological findings for ITCs

pN0(mol-)(sn) No sentinel lymph node metastasis histologically; negative non-morphological findings for ITCs

pN0(mol+)(sn) No sentinel lymph node metastasis histologically; positive non-morphological findings for ITCs

Multiple Primary Tumors

The suffix "m," in parentheses, is used to indicate the presence of multiple primary tumors at a single site.

Classification Following Multimodality Therapy

The prefix "y" is used to categorize tumors examined following multimodality therapy. This indicates the extent of tumor present at the time of that examination and is not an estimate of the extent of tumor prior to multimodality therapy.

Recurrent Tumors

Recurrent tumors classified after a disease-free interval are identified by the "r" prefix.

Classification at Autopsy

The prefix "a" indicates that classification is first determined at autopsy.

Lymphatic Invasion: L

LX Lymphatic invasion cannot be assessed.

L0 No lymphatic invasion.

L1 Lymphatic invasion.

Venous Invasion: V

VX Venous invasion cannot be assessed.

V0 No venous invasion.

V1 Microscopic venous invasion.

V2 Macroscopic venous invasion.

Note: Macroscopic involvement of the wall of veins (with no tumor within the lumen of the veins) is classified as V2.

Perineural Invasion: Pn

PnX Perineural invasion cannot be assessed.

Pn0 No perineural invasion.

Pn1 Perineural invasion.

Appendix 4: Frozen Section Analysis in Cervical Carcinoma

In certain instances, surgical specimens in patients with cervical carcinoma may require intraoperative assessment by frozen section (FS) analysis. Here we will briefly review the approach and results of FS analyses in conization specimens, simple and radical trachelectomies and radical hysterectomies, as well as lymph node specimens.

Conization Specimens

There may be two scenarios for FS analyses in conization specimens:

1. To diagnose the lesion

2. To examine the excision margins

In patients with non-visible lesions, a loop electrosurgical excisional procedure (LEEP) or cone biopsy must be performed to make a definitive diagnosis of invasive cancer and to evaluate the size of the carcinoma and its depth of invasion. To reduce the time for establishing the definitive diagnosis and for reduction of the waiting time for the patient in case of additional surgery, some institutions offer FS to determine lesion size and depth of invasion followed by simple or radical hysterectomy with or without lymphadenectomy performed at the same surgery [9-12]. For FS, the cone specimen may be entirely submitted as radial sections aided by inking the endocervical and ectocervical margins with different colors, performing one or two sections per slide [12]. An accuracy of 75–100% in distinguishing dysplasia from invasive carcinoma and for the diagnosis of microinvasive carcinoma has been reported [9–13].

Other institutions examine only the endocervical margin by transverse (en face) section on FS to guide additional surgery in cases of involvement [13, 14]. The reported accuracy ranges between 87 and 100%.

Trachelectomy Specimens

Simple or radical trachelectomy (TE) is the fertility preserving approach in patients with cervical cancer of FIGO stage IA2/IB1 or more. One important parameter of successful treatment is the absence of disease at surgical margins [15], in particular the isthmic/endocervical margin. At present, there is no consensus regarding the best approach for FS [16 , 17]. Performing a transverse or en face section tangential to the endocervical margin allows the examination of the entire margin surface. The disadvantage may be that the exact distance between the invasive growing tumor and the

endocervical margin cannot be given. Longitudinal section(s) from the (inked) endocervical margin in the direction of the invasive growing tumor (to the ectocervix) allows the exact measurement of the distance between the tumor edge and the endocervical margin. But the whole circumference of the endocervical margin will not be covered by this method [16 , 18]. Some studies examine the margin by longitudinal sections with complete embedding as radial sections aided by inking [19].

The best way may be the combination of both approaches [17 , 20] by performing a transverse section of about 0.2–0.3 cm thickness and an additional perpendicular section from the leading edge of the tumor in the direction of the endocervical margin to measure the distance between the tumor and the margin. The final distance between the invasive front and the endocervical margin is calculated by adding the distance from the tumor to the edge of the perpendicular section to the thickness of the tangential section. An accuracy of up to 100% has been reported for combined transverse and perpendicular sections [17].

Radical Hysterectomies

Intraoperative examination of radical hysterectomy for cervical cancer is rarely indicated [21 , 22]. The distal vaginal resection margin may be examined using tangential sections. Additionally, cases with close posterior, anterior, or parametrial resection margins (i.e., in the direction of the mesorectum, urinary bladder, or site of parametrial infiltration) may be examined using perpendicular sectioning with inking of the margins. Very rarely, assessment of involvement of the lower uterine segment/uterine corpus may be requested [23].

Lymph Nodes

Frozen section examination of pelvic (and rarely para-aortic) lymph nodes may be requested intraoperatively. The examination of lymph nodes in cervical cancer may be challenging, and accuracy of up to 33% has been reported [24-26]. The tissue received should be measured and carefully dissected and palpated to identify small lymph nodes. Lymph nodes up to 0.3 cm may be embedded completely; larger nodes should ideally be sliced perpendicular to their longest axis and processed completely [27]. Two to three step sections from the frozen block should be performed to increase the detection of small metastatic deposits.

References

Appendix 1 : Surgical Cut Up of Cervical Specimens

1. Hirschowitz L, Ganesan R, Singh N, McCluggage WG. Dataset for histological reporting of cervical neoplasia. 3rd ed. London: The Royal College of Pathologists; 2011.

2. NHS Cancer Screening Programmes. Histopathology reporting in cervical screening : an integrated approach. 2nd ed. Sheffield: NHS Cancer Screening Programmes; 2012.

3. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynaecol Obstet. 2009;105(2):107–8.
[Crossref][PubMed]

4. Sobin LH, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. 7th ed. Oxford: Wiley-Blackwell; 2010.

5. Castanon A, Landy R, Brocklehurst P, Evans H, Peebles D, Singh N, et al. Risk of preterm delivery with increasing depth of excision for cervical intraepithelial neoplasia in England: nested case-control study. BMJ. 2014;349:g6223.
[Crossref][PubMed][PubMedCentral]

Appendix 2 : Dataset for Reporting Cervical Neoplasia

6. McCluggage WG, Hirschowitz L, Rous B, AlvaradoCabrero I, Duggan MA, Horn LC, Hui P, Ordi J, Otis CN, Park KJ, Plante M, Stewart CJR, Wiredu EK. Carcinoma of the Cervix. Histopathology Reporting Guide. International Collaboration on Cancer Reporting. http://www.iccrcancer.org/datasets/publisheddatasets/femalereproductiveorgans/cervicalcarcinoma . Accession date 22 April 2017.

Appendix 3 : TNM AND FIGO Staging of Cervical Carcinoma (ICD-O C53)

7. Brierley JD; Gospodarowicz MK; Wittekind C. TNM Classification of Malignant Tumours. 8th ed: Wiley Blackwell; 2016.

8. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103–4.
[Crossref][PubMed]

Appendix 4 : Frozen Section Analysis in Cervical Carcinoma

9. Giuntoli 2nd RL, Winburn KA, Silverman MB, Keeney GL, Cliby WA. Frozen section evaluation of cervical cold knife cone specimens is accurate in the diagnosis of microinvasive squamous cell carcinoma. Gynecol Oncol. 2003;91(2):280–4.

10. Gu M, Lin F. Efficacy of cone biopsy of the uterine cervix during frozen section for the evaluation of cervical intraepithelial neoplasia grade 3. Am J Clin Pathol. 2004;122(3):383–8.

11. Hasanzadeh M, Sharifi N, Yusefi Z, Saghafe N, Moghiman T, Tetedeg V. Role of frozen sections in the evaluation of moderate to severe dysplasia during uterine cervix conization. Asian Pac J Cancer Prev. 2010;11(3):731–4.

12.

Martinelli F, Schmeler KM, Johnson C, Brown J, Euscher ED, Ramirez PT, Frumovitz M. Utility of conization with frozen section for intraoperative triage prior to definitive hysterectomy. Gynecol Oncol. 2012;127(2):307–11.

13. Rouzier R, Feyereisen E, Constancis E, Haddad B, Dubois P, Paniel BJ. Frozen section examination of the endocervical margin of cervical conization specimens. Gynecol Oncol. 2003;90(2):305–9.

14. Behtash N, Karimi Zarchi M, Hamedi B, Azmoode Ardalan F, Tehranian A. The value of frozen sectioning for the evaluation of resection margins in cases of conization. Arch Gynecol Obstet. 2007;276(5):529–32.

15. Schneider A, Erdemoglu E, Chiantera V, Reed N, Morice P, Rodolakis A, Denschlag D, Kesic V. Clinical recommendation radical trachelectomy for fertility preservation in patients with early-stage cervical cancer. Int J Gynecol Cancer. 2012;22(4):659–66.

16. Tanguay C, Plante M, Renaud MC, Roy M, Têtu B. Vaginal radical trachelectomy in the treatment of cervical cancer: the role of frozen section. Int J Gynecol Pathol. 2004;23(2):170–5.

17. Zhang D, Ge H, Li J, Wu X. A new method of surgical margin assuring for abdominal radical trachelectomy in frozen section. Eur J Cancer. 2015;51(6):734–41.

18. Chênevert J, Têtu B, Plante M, Roy M, Renaud MC, Grégoire J, Grondin K, Dubé V. Indication and method of frozen section in vaginal radical trachelectomy. Int J Gynecol Pathol. 2009;28(5):480–8.

19. Ismiil N, Ghorab Z, Covens A, Nofech-Mozes S, Saad R, Dubé V, Khalifa MA. Intraoperative margin assessment of the radical trachelectomy specimen. Gynecol Oncol. 2008;110(3):316–23.

20. Park KJ, Soslow RA, Sonoda Y, Barakat RR, Abu-Rustum NR. Frozen-section evaluation of cervical adenocarcinoma at time of radical trachelectomy: pathologic pitfalls and the application of an objective scoring system. Gynecol Oncol. 2009;113(1):42–6.

21. Baker P, Oliva E. A practical approach to intraoperative consultation in gynecological pathology. Int J Gynecol Pathol. 2008;27(3):353–65.

22. Horn LC, Wagner S. Frozen section analysis of vulvectomy specimens: results of a 5-year study period. Int J Gynecol Pathol. 2010;29(2):165–72.

23. Höckel M, Hentschel B, Horn LC. Association between developmental steps in the organogenesis of the uterine cervix and locoregional progression of cervical cancer: a prospective clinicopathological analysis. Lancet Oncol. 2014;15(4):445–56.

24. Bjornsson BL, Nelson BE, Reale FR, Rose PG. Accuracy of frozen section for lymph node metastasis in patients undergoing radical hysterectomy for carcinoma of the cervix. Gynecol Oncol. 1993;51(1):50–3.

25. Fader AN, Edwards RP, Cost M, Kanbour-Shakir A, Kelley JL, Schwartz B, Sukumvanich P, Comerci J, Sumkin J, Elishaev E, Rohan LC. Sentinel lymph node biopsy in early-stage cervical cancer: utility of intraoperative versus postoperative assessment. Gynecol Oncol. 2008;111(1):13–7.

26. Garg G, Shah JP, Toy EP, Field JB, Bryant CS, Liu JR, Morris RT. Intra-operative detection of nodal metastasis in early stage cervical cancer: a survey of the practice patterns of SGO members. Gynecol Oncol. 2011;121(1):143–7.

27. Rosai J, editor. Rosai and Ackerman's Surgical Pathology. Edinburgh/London/New York/Oxford/Philadelphia/St. Louis/Sydney/Toronto: Elsevier; 2011. p. 2553–4, 2579–83, 2633–6.

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