Chapter 10
Female Genital Tract

Rosemary Tambouret

10.1 Cervix

10.1.1 Epidemiology of Cervical Cancer

Almost all cervical cancers are associated with persistent infection with high risk human papillomavirus (hrHPV), however, HPV infection is vastly more common than cervical cancer. The lifetime risk of HPV genital infection is high for sexually active women as evidenced by prevalence studies [1, 2]. But of the 40 HPV types known to cause genital infection, less than half are considered high risk, that is, capable of causing a persistent infection with an increased risk of cervical cancer [3]. The majority of hrHPV infections cause low grade cytologic abnormalities that regress spontaneously [4]. The highest risk is associated with hrHPV types 16 and 18 that are associated with 50 and 20 % of cervical cancers, respectively. The estimated number of cases of cervical cancer in the United States in 2012 was 12,170 [5]. According to the population studied, multiple factors influence the epidemiology of cervical cancer including the prevalence of hrHPV, the distribution of the different types of hrHPV, the availability of screening, and treatment for premalignant and malignant lesions of the cervix. The cultural make-up of the population also impacts the rate of cervical cancer in that the number of sexual partners influences the exposure to hrHPV [6]. Finally, in the future, widespread use of HPV vaccines is anticipated to change the prevalence of infection and ultimately, of cervical cancer.

R. Tambouret (✉)
Department of Pathology, Massachusetts General Hospital,
Warren 105/55 Fruit Street, Boston, MA 02114, USA
e-mail: RTAMBOURET@PARTNERS.ORG

R. Nayar (ed.), *Cytopathology in Oncology*,
Cancer Treatment and Research 160, DOI: 10.1007/978-3-642-38850-7_10,

10.1.2 Overview of Methods Used to Screen for Cervical Cancer

In many countries, cervical–vaginal cytology (CVC) is the mainstay of cervical cancer screening programs. Approximately, 60 million CVC tests are performed annually in the US [7]. The cytologic method to sample the cervix was first published in a French language medical journal, Presse Medicale, by Babes in 1927, however cervical cytology did not become widely known until publication in the English language in 1934 by George Papanicolaou, after whom the more common name for CVC, the Pap test, is derived [8]. With the institution of Pap test screening programs, the rate of cervical cancer rapidly declined in countries in which it was adopted. Currently, in countries with well established screening programs, more than half of all cases of cervical cancer occur in women who were never screened or who were inadequately screened [9].

However, to this day in many countries the necessary resources to set up screening programs are lacking. For this reason, various international health organizations have promoted other methods for detection of cervical cancer and precursor lesions. The aim is to find a test that is as simple and inexpensive as the Pap test, with similar or superior performance parameters, but which does not demand the highly trained cytotechnologist to read the test. Primary testing for hrHPV is a possibility because of its high sensitivity but the specificity is much lower than that of the Pap test such that many more women would require follow-up testing to identify women with lesions [10]. Also the currently available FDA approved assays for hrHPV testing and/or genotyping are either patented and/or proprietary and thus too expensive for low-resource countries (for example, Digene Hybrid Capture 2, Qiagen, Inc.; Cervista^{TM}HPV, Hologic, Inc., Roche's COBAS, Gen-Probe's APTIMA) or although quite inexpensive, are too technically challenging (for example, polymerase chain reaction (PCR) using generic reagents). Until a simplified, accurate, less costly hrHPV test is developed, cytology will remain the most cost effective screening method. Direct visualization of the cervix after application of acetic acid (VIA) has been promoted as a simple test that can be performed by health care workers with little training [11]. If cervical abnormalities are identified, cryotherapy is performed to ablate the transformation zone. One problem with the VIA test is the similarity in appearance of non-precursor inflammatory lesions to cancer precursor lesions (squamous intraepithelial lesions or SIL). Also treatment may distort the cervix such that subsequent examination of the cervix is difficult. Another potential problem is the inadvertent, inadequate treatment of an invasive cancer.

10.2 Cervical Cytology

10.2.1 Collection and Processing

CVC samples are obtained by direct visualization of the cervix after expanding the vagina with a speculum. The goal is to scrap off easily exfoliated cells from the ectocervix, endocervical canal, and the junction between these two regions, the transformation zone, which is the region most susceptible to HPV infection. Sampling devices include the spatula, the brush, and the broom (Fig. 10.1). Wooden or plastic spatulas usually have a double convexity of the tip such that both cervical os and ectocervix can be sampled with a 360° sweep of the cervix; wooden spatulas should not be used with liquid-based preparations. The endocervical sample can be enhanced when the endocervical brush is used to obtain a separate endocervical sample [12]. An abundance of endocervical cells, including large cell groups, is obtained with the friction of the short bristles of the endocervical brush. The brush, introduced into the endocervical canal such that the bristles nearest the examiner are at the level of the external os, is rotated 180°. Use of the endocervical brush before the spatula has been found to be associated with significantly more blood, so order of sampling the endocervix and ectocervix is important. The extended tip spatula has been found to provide optimum samples when used with the endocervical brush [13]. The entire ectocervix and the endocervical canal are sampled simultaneously with the broom, commercially known as the Cervex-brush™ [14]. The bristles on the broom are longest in the center in order to extend into the endocervical canal. The bristles are oriented in such a way that when the device is turned in a clockwise direction, the angled bristles scrape of the superficial epithelial cells. If the broom is turned in a counterclockwise direction, the smooth edge of the bristles will not pick up cells. Cotton swabs should not be used to sample the cervix for cytology because fewer endocervical cells are obtained and SIL is detected less readily [15, 16].

For a conventional smear (CS), the CVC sample is immediately spread as thinly as possible on a glass slide, followed by immediate immersion of the slide in 95 % ethanol or by misting the slide with an ethanol-based spray fixative. The rapid

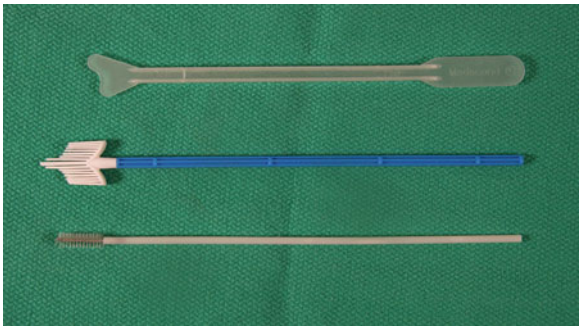

Fig. 10.1 Sampling devices used to obtain cervical-vaginal cytology

fixation is necessary in order to avoid air drying of the cells which leads to distortion of the cellular morphology. Since the late 1990s, an alternative method for preparation of CVC samples, generically known as liquid-based cytology (LBC), has become available. LBC methods were developed in order to facilitate computer assisted screening of cytology slides. Currently in the U.S., two LBC methods are FDA approved: ThinPrep™ (Hologic, Inc) and BD SurePath™ (Becton Dickinson, Inc). Both methods produce a homogenously distributed thin layer of cells restricted to a much smaller area of the slide than the CS (Fig. 10.2). The collection devices for both methods are either a combination of the endocervical brush and a plastic spatula or the broom (Cervex-Brush®). ThinPrep (TP) uses a methanol-based fixative into which the collection devices are rinsed and removed. In the case of SurePath (SP), the collection devices have modified scored handles so that the sampling head can be broken off into the ethanol fixative solution to allow for maximum cell recovery by the time the sample is processed. Some clinicians use a combination of the broom and the endocervical brush. This method increases the cost of disposables, but has not been shown to increase cellular yield or to enhance diagnostic utility [17].

Processing of TP entails vortexing to create a uniform suspension of cells followed by vacuum suction of the sample through a fine mesh membrane to which the cells adhere. The cells are transferred by gently pressing the membrane to the glass slide. The SP process entails vortexing and agitation of the sample, followed by centrifugation with a density gradient medium to remove a most of the potentially obscuring erythrocytes and white blood cells. An aliquot of resultant cell pellet is allowed to settle by gravity onto the poly-lysine coated slide.

All CVC samples, both CS and LBC, are stained with the Papanicolaou stain, a mixture of several pigments, designed to provide crisp nuclear staining with hematoxylin and variably colored cytoplasm according to the degree of keratinization (Fig. 10.3).

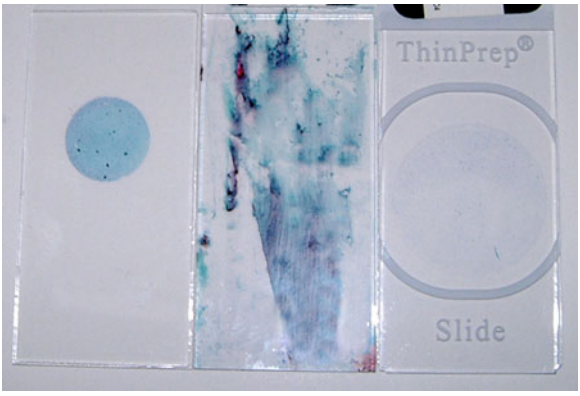

Fig. 10.2 Glass slides from conventional smear, ThinPrep, and SurePrep

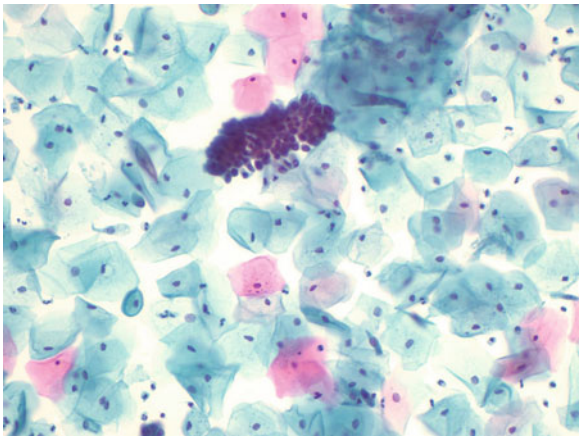

Fig. 10.3 Papanicolaou stained cytology with normal squamous cells and endocervical cells designated as "negative for intra-epithelial malignancy or malignancy"

10.2.2 Slide Evaluation and Interpretation

Cervical cytology screening is a high volume test by virtue of the large number of samples needed to screen the entire adult female population. In most cytology labs, the screening is done by cytotechnologists (CT) who hold a bachelor's degree and have 1 year specialized training in cytopathology. The CTs are legally permitted to result cervical cytology cases deemed negative for intraepithelial lesion or malignancy (NILM) without further analysis. Any slides found to have abnormal cells must be passed on to the cytopathologist for review. Depending on the result, the report will have the name of CT screener and potentially, the cytopathologist. Although the prevalence of abnormal cervical cytology samples will vary with the population served, the majority of slides submitted for screening are normal. Thus, the CTs do the bulk of the work for gynecologic cytology.

Recently, computer assisted screening has been introduced into the cytology lab and is intended to maintain or increase the sensitivity of the screening process while easing the manpower shortages affecting the cytotechnology community. Two types of systems are currently available. Using different approaches, both systems perform image analysis on CVC slides prior to evaluation of selected fields of view by the CT. The coordinates of the fields of view are relayed from the computer to the CT via a hard-wired link to a motorized stage on the microscope. If no abnormal cells are identified in the computer selected fields of view, the CT may result the case as NILM. The most widely used machine is the ThinPrep^{TM} imaging system which relies on a proprietary modification of the hematoxylin stain component of the Papanicolaou stain, such that the nuclei of cells are stained in a "Feulgen-like" manner. The Feulgen reaction has long been used in image analysis to produce a stoichiometric measurement of the DNA present in cells evaluated by optical density. The ThinPrep^{TM} imaging system identifies potentially abnormal cells in the CVC sample with increased DNA and presents 22 fields of view, or about one-third of the slide, for evaluation by the CT. The second system,

marketed by Becton–Dickinson for use with SP, relies on a complex algorithm of thousands of images of normal and abnormal cells stored within the computer that are compared to the cells on the cytology sample. No stain modification is used. The computer stores not only the coordinates to localize the fields of view harboring the most potentially abnormal cells but also retains black and white images of these fields of view.

10.2.3 Reporting Terminology

In the early years of cervical cancer screening, the terminology used to report the results of cervical cytology was not standardized. The most commonly used method was known as the Papanicolaou classification system which consisted of five categories or classes ranging from class I, absence of abnormal cells to class V, cytology conclusive for malignancy. However, classes II–IV were not uniformly used. The cytology community recognized the need for a standardized, reproducible system of interpretation and reporting, and so a consensus conference was held in 1987 at the National Cancer Institute. The Bethesda System for Reporting Cervical Cytology, first published in 1988 and subsequently revised in 1991 and 2001, was the result of the NCI meeting. Besides delineation of the required and optional elements of the cervical cytology report, the Bethesda system provided a written discussion of morphologic criteria as well as photographic print and online atlases to illustrate each cellular entity [18]. The atlases have proven to be an invaluable educational tool for CT and cytopathologists. Required elements of the report are listed in Table 10.1. A brief explanation follows herein.

Specimen type is self explanatory, either CS or one of the liquid-based preparations (ThinPrep™ or SurePath™).

Specimen adequacy is either satisfactory or unsatisfactory. There are two types of unsatisfactory samples. The first type is recognized as unsatisfactory up-front such that the sample is not processed. Examples of reasons for sample rejection include lack of patient identification or an empty specimen container. On the other hand, if the specimen is processed but is found to have too few squamous cells or squamous cells obscured by blood or inflammation, the sample is deemed unsatisfactory. When there are less critical issues with the quality of the sample, the specimen is processed and resulted but the issues, such as absence of endocervical cells, are listed under the subheading of **"quality indicators"**. In the past, lack of endocervical cells was felt to indicate a compromised sample, but more recent studies have not found this to be the case.

Interpretation/Result allows for a variety of options.

The sample will be interpreted as NILM when the epithelial cells appear normal or show "reactive" features (Fig. 10.3). The cause of the reactive changes may be obvious, for example an organism such as *Candida* sp., radiation change, intrauterine device, but the cause may not be evident. The risk of subsequent epithelial

Table 10.1 Required elements of report

Specimen type	Specimen adequacy	Interpretation	Ancillary testing	Automated review	Educational notes
(1) Conventional smear (2) Liquid-based prep	(1) Satisfactory for evaluation (2) Unsatisfactory for evaluation (a) Specimen not processed (b) Specimen processed	(1) Negative for intraepithelial lesion or malignancy (NILM) (a) Organisms (i) Trichomas vaginalis (ii) c/w Candida spp. (iii) Shift in vaginal flora (iv) c/w Actinomyces spp. (v) c/w Herpes simplex virus (b) Other non-neoplastic findings (i) Reactive cellular changes (ii) Glandular cells post hysterectomy (iii) Atrophy (2) Other (i) Endometrial cells in woman over 40 years of age (3) Epithelial cell abnormalities (a) Squamous cell (i) Atypical squamous cells of un-determined significance(ASCUS) (ii) Cannot exclude HSIL (ASC-H) (iii) LSIL (iv) HSIL (v) Squamous cell carcinoma (b) Glandular cell (i) Atypical (I) Endocervical cells, NOS (II) Endometrial cells, NOS (III) Glandular cells, NOS (IV) Endocervical cells, favor neoplastic (V) Glandular cells, favor neoplastic (ii) Endocervical adenocarcinoma in situ (iii) Adenocarcinoma (I) Specify type or designate NOS (4) Other malignant neoplasms	High risk HPV test results	(1) ThinPrep image analyzer (2) Focal point imager (3) Focal point GS imager	ASCCP guidelines

lesions has been found in small studies of patients with reactive changes to be slightly increased, but not sufficiently increased to warrant closer follow-up or ancillary testing of the sample [19–21]. Other findings that are covered under the NILM category include benign glandular cells identified in a posthysterectomy sample taken from the vaginal vault and changes associated with atrophy. Benign glandular cells in a posthysterectomy sample can result from glandular metaplasia (acquired vaginal adenosis) or if only the uterus has been removed, prolapse of a fallopian tube through the vaginal vault incision [22]. The cytologist must be careful to exclude recurrent tumor if the hysterectomy was performed for an adenocarcinoma.

A special "other" category is used to alert the clinician for the presence of benign appearing exfoliated endometrial cells identified in woman older than 40 years of age (Fig. 10.4). In younger women, shed endometrial cells rarely are associated with underlying abnormalities of the endometrium, but as women approach menopause and beyond, even benign endometrial cells can be a sign of endometrial hyperplasia or adenocarcinoma; it is left to the clinician to see if further investigation is warranted [23–25]. Although debated in the past due to their presence in biopsies of endometrial carcinoma, the presence of foamy histiocytes without accompanying endometrial cells in CVC are not considered to be clinically significant [26, 27]. Providing the laboratory with the date of the last menstrual period (LMP) and patient history is important to put endometrial cells as well as other findings into the correct context and thus the most appropriate Bethesda category.

Epithelial cell abnormalities are divided into squamous and glandular. Squamous abnormalities range from atypical squamous cells to SIL, either low or high grade, to squamous cell carcinoma. The atypical squamous cell category consists of two types: Atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells, cannot exclude HSIL (ASC-H). The cytologic changes of ASC-US are suggestive of low grade squamous intraepithelial lesion (LSIL) but of

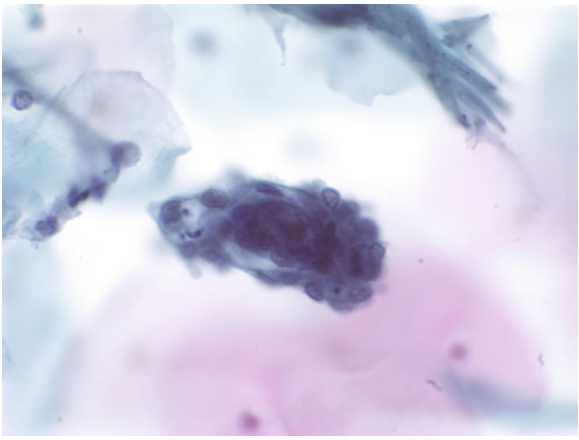

Fig. 10.4 Cluster of shed endometrial cells as may be seen during the first part of the menstrual cycle in reproductive age women

insufficient quantity or quality to warrant the interpretation. Cells designated as ASC-H are smaller cells in the range of immature squamous metaplastic cells. Cytologic features of LSIL consist of HPV cytopathic effects in the nucleus and cytoplasm (koilocytosis) of an intermediate type squamous cell with a modest increase in the nuclear to cytoplasmic ratio (Fig. 10.5). HSIL is identified in generally smaller metaplastic type squamous cells with a significantly increased nuclear to cytoplasmic ratio (Fig. 10.6). Squamous cell carcinoma implies more markedly abnormal cells in greater quantities. Invasion may be suspected if evidence of tissue breakdown, known as "tumor diathesis" (Fig. 10.7). Accurate grading of squamous abnormalities may not be possible if the squamous cells are fully keratinized.

Glandular cell abnormalities imply that the cellular changes are recognized in cells with cytoplasmic features of glandular cells [28]. If possible, the origin of the glandular cells will be indicated, either endocervix or endometrium, but at times,

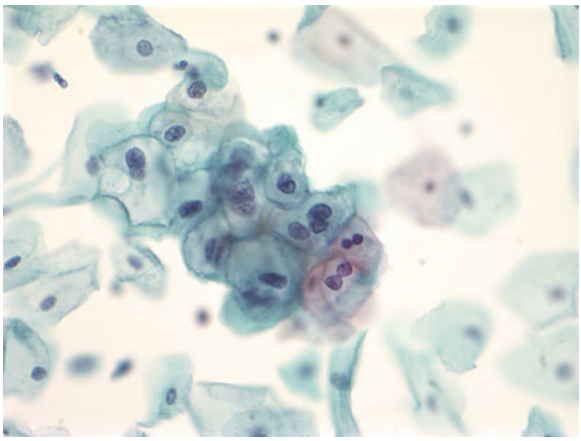

Fig. 10.5 Squamous cells with features of "low grade squamous intraepithelial lesion" as manifested by nuclear enlargement and cytoplasmic cavitation (koilocytosis)

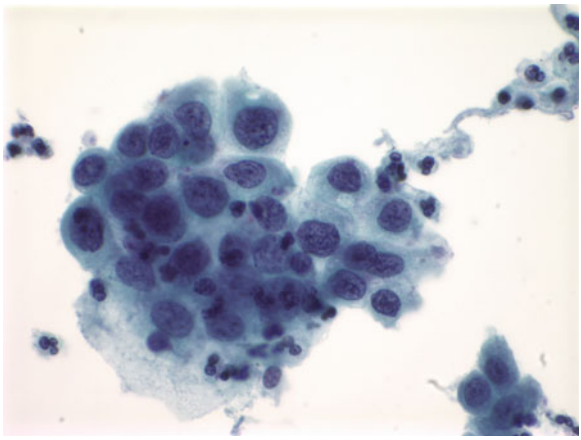

Fig. 10.6 Squamous cells with abnormal, hyperchromatic, irregular nucleus, and scant cytoplasm, features of "high grade squamous intraepithelial lesion"

the abnormal glandular cells cannot be further classified [29, 30]. The degree of abnormality is graded as either "not otherwise specified", to "cannot exclude neoplasia". The cytologic features of endocervical adenocarcinoma-in situ (AIS) are well recognized and permit a precise diagnosis of AIS [31, 32] (Fig. 10.8). AIS must be differentiated from sheets of HSIL cells that mimic AIS [33, 34]. If possible, when invasive adenocarcinoma is identified, the probable site of origin is also indicated [18] (Fig. 10.9). Supracervical or extrauterine origin of adenocarcinoma can also be suspected by the cytomorphology [35] (Fig. 10.10). For instance, cells of serous carcinoma of the adnexa or peritoneum may be shed and transported via the fallopian tube to the endometrial cavity and the endocervical canal to be detected in the CVC sample. An extrauterine origin of the malignancy is often suspected when no or little inflammatory response or tumor diathesis is identified. The malignant cells of serous carcinoma may be accompanied by

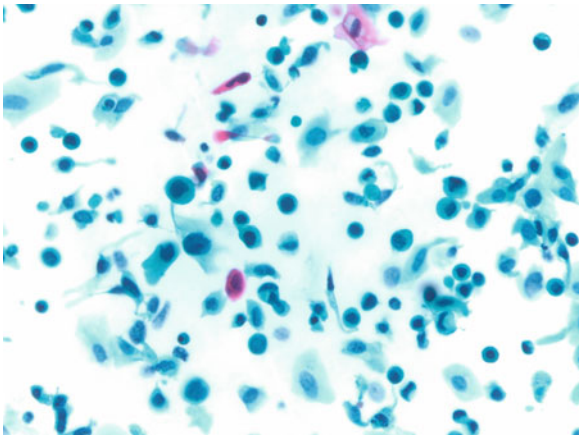

Fig. 10.7 Malignant squamous cells, some with bizarre shapes and keratinized, deeply *red-orange* cytoplasm, features of "squamous cell carcinoma"

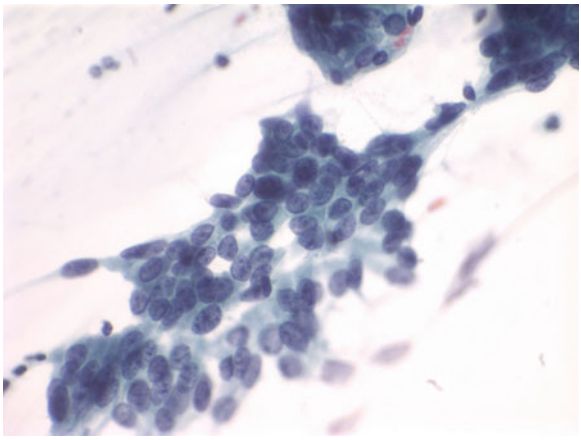

Fig. 10.8 Endocervical cells in a cluster with features of adenocarcinoma-in situ consisting of hyperchromatic, elongate, enlarged nuclei

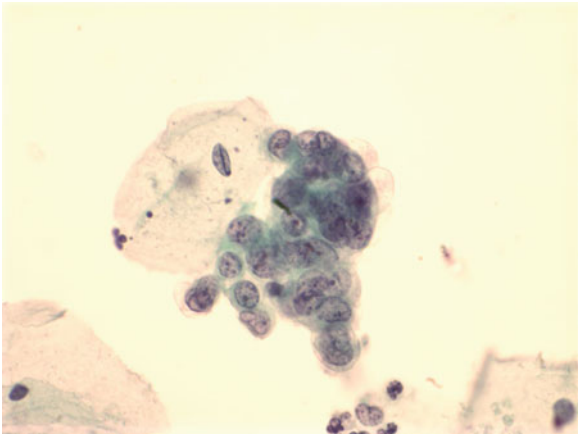

Fig. 10.9 Endocervical cells with large nuclei with irregularly distributed chromatin, large nucleoli, and vacuolated cytoplasm, features of endocervical adenocarcinoma

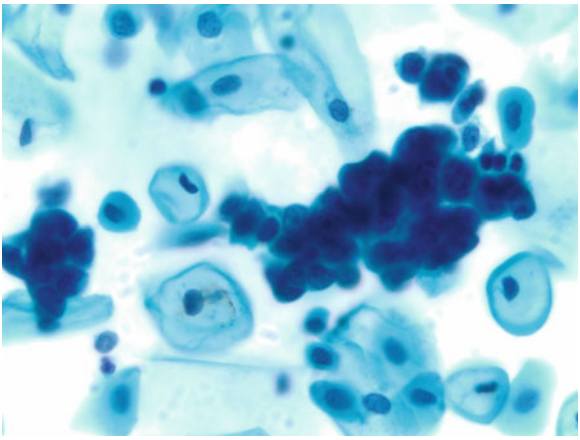

Fig. 10.10 Cluster of tightly aggregated irregular glandular cells with enlarged, irregular nuclei, and focally prominent nucleoli, features of endometrial adenocarcinoma

psammoma bodies, but the presence of psammoma bodies unassociated with glandular cells is most often a benign finding. All methods for preparation of CVC samples perform similarly in regards to detection of glandular abnormalities [36].

Rarely, unusual primary tumors, such as lymphoma, or sarcoma, may be cytologically identified [37, 38]. Likewise, metastatic malignant cells may be found, but in most instances, the primary tumor is known [35]. Initial diagnosis of a malignancy by metastatic tumor cells in the Pap is unusual [39]. Metastatic carcinomas to the cervix can mimic primary cervical adenocarcinoma [40]. **Ancillary testing** currently is high risk HPV testing, but in the future will most certainly include other tests. See the discussion below.

Automated review implies that either the Hologic system or BD system for image analysis is used as the initial screen.

Educational notes and suggestions are optional and refer to published guidelines.

10.2.4 Ancillary Testing

1. Testing for high risk HPV

Following the demonstration in 1976 of HPV as the cause of viral cytopathic effect in abnormal cervical cytology samples, increasingly sensitive tests lead to the conclusion that HPV is associated with nearly all cervical cancers. The results of small published trials suggested cervical cytology interpreted as ASC-US could be triaged to colposcopy according to the HPV test results [41, 42]. Two large studies were undertaken to provide enough data to support a change in management of abnormal cervical cytology [43, 44]. The first study, the ASC-US-LSIL Triage Study (ALTS), funded by the National Cancer Institute, was a randomized trial to compare three types of follow-up management for ASC-US: Immediate colposcopy, triage to colposcopy based on either testing for high risk HPV or by a repeat cervical cytology [44]. The 3,488 enrolled women had an ASC-US interpretation in the community and were assigned to a management arm and then followed every 6 months for 2 years with colposcopy at the last visit. By the end of the trial, CIN3 was diagnosed with approximately equal frequency in all arms (8–9 %) but the rate of referral to colposcopy and the number of additional visits and tests needed to diagnose CIN3 varied with the study arm:

- Only 53.6 % of the cumulative cases of CIN3 were detected at the initial immediate colposcopy;
- in the HPV triage arm, 72.3 % of the cumulative CIN3 were identified at the initial colposcopy, but only 55.6 % of women in the HPV arm were referred to colposcopy; in fact, 92.4 % of women with CIN3 were identified by the HPV test at enrollment;
- repeat cytology with triage at the ASC-US level identified 95.4 % of women with CIN3 but referred 67.1 % to colposcopy and required two visits.

The conclusion of ALTS trial was that reflex testing for HPV from the remaining sample from a liquid-based cervical cytology was the most efficient method for detection of CIN3. The trial also evaluated a separate group of women with LSIL cytology but found that HPV testing did not help in triage since 83 % of the 642 women were high risk HPV positive.

The second large study, begun in 1996 around the same time as the ALTS trial, compared the sensitivity for CIN2+ by reflex HPV testing from LBC to repeat conventional cytology in the triage of 995 women with ASC-US [43]. The sensitivity of HPV testing compared to cytology was 89.2 % versus 75.8 % with a positive predictive value of 15.2 versus 13.0.

In 2001, following these large studies, the American Society for Colposcopy and Cervical Pathology (ASCCP) published guidelines for management of women with cervical cytologic and histologic abnormalities, including HPV testing for women with ASC-US on LBC [45]. In 2007, the ASCCP published revised guidelines for adolescents, incorporation of HPV testing into the work-up

algorithm of atypical glandular cells (AGC) and the addition of HPV testing to the cervical cytology screening of women over 30 years of age [46].

Following additional large scale studies that explored the use of hrHPV co-testing with cytology or as a primary screening method [10, 47–50], major revisions to the guidelines were made in 2011 by the U.S. Preventive Services Task Force (USPSTF) [51] and in 2012 jointly by the American Cancer Society (ACS), the ASCCP and the American Society of Clinical Pathology (ASCP) [52].These and other guidelines are discussed in more detail below.

There are several methods used to test for the presence of high risk HPV. The Hybrid Capture 2 (HC2) (Digene) is FDA approved and was the test used in the ALTS trial, thus considerable data support the use of this test [44]. The HC2 is a signal amplification nucleic acid hybridization test for 13 hrHPV types that uses an RNA probe to detect the HPV DNA. The RNA–DNA hybrid is then "captured" by anti-RNA–DNA antibodies fixed to the well of a microtitre plate. Chemiluminescent-tagged antibodies attach to the fixed hybrids and the signal is amplified. The result is semi-quantitative but is reported simply as positive or negative. The cutoff for a positive test is 1 pg hrHPV DNA/ml which corresponds to 5,000 DNA copies. Lacking a measure of the number of cells in the test sample, the test cannot be rendered quantitative. In other words, the viral load cannot be accurately determined. The test is sold as a relatively easy to use kit.

The Cervista™ HPV HR test (Hologic, Inc), also FDA approved, is a signal amplified nucleic acid hybridization test that relies on an invader reaction in order to test for 14 high-risk HPV types. The reaction was described in 1993 as the enzymatic cleavage of single stranded DNA by structure, rather than sequence, recognition [53]. The reaction was first used to detect single nucleotide polymorphisms [54]. In the Cervista test, 14 hrHPV types are detected by three sets of pooled reagents. The probes target 2–3 separate regions of the HPV genome in order to avoid potential false negative tests due to the presence of loss of HPV DNA during integration into the human genome. The test also detects the presence of human DNA as an internal control, so as to provide a semi-quantitative result [55]. Studies have shown the Cervista test to perform comparably to the HC2 [56, 57].

HPV type specific tests will become increasingly important in the era of HPV vaccination and for epidemiological studies. Hologic offers a second separate test, Cervista HPV 16/18, to determine if a positive Cervista test for hrHPV is due to these particularly high risk types. The test result is reported as positive for HPV 16/18. The test has been analytically and clinically validated [58, 59].

Many variations of the PCR exist and many studies of HPV testing to screen for cervical cancer, especially those conducted in Europe have used PCR assays [60, 61]. The Roche Cobas® HPV test, the only FDA approved PCR test, is an almost fully automated real time PCR test using primer pairs to a polymorphic region of the L1 gene to detect 14 hrHPV types (16,18,31,33,35,39,45,51,52,56, 58,59,66,68) and to ß-globin gene. The resultant amplified product(s) are detected by four fluorescent dye labeled probes to HPV16, HPV18, the other 12 HPV types, and the ß-globin gene. Thus the results will indicate the presence of hrHPV with genotyping for HPV16 and HPV18 as well as confirmation of the presence of

human cells in the sample (ß-globin). The rationale for genotyping HPV 16 and HPV 18 is their strong association with cervical cancer (HPV 16 in 55–60 % and HPV 18 in 10–15 %) [3, 62, 63]. The test has been analytically and clinically validated [64–66] and has been extensively compared to the HC2 test [67–70]. In addition, a large prospective multicenter clinical trial entitled, Addressing the Need for Advanced HPV Diagnostics (ATHENA) is nearing completion. The study aims to evaluate the performance of the Cobas HPV test as a triage for women with abnormal cytology results (ASC-US), as an adjunctive test in women with negative cytology results and as a primary screening test. A total of 47,208 women aged 21 years and older are enrolled for baseline evaluation including cytology and the cobas HPV test. All women with double negative results, except a subset selected for the follow-up of 3 years longitudinal study with colposcopy, exit the study. All women with any abnormal results are entered into the longitudinal study. Pregnant women are excluded from the longitudinal study. Overall the prevalence of hrHPV was found to be 6.7 % with the prevalence of HPV types 16/18 being 1.2 %. The prevalence of cervical intraepithelial neoplasia 2 or worse ($\geq$CIN2) was 1.2 %. The absolute risk of $\geq$CIN2 in women positive for HPV 16/18 was 11.4 % compared to 6.1 % for other hrHPV types and 0.8 % in women negative for hrHPV [71]. In women with cytology categorized as ASC-US (4.1 %), the Cobas HPV test performed similarly to HC2 in the ALTS trial. HPV 16/18 genotyping was associated with a 24.4 % risk of biopsy proven $\geq$CIN2 as compared to the finding of other hrHPV (14.0 %) or no evidence of hrHPV (0.8 %) [72]. Genotyping was found to be useful in risk assessment by age stratification and when integrated into screening strategies provide more efficient referral to colposcopy [73–75].

The FDA recently approved an alternative type of test for high risk HPV, the Gen-Probe APTIMA test which evaluates the presence of hrHPV mRNA in CVC samples. APTIMA is a qualitative multiplex nuclei acid amplification test for E6/E7 viral mRNA from 14 hrHPV types. The rationale for the test is that biopsy proven $\geq$CIN2 is more likely to be associated with integration of the hrHPV into the human genome with subsequent over expression of the E6/E7 genes that tend to further dysregulate the cell cycle. The idea is that transient innocuous hrHPV infections would not be detected, thus significantly increasing the specificity of the hrHPV test. The APTIMA test has been analytically and clinically validated [76–80]. The test results are reported as positive or negative for the presence of hrHPV E6/E7 mRNA.

2. Other ancillary tests

A number of other tests have been investigated in the hope of increasing the specificity in order to detect the HPV infection most likely to progress to full blown cancer if left untreated.

Tests for human cellular dysregulation include abnormal cell cycle proteins, most notably p16, amplification of human telomerase gene TERC on chromosome 3q, and determination of the methylation status of the human DNA or of the HPV DNA hold promise but larger, more rigorous studies are needed before these tests

could be used in screening [81–84]. An immunocytochemical test for p16 and Ki67 (the cell cycle protein detected in actively cycling cells), commercially known as CINtec, is used on CVC samples in Europe and may become available in North America [85–87].

10.2.5 Diagnostic Performance

As a screening test, CVC has been criticized for the variable sensitivity (55–90 %), albeit high specificity (90 %), for SIL [88]. In unscreened populations, well developed precursor lesions are readily picked up by CVC. Treatment of these lesions leads to the rapid decline of cervical cancer as has been demonstrated in many populations. In more well screened populations, such as countries of North America and Western Europe, precursor lesions are less frequent and cytologic abnormalities are often more subtle. For this reason, in order to maintain a robust sensitivity, the cutoff for an abnormal CVC interpretation is usually at the level of ASC-US.

The risk of cancer or a high grade precursor lesion of cancer in the cervix varies with the cytologic interpretation and, if performed, the risk is modified by the results of hrHPV testing.

NILM: The sensitivity of a single CVC cytology is difficult to assess due to the variation in methods used in studies to investigate sensitivity. A wide range has been reported, from a low of 50 % to as high as 94 % [88]. Repeating the CVC on an annual basis compensates for the potentially low sensitivity. When coupled with hrHPV testing (as in women over 30 years), the sensitivity increases to 100 % but specificity is somewhat diminished [89]. Most importantly, a combined NILM CVC and negative test for hrHPV has close to a 100 % negative predictive value for high grade disease.

ASC-US: This is the most common CVC abnormality in the United States, reported in about 5 % of samples. When hrHPV testing is used to triage ASC-US, biopsy-proven CIN2-3 is found in 4.3–26.7 % of women. In fact, most biopsy proven CIN2-3 is preceded by an ASC-US CVC.

ASC-H: The interpretation of ASC-H is about 10 times less common than ASC-US. However, the risk of subsequent biopsy-proven CIN2-3 is significantly higher than for ASC-US, about 40 %. Studies have suggested that testing for hrHPV may help better define the risk. However, per current guidelines, women with ASC-H should go directly to colposcopy.

LSIL: This interpretation is made in about 2.5 % of all CVC, but the incidence varies with the age group studied. Subsequent biopsy-proven CIN2-3 occurs in 12–16 % of women.

HSIL: About 0.7 % of CVC samples are interpreted as HSIL. The rate varies with the age group, being less common in older women. Subsequent biopsy-proven CIN2-3 is identified in 84–97 % of women with an HSIL CVC.

AGC: Similar to HSIL, about 0.7 % of CVC samples are interpreted as AGC (all degrees together). The interpretation of AGC can encompass both HPV-related

disease and non-HPV-related disease. Many studies indicate a benefit from performing hrHPV testing to alert the practitioner to the possibility of HPV-related disease (CIN2/3 and/or adenocarcinoma in situ of the endocervix). In women with hrHPV negative AGC, noncervical glandular neoplasia remains a possibility, especially in older women.

10.2.6 Regulatory Issues and Quality Assurance

Gynecologic cytology screening is highly regulated by the Federal Government. The Clinical Laboratory Improvement Amendment of 1988 (CLIA'88) spells out in exquisite detail the regulations by which the lab must abide by (http://wwwn.cdc.gov/clia/regs/toc.aspx) [90]. 10 % of CVC samples interpreted as negative must be rescreened, cytology–histology correlation must be evaluated, following a new interpretation of HSIL all CVC samples interpreted as NILM for the prior 5 years must be reviewed.

10.2.7 Guidelines for Screening Interval and Follow-Up

In the United States, several professional organizations regularly issue updated guidelines screening for cervical cancer and for follow-up of cytological abnormalities as new data becomes available. The most recent guidelines for screening were published in 2011 by the USPSTF (www.ahrq.gov) [51] and in 2012 by a consortium of the ACS, ASCCP, and the ASCP [52]. In response to the updated guideline, the American College of Obstetrics and Gynecology (ACOG) published an updated practice bulletin with up-dated screening recommendations [91].

The recommendations by all organizations are in substantial agreement that screening be stratified according to patient age and risk factors as follows:

1. Women under 21 years should not be screened
2. Women between the ages of 21 and 29 should have cervical cytology (Pap test) alone every 3 years. They should not be tested for HPV unless as triage following an abnormal Pap test result (ASC-US).
3. Women between the ages of 30 and 65 should be screened with a Pap test and an HPV test ("co-testing") every 5 years (the preferred approach) or a Pap test alone every 3 years (acceptable).
4. Women over age 65 who have had regular screenings with normal results should not be screened for cervical cancer. Women who have been diagnosed with cervical pre-cancer (CIN2 or more severe) should continue to be screened for 20 years.
5. Women who have had a hysterectomy with the cervix removed and have no history of cervical cancer or precancer should not be screened.

6. Women who have had the HPV vaccine should be screened according to recommendations for their age group.
7. Women who are at high risk for cervical cancer may need to be screened more often or alternatively. Women at high risk might include those with a history of cervical cancer or immunocompromised (e.g., infection with human immunodeficiency virus or status-post organ transplant), or exposure in utero to the drug diethylstilbestrol (DES).

In summary, the ACS and the other organizations no longer recommend annual screening for cervical cancer, because an analysis of the benefits of screening weighed against the harm of overtreatment favored a prolongation of the screening interval [52]. The development of cervical carcinoma is generally long, as long as 10–20 years, so that even with the longer screening interval of 5 years, invasive cervical cancer is unlikely to develop.

The ASCCP previously published guidelines for the management of women with abnormal cervical cancer screening tests in 2007 [6, 74]. The ASCCP convened a conference in the fall of 2012 to discuss updated guidelines which were released in early 2013 [92]. The 2006 guidelines propose choices for management of ASC-US that include testing for hrHPV, repeat cervical cytology or colposcopy. However, when residual cells from a LBC sample are available, "reflex" hrHPV testing is preferred. Women who are hrHPV negative should have a repeat cytology in 12 months (this interval will probably be lengthened to 3 or 5 years depending on the age of the woman). Women who test positive for hrHPV have the equivalent of LSIL and should be referred for colposcopy. If no lesion is identified on the exocervix at colposcopy, blind endocervical sampling should be performed. If the colposcopy is negative, hrHPV testing should be repeated in 1 year (never less than 1 year) or cytology should be repeated at 6 and 12 months.

The recommended management of ASC-H is colposcopy. If CIN2+ is not identified, hrHPV testing should be repeated in 12 months, or cytology should be repeated at 6 and 12 months. LSIL is managed with colposcopy; if CIN2+ is not identified, hrHPV testing should be repeated at 1 year or cytology repeated at 6 and 12 months. In postmenopausal women with LSIL, "reflex" hrHPV testing may be performed or repeat cytology testing at 6 and 12 months can be done in lieu of colposcopy. If either hrHPV testing is positive or cytology is $\geq$ASC-US, colposcopy is recommended. Women with HSIL on cervical cytology should be referred for colposcopy. Post-excision/ablation of CIN2+, follow-up is intended to detect persistent or recurrent disease. The ASCCP recommends either cervical cytology alone or with colposcopy every 4–6 months until three negative cytologies or a single hrHPV test at 6 months.

Women with AGC need multiple tests including colposcopy, endocervical evaluation and sampling, HPV testing, and endometrial evaluation. The 2012 revised ASC-ASCCP-ASCP guidelines for cervical cancer screening also made some recommendations on management. One particularly challenging problem occurs in co-testing of women over 30 years old who have a negative cytology result but a positive test for hrHPV. Two management options were recommended:

1. Repeat co-testing in 12 months; if the co-test is positive, the woman is referred to colposcopy; if the test is negative, the woman may return to 5 year screening interval.
2. immediate, HPV genotype-specific testing for HPV 16 and/or HPV 18; women testing positive go directly to colposcopy; if negative, repeat co-testing in 12 months; if negative (that is HPV negative AND ASC-US or negative cytology), return to 5 year interval but if the co-test is positive (HPV positive OR LSIL or more severe cytology), refer to colposcopy.

Women testing hrHPV positive and cytology negative should not be referred directly to colposcopy, should not be genotyped for hrHPV other than 16 or 18, and should not be tested with other non-HPV biomarkers.

Women between 30 and 65 years with ASC-US cytology results and negative hrHPV testing should continue to be screened by either co-testing at 5 years or by cytology alone at 3 years.

Essential Changes in ASCCP 2012 Guidelines From Prior 2006 Management Guidelines [92]

- Cytology reported as negative but lacking endocervical cells can be managed without early repeat.
- CIN 1 on endocervical curettage should be managed as CIN 1, not as a positive ECC.
- Cytology reported as unsatisfactory requires repeat even if HPV negative.
- Genotyping triages HPV-positive women with HPV type 16 or type 18 to earlier colposcopy only after negative cytology; colposcopy is indicated for all women with HPV and ASC-US, regardless of genotyping result.
- For ASC-US cytology, immediate colposcopy is not an option. The serial cytology option for ASC-US incorporates cytology at 12 months, not 6 months and 12 months, and then if negative, cytology every 3 years.
- HPV-negative and ASC-US results should be followed with co-testing at 3 years rather than 5 years.
- HPV-negative and ASC-US results are insufficient to allow exit from screening at age 65 years.
- The pathway to long-term follow-up of treated and untreated CIN 2+ is more clearly defined by incorporating co-testing.
- More strategies incorporate co-testing to reduce follow-up visits. Pap-only strategies are now limited to women younger than 30 years, but co-testing is expanded even to women younger than 30 years in some circumstances. Women aged 21-24 years are managed conservatively.

CIN, cervical intraepithelial neoplasia; ECC, endocervical curettage; HPV, human papil-lomavirus; ASC-US, atypical squamous cells of undetermined significance. *Prior management guidelines were from the ‘‘2006 Consensus Guidelines for the Management of Women With Abnormal Cervical Screening Tests’’ (6). Prior guidelines not changed were retained.

10.3 Cytology of the Endometrium

10.3.1 Epidemiology of Endometrial Cancer

Endometrial adenocarcinoma (EMCa) is the most common gynecologic cancer and corresponds to approximately 6 % of all cancers in women [93]. Most cases are diagnosed at an early stage. Epidemiologic and prognostic factors suggest that there exist two forms of endometrial cancer [94]:

- Type I, estrogen-related, low grade endometrioid-type, often arising in a background of endometrial hyperplasia. Risk factors for type I include obesity, nulliparity, estrogen excess, either exogenous (tamoxifen, hormone replacement therapy) or endogenous, diabetes melitis, and hypertension. Type 1 makes up approximately 80 % of EMCa.
- Type II, unrelated to estrogen, tends to be of higher grade, of poorer prognostic type, and includes serous carcinoma and clear cell carcinoma. Type II does not arise in association with hyperplasia and the patients do not share the risk factors of type I.

Most cases of EMCa are diagnosed after menopause, usually over the age of 60. Twenty-five percent are diagnosed before menopause with only 5–10 % before 40. A hereditary form, part of the Lynch syndrome associated with hereditary non-polyposis colorectal cancer, occurs in a minority of patients [95].

Screening of the general population is not advocated. Most cases are detected without screening by the presence of abnormal uterine bleeding in 90 % of patients [96]. Even a minimal amount of blood in a postmenopausal patient is cause for exploration and while atrophy of the endometrium is the most usual case of bleeding in this age group, the further one is from menopause, the more likely is EMCa. Overall, 5–20 % of postmenopausal bleeding is related to EMCa. Abnormal uterine bleeding should be investigated by physical exam and Pipelle endometrial biopsy, a technique that can be performed in the office without anesthesia. The Pipelle biopsy sample permits examination of the cytology and architecture of the endometrium. Transvaginal ultrasound is often added as part of the diagnostic work-up.

10.3.2 Cytology of Endometrial Cells Obtained from the Endometrial Cavity

Diagnosis of endometrial lesions by cytology can be made by two methods. First, endometrial cells may be fortuitously exfoliated in cervical cytology, providing cells for evaluation even though CVC is not designed to sample the endometrium. The sensitivity of finding EMCa in CVC has been calculated to be 40–55 % on

CSs and 60–65 % on LBC [97, 98]. Despite the Pap test's unpredictability for detecting EMCa, the presence of endometrial cells may be significant. For this reason, in women 40 years old or older, when benign appearing endometrial cells are identified, they are mentioned in the cytology report under "other" [18]. The clinician should correlate with the menstrual status and determine if follow-up is warranted [99]. If the endometrial cells are cytologically atypical, they will be reported under "Epithelial cell abnormality—Glandular".

The second method, direct sampling of the endometrium for cytology, can be fortuitous or deliberate. Fortuitous abrasion of endometrial glandular and stromal cells or lower uterine segment cells occurs not infrequently with thorough sampling with an endocervical brush, especially in women who have undergone LEEP or cone biopsy of the cervix that shortens the endocervical canal [100]. Abraded, directly sampled benign appearing endometrium is not associated with increased risk of EMCa and is not reported in a Pap test report [101]. Deliberate, direct sampling of the endometrium has been advocated by cytologists for a number of years. A variety of sampling devices that brush or aspirate the endometrium have been developed, most recently the Tao brush. The sample is smeared thinly on a glass slide, stained with Papanicolaou stain, and read in a manner similar to CVC slide or the sample is suspended in alcohol [102]. The technique has not caught on as a routine test probably due to the number of inadequate smears obtained, from 8 to 20 %, the learning curve needed to skillfully read a slide and the amount of time needed to adequately screen the slide. In the end, the Pipelle biopsy is simpler to obtain and simpler to read [103].

10.3.3 Peritoneal Wash Cytology and Staging Endometrial Cancer

Surgical staging of cancer of the uterine corpus has been recommended for more than 20 years by the International Federation of Gynecology and Obstetrics (FIGO). The original staging system included peritoneal wash cytology (PWC), but FIGO revised the staging system in 2009 and eliminated PWC as a variable dictating a more advanced stage when positive [104]. The reason given was the uncertain prognostic significance of isolated positive PWC [105]. According to the committee, there is evidence that positive PWC is not an independent predictor of poor outcome in the absence of other evidence of aggressive disease. However, discovery of occult primary tumors following positive PWC have been reported [106]. The method of PWC collection entails instillation of 100 ml of sterile saline into the peritoneal cavity immediately after entry into the peritoneum and lysis of adhesions. The fluid is then aspirated and sent to cytology. Differentiation of benign mesothelial cells from malignant glandular cells is often straightforward, but pitfalls can be encountered, such as hyperplastic mesothelial cells from adhesions or cells derived from endometriosis or endosalpingiosis (Fig. 10.11).

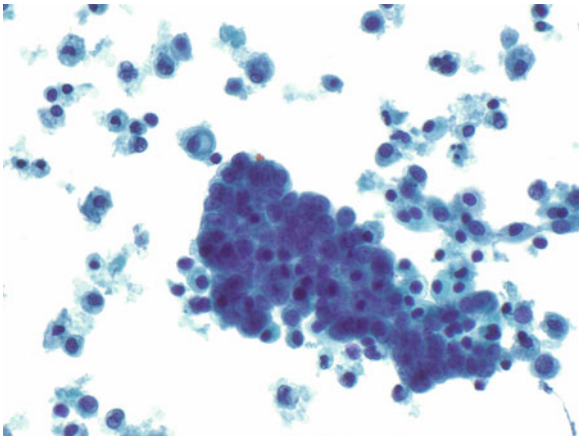

Fig. 10.11 Pelvic washing with benign, uniform mesothelial cells on the *left* contrasted with a cluster of serous adenocarcinoma on the *right*

The cytologist should correlate the PWC findings with surgical pathology findings if any question as to the origin of the cells in the sample arises [107].

10.3.4 Diagnosis of Recurrent Endometrial Adenocarcinoma in the Vaginal Cuff

The National Comprehensive Cancer Network has developed posttreatment surveillance consensus-based guidelines for EMCa that includes vaginal cytology every 6 months for 2 years, then annually (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp).

However, studies indicate that the use of vaginal cuff cytology may not be cost effective [108, 109].

10.4 Cytology of the Female Adnexa

The female adnexa include the ovaries, fallopian tubes, and accompanying ligaments and soft tissues. Cysts and mass lesions in the adnexa occur at all ages and most adnexal masses arise in the ovary [110]. The lesions may be symptomatic or may be found incidentally on physical exam or imaging. The risk of malignancy depends on the several factors, being higher in prepubertal girls and in postmenopausal women. If the mass is solid or is a complex cyst with solid components, if the woman has a history of malignancy elsewhere or if ascites is present, the risk is also elevated. Besides a good history and physical exam, the most important diagnostic tool in the evaluation of an adnexal mass is pelvic ultrasound (US). Certain benign lesions (simple cyst, dermoid cyst, hemorrhagic cyst, and

endometrioma) produce characteristic US features [111]. Watchful waiting is usually advocated for simple cysts less than 10 cm in premenopausal women and less than 5 cm in the postmenopausal woman. Surgical exploration, by laparoscopy or laparotomy, is advised for larger cysts or if other worrisome features are present, such as a solid component [112]. Two types of cytology samples are used as part of the diagnostic work-up of adnexal masses: Peritoneal washings and fine needle aspiration (FNA) [113].

10.4.1 Peritoneal Washings

Peritoneal washing is the technique used to sample a large surface area of the pelvis at the time of laparotomy or laparoscopic surgery. The findings compliment visualization of the pelvic and abdominal organs by the surgeon and histologic examination of the tissues by the pathologist. In case of malignant tumors of the ovary, the findings of the peritoneal washing will be taken into account in the FIGO staging. In tumors confined to the ovary, positive peritoneal washings will upstage the tumor to IC.

10.4.1.1 Collection Technique, Processing and Interpretation

The pelvic cavity can be accessed by the laparotomy incision or by the laparoscopic trocar. Sterile saline is washed over the pelvic organs prior to disruption by surgery. The saline dislodges mesothelial cells in sheets and as single cells, along with inflammatory cells, red blood cells and if present, cells extraneous to the peritoneal lining. The extraneous cells may be benign, such as cells from endometriosis, or malignant, such as serous carcinoma of the ovary. The wash fluid is aspirated into a collection container. In the cytology laboratory, the fluid is centrifuged. The cell rich pellet is used to make smears, LBC slides and if sufficiently cellular, a cell block for histology. A cell block can also be made from the residual of the LBC suspension and is particularly useful if the origin of cells is in question. The usual question is whether epithelioid-appearing cells are of mesothelial, histiocytic, or glandular origin. A panel of antibodies including pan-cytokeratin, calretinin, CD68, BerEP4, B72.3, CEA, and MOC31 can be used [107, 114, 115].

Although not a washing, aspiration of small amounts of fluid that accumulate in the pouch of Douglas by cul-de-sac during many benign or malignant processes can be obtained by culdocentesis. The fluid is processed in a manner similar to the peritoneal wash.

Correlation of the cytology sample with the histology is mandatory in order to interpret the peritoneal washing. For example, atypical cells may be found in histologically benign lesions such as torsion, acute salpingitis, or endometriosis that provoke serosal adhesions with reactive hyperplasia of the mesothelium [116]. Unless the cytology sample is compared with the histology, there is a potential for

a false positive diagnosis. Another example is the case of malignant appearing cells with no histologic evidence of tumor on the serosal surface of the adnexa or uterus. In this case, comparison to the primary tumor will confirm the origin of the abnormal cells in the pelvic washing.

10.4.1.2 Diagnostic Performance

In ovarian cancer, the sensitivity of peritoneal washing when the peritoneal surface is found to be histologically involved by tumor has been found in several studies to vary from 73 to 82.9 % [117–119]. However, one more recent study found a sensitivity of only 25 % for all malignant ovarian tumors; but, the sensitivity varied with the tumor type [120]. For the five most common tumors of epithelial origin the overall sensitivity was 51 % with a specificity of 93 %. Among these five types, the PWC was most sensitive for serous type at 71 % and lowest for clear cell type at 20 %. The sensitivity for malignancy has also been found to be lower at posttreatment "second look" [117] but despite this, peritoneal washing has been deemed an important test because women are identified with tumors are more likely to recur [121].

10.4.2 Fine Needle Aspiration of Adnexal Masses

FNA has been advocated by some cytopathologists as an aid in diagnosis of adnexal masses in women. FNA is a low risk procedure that offers the possibility of avoiding surgery on the ovary [122]. The main objections to FNA of an adnexal mass include inadvertent rupture of a malignant cyst and concerns about the accuracy of an FNA diagnosis.

10.4.2.1 Procedure, Cytology Preparation and Interpretation

Aspiration of adnexal masses may be performed by the transvaginal or transrectal route or percutaneously through the abdominal wall, with or without imaging guidance [123]. An FNA can also be performed during laparotomy or laparoscopic surgery. By whatever route chosen, a 14- to 22-gauge needle is used. If a cyst is aspirated, all of the aspirated fluid should be sent to cytology for centrifugation. The cell pellet is used to make smears, liquid-based preparations or cell blocks. If a solid lesion is aspirated, the material is smeared on glass slides by the operator and the slides are either air dried for Wright-Giemsa staining (the same stain as is used for peripheral blood smears) or fixed immediately by spray or immersion in 95 % ethanol. The aspirate may also be expressed into a liquid fixative/preservative or saline for transport to the cytology laboratory for processing as a liquid-based slide.

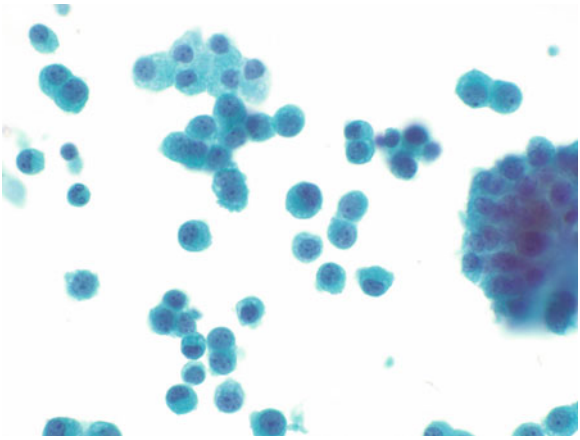

Fig. 10.12 Follicular cells aspirated from a functional, follicular cyst of the ovary

Interpretation of the FNA sample depends on the type of mass sampled. Unilocular ovarian cysts may be functional, non-neoplastic cysts such as preovulatory follicle cysts or postovulatory corpus luteum cysts (Fig. 10.12). Cysts of less than 3 cm in diameter are considered physiologic. Aspiration of functional cysts are variably cellular, from highly cellular with many diagnostic granulosa cells, to nondiagnostic paucicellular samples comprised of only macrophages. In follicle cysts, the granulosa cells can be shed singly or in clusters. The cells often have scant cytoplasm, dark round to oval, occasionally creased nuclei. Mitoses may be identified. The differential diagnosis includes unilocular cystic neoplasms including cystic granulosa cell tumor, carcinoid tumor, and serous tumors. Aspiration of a corpus luteum cyst will produce granulosa cells with abundant luteinized, foamy, eosinophilic cytoplasm.

Aspiration of an endometriotic cyst usually contains almost only hemosiderin laden macrophages and lysed blood. Benign epithelial cysts can be found in the ovary or paratubal tissue. Hydrosalpinx can appear to be a cyst. The FNA sample is usually sparsely cellular, containing mainly macrophages, but also low cuboidal epithelial cells, sometimes ciliated. The epithelial cells can appear atypical due to degenerative changes [124]. The presence of benign appearing mucinous cells may be derived from a mucinous cystadenoma.

Aspiration of complex solid and cystic lesions or entirely solid lesions of the adnexa is not usually recommended. The more common lesions, such as mature cystic teratomas, are usually diagnosed by ultrasound. If there is any clinical suspicion of malignancy, aspiration is not recommended [125].

However, the cytopathologist will be expected to recognize the cytologic appearance of metastatic ovarian tumors, some of which may be difficult. For instance, a granulosa cell tumor is a potentially malignant neoplasm that has a predilection to metastasize long after the primary tumor has been removed.

10.4.2.2 Diagnostic Performance

Most series of adnexal mass FNA considered only cysts [124–126]. And most of the cyst aspirates in these studies were done, at least for some of the cases in each series, not in vivo, but after surgical removal of cyst, creating a somewhat artificial scenario. The results of these studies were variable. In the studies that commented on the number of samples without diagnostic cells, the rate varied from 11 to 46 % [125–127]. The sensitivity for malignancy varied from 25 to 75 % [123, 125]. The false positive rate varied from 0 to 73 % and the false negative rate varied from 12 to 35 %. The problem of nondiagnostic samples and false negative cases appears to be due to the presence of only macrophages and other inflammatory cells in many cyst fluid samples. Conversely, the cells lining non-neoplastic cysts or benign cysts can become atypical when shed into the cyst fluid, leading to false positive interpretations.

Only a few studies have dealt with real time aspiration of both solid and cystic ovarian lesions. One comprehensive study from 1972 in Sweden obtained FNA samples from palpable adnexal masses using either a transvaginal or a transrectal route [128]. Categorization of 80 samples into benign or malignant categories was highly accurate with a false positive and false negative rate of 5 and 7 %, respectively. A separate group of 52 malignant ovarian tumors were reliably categorized in almost each case with an unsatisfactory rate of only 6 %.

A more recent study of 235 cystic ovarian lesions sampled by FNA under ultrasound guidance found the nondiagnostic rate to be much higher at 56 %. An elevated cyst fluid estradiol was measured to help identify follicular cysts. The sensitivity was lowest (36 %) for endometriotic cysts, presumably due to excess blood. The highest sensitivity was found for proliferating and malignant neoplasms including surface epithelial tumors, granulosa cell tumor, and an immature teratoma. Specificity for all lesions approached 100 % except for paratubal cysts (83 %) [129].

As evidenced by the cited studies, the accuracy of cytology results varies widely. Therefore, if the practitioner wishes to use cytology in the work-up and treatment of adnexal masses, communication with the cytopathologist is advised to determine their level of experience with adnexal samples. Some clinicians have employed cytology in conjunction with other parameters, such as ultrasound findings and estradiol measurement to more selectively aspirate cystic lesions [130–132]. However, when benign appearing cysts are aspirated, a recurrence rate as high as 54 % has been found [133]. A single randomized controlled study of cyst aspiration including 278 women aged 14–81 years with unilocular cysts 4–7 cm found no difference in outcome between women undergoing cyst puncture and those managed expectantly after 6 months of observation [134]. Some clinicians have used cyst aspiration in conjunction with sclerotherapy to treat benign non-neoplastic cysts [135–137].

References

1. Bosch FX, Burchell AN, Schiffman M et al (2008) Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 26(Suppl 10):K1–K16
2. Dunne EF, Unger ER, Sternberg M et al (2007) Prevalence of HPV infection among females in the United States. JAMA 297:813–819
3. Munoz N, Bosch FX, de Sanjose S et al (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348:518–527
4. Wright TC Jr, Schiffman M (2003) Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 348:489–490
5. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62:10–29
6. Wellings K, Collumbien M, Slaymaker E et al (2006) Sexual behaviour in context: a global perspective. Lancet 368:1706–1728
7. Sirovich BE, Welch HG (2004) The frequency of Pap smear screening in the United States. J Gen Intern Med 19:243–250
8. Diamantis A, Magiorkinis E, Androutsos G (2009) What's in a name? Evidence that Papanicolaou, not Babes, deserves credit for the PAP test. Diagn Cytopatho l38(7):473–476)
9. Janerich DT, Hadjimichael O, Schwartz PE et al (1995) The screening histories of women with invasive cervical cancer, Connecticut. Am J Public Health 85:791–794
10. Koliopoulos G, Arbyn M, Martin-Hirsch P, Kyrgiou M, Prendiville W, Paraskevaidis E (2007) Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non-randomized studies. Gynecol Oncol 104:232–246
11. Wright TC Jr (2003) Cervical cancer screening using visualization techniques. J Natl Cancer Inst Monogr 31:66–71
12. Marchand L, Mundt M, Klein G, Agarwal SC (2005) Optimal collection technique and devices for a quality pap smear. WMJ 104:51–55
13. Martin-Hirsch P, Jarvis G, Kitchener H, Lilford R (2000) Collection devices for obtaining cervical cytology samples. Cochrane Database Syst Rev 2:CD001036
14. Ferris DG, Berrey MM, Ellis KE, Petry LJ, Voxnaes J, Beatie RT (1992) The optimal technique for obtaining a Papanicolaou smear with the Cervex-Brush. J Fam Pract 34:276–280
15. Harrison DD, Hernandez E, Dunton CJ (1993) Endocervical brush versus cotton swab for obtaining cervical smears at a clinic. A cost comparison. J Reprod Med 38:285–288
16. Arbyn M, Herbert A, Schenck U et al (2007) European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology. Cytopathology 18:133–139
17. Day SJ, O'Shaughnessy DL, O'Connor JC, Freund GG (2004) Additional collection devices used in conjunction with the SurePath Liquid-Based Pap Test broom device do not enhance diagnostic utility. BMC Womens Health 4:6
18. Solomon D, Nayar R (2004) The Bethesda System for reporting cervical cytology. Springer, New York
19. Barr Soofer S, Sidawy MK (1997) Reactive cellular change: is there an increased risk for squamous intraepithelial lesions? Cancer 81:144–147
20. Malik SN, Wilkinson EJ, Drew PA, Hardt NS (2001) Benign cellular changes in Pap smears: causes and significance. Acta Cytol 45:5–8
21. Moriarty AT, Young NA (2003) The four R's: reactive/repair, reporting, and regulations. Diagn Cytopathol 29:1–3
22. Tambouret R, Pitman MB, Bell DA (1998) Benign glandular cells in posthysterectomy vaginal smears. Acta Cytol 42:1403–1408

23. Bean SM, Connolly K, Roberson J, Eltoum I, Chhieng DC (2006) Incidence and clinical significance of morphologically benign-appearing endometrial cells in patients age 40 years or older: the impact of the 2001 Bethesda System. Cancer 108:39–44
24. Chang A, Sandweiss L, Bose S (2001) Cytologically benign endometrial cells in the papanicolaou smears of postmenopausal women. Gynecol Oncol 80:37–43
25. Gray JA, Nguyen GK (1999) Cytologic detection of endometrial pathology by Pap smears [letter]. Diagn Cytopathol 20:181–182
26. Nguyen TN, Bourdeau JL, Ferenczy A, Franco EL (1998) Clinical significance of histiocytes in the detection of endometrial adenocarcinoma and hyperplasia. Diagn Cytopathol 19:89–93
27. Tambouret R, Bell DA, Centeno BA (2001) Significance of histiocytes in cervical smears from peri/postmenopausal women. Diagn Cytopathol 24:271–275
28. Ashfaq R, Gibbons D, Vela C, Saboorian MH, Iliya F (1999) ThinPrep Pap Test: accuracy for glandular disease. Acta Cytol 43:81–85
29. Bose S, Kannan V, Kline TS (1994) Abnormal endocervical cells. Really abnormal? Really endocervical? Am J Clin Pathol 101:708–713
30. DiTomasso JP, Ramzy I, Mody DR (1996) Glandular lesions of the cervix. Validity of cytologic criteria used to differentiate reactive changes, glandular intraepithelial lesions and adenocarcinoma. Acta Cytol 40:1127–1135
31. Betsill WL Jr, Clark AH (1986) Early endocervical glandular neoplasia. I. Histomorphology and cytomorphology. Acta Cytol 30:115–126
32. Lee KR, Minter LJ, Granter SR (1997) Papanicolaou smear sensitivity for adenocarcinoma in situ of the cervix. A study of 34 cases. Am J Clin Pathol 107:30–35
33. Drijkoningen M, Meertens B, Lauweryns J (1996) High grade squamous intraepithelial lesion (CIN 3) with extension into the endocervical clefts. Difficulty of cytologic differentiation from adenocarcinoma in situ. Acta Cytol 40:889–894
34. Selvaggi SM (2002) Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep cytology. Diagn Cytopathol 26:181–185
35. Gupta D, Balsara G (1999) Extrauterine malignancies. Role of Pap smears in diagnosis and management. Acta Cytol 43:806–813
36. Belsley NA, Tambouret RH, Misdraji J, Muzikansky A, Russell DK, Wilbur DC (2008) Cytologic features of endocervical glandular lesions: comparison of SurePath, ThinPrep, and conventional smear specimen preparations. Diagn Cytopathol 36:232–237
37. Hanley KZ, Tadros TS, Briones AJ, Birdsong GG, Mosunjac MB (2009) Hematologic malignancies of the female genital tract diagnosed on liquid-based Pap test: cytomorphologic features and review of differential diagnoses. Diagn Cytopathol 37:61–67
38. Pasternak S, MacIntosh R (2006) Uterine adenosarcoma detected by Papanicolaou smear: a case report. Diagn Cytopathol 34:495–498
39. McGill F, Adachi A, Karimi N et al (1990) Abnormal cervical cytology leading to the diagnosis of gastric cancer. Gynecol Oncol 36:101–105
40. McCluggage WG, Hurrell DP, Kennedy K (2010) Metastatic carcinomas in the cervix mimicking primary cervical adenocarcinoma and adenocarcinoma in situ: report of a series of cases. Am J Surg Pathol 34:735–741
41. Reid R, Greenberg MD, Lorincz A et al (1991) Should cervical cytologic testing be augmented by cervicography or human papillomavirus deoxyribonucleic acid detection? Am J Obstet Gynecol 164:14611469; discussion 9–71
42. Cox JT, Schiffman MH, Winzelberg AJ, Patterson JM (1992) An evaluation of human papillomavirus testing as part of referral to colposcopy clinics. Obstet Gynecol 80:389–395
43. Manos MM, Kinney WK, Hurley LB et al (1999) Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results [see comments]. JAMA 281:1605–1610
44. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical

Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. J Natl Cancer Inst 92:397–402 (2000)
45. Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ (2002) 2001 consensus guidelines for the management of women with cervical cytological abnormalities. JAMA 287:2120–2129
46. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D (2007) 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 197:346–355
47. Kitchener HC, Almonte M, Wheeler P et al (2006) HPV testing in routine cervical screening: cross sectional data from the ARTISTIC trial. Br J Cancer 95:56–61
48. Naucler P, Ryd W, Tornberg S et al (2007) Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 357:1589–1597
49. Mayrand MH, Duarte-Franco E, Rodrigues I et al (2007) Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 357:1579–1588
50. Ronco G, Giorgi-Rossi P, Carozzi F et al (2010) Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol 11:249–257
51. Kulasingam SL, Havrilesky L, Ghebre R, Myers ER (2011) Screening for cervical cancer: a decision analysis for the U.S. Preventive Services Task Force [Internet]. Agency for Healthcare Research and Quality (US), Rockville
52. Saslow D, Solomon D, Lawson HW et al (2012) 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 137:516–542
53. Lyamichev V, Brow MA, Dahlberg JE (1993) Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases. Science 260:778–783
54. Hsu TM, Law SM, Duan S, Neri BP, Kwok PY (2001) Genotyping single-nucleotide polymorphisms by the invader assay with dual-color fluorescence polarization detection. Clin Chem 47:1373–1377
55. Stillman MJ, Day SP, Schutzbank TE (2009) A comparative review of laboratory-developed tests utilizing Invader HPV analyte-specific reagents for the detection of high-risk human papillomavirus. J Clin Virol 45(Suppl 1):S73–S77
56. Johnson LR, Starkey CR, Palmer J et al (2008) A comparison of two methods to determine the presence of high-risk HPV cervical infections. Am J Clin Pathol 130:401–408
57. Ginocchio CC, Barth D, Zhang F (2008) Comparison of the Third Wave Invader human papillomavirus (HPV) assay and the digene HPV hybrid capture 2 assay for detection of high-risk HPV DNA. J Clin Microbiol 46:1641–1646
58. Bartholomew DA, Luff RD, Quigley NB, Curtis M, Olson MC (2011) Analytical performance of Cervista HPV 16/18 genotyping test for cervical cytology samples. J Clin Virol 51:38–43
59. Einstein MH, Martens MG, Garcia FA et al (2010) Clinical validation of the Cervista HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. Gynecol Oncol 118:116–122
60. Cuzick J, Arbyn M, Sankaranarayanan R et al (2008) Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 26(Suppl 10):K29–K41
61. Rozendaal L, Walboomers JM, van der Linden JC et al (1996) PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. Int J Cancer 68:766–769
62. Walboomers JM, Jacobs MV, Manos MM et al (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19
63. de Sanjose S, Quint WG, Alemany L et al (2010) Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 11:1048–1056

64. Castle PE, Porras C, Quint WG et al (2008) Comparison of two PCR-based human papillomavirus genotyping methods. J Clin Microbiol 46:3437–3445
65. Castle PE, Sadorra M, Lau T, Aldrich C, Garcia FA, Kornegay J (2009) Evaluation of a prototype real-time PCR assay for carcinogenic human papillomavirus (HPV) detection and simultaneous HPV genotype 16 (HPV16) and HPV18 genotyping. J Clin Microbiol 47:3344–3347
66. Heideman DA, Hesselink AT, Berkhof J et al (2011) Clinical validation of the cobas 4800 HPV test for cervical screening purposes. J Clin Microbiol 49:3983–3985
67. Lapierre SG, Sauthier P, Mayrand MH et al (2012) Human papillomavirus (HPV) DNA triage of women with atypical squamous cells of undetermined significance with cobas 4800 HPV and Hybrid Capture 2 tests for detection of high-grade lesions of the uterine cervix. J Clin Microbiol 50:1240–1244
68. Martinez SB, Palomares JC, Artura A et al (2012) Comparison of the Cobas 4800 Human Papillomavirus test against a combination of the Amplicor Human Papillomavirus and the Linear Array tests for detection of HPV types 16 and 18 in cervical samples. J Virol Methods 180:7–10
69. Lindemann ML, Dominguez MJ, de Antonio JC et al (2012) Analytical comparison of the cobas HPV Test with Hybrid Capture 2 for the detection of high-risk HPV genotypes. J Mol Diagn 14:65–70
70. Gage JC, Sadorra M, Lamere BJ et al (2012) Comparison of the cobas Human Papillomavirus (HPV) test with the hybrid capture 2 and linear array HPV DNA tests. J Clin Microbiol 50:61–65
71. Wright TC Jr, Stoler MH, Sharma A, Zhang G, Behrens C, Wright TL (2011) Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV+ cytology-negative results. Am J Clin Pathol 136:578–586
72. Stoler MH, Wright TC Jr, Sharma A, Apple R, Gutekunst K, Wright TL (2011) High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol 135:468–475
73. Castle PE, Stoler MH, Wright TC Jr, Sharma A, Wright TL, Behrens CM (2011) Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol 12:880–890
74. Stoler MH, Wright TC Jr Sharma A et al (2012) The interplay of age stratification and HPV testing on the predictive value of ASC-US cytology. Results from the ATHENA HPV study. Am J Clin Pathol 137:295–303
75. Cox JT, Castle PE, Behrens CM, Sharma A, Wright TC Jr, Cuzick J (2013) 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 208(3):e181–184
76. Dockter J, Schroder A, Eaton B et al (2009) Analytical characterization of the APTIMA HPV Assay. J Clin Virol 45(Suppl 1):S39–S47
77. Getman D, Aiyer A, Dockter J, Giachetti C, Zhang F, Ginocchio CC (2009) Efficiency of the APTIMA HPV Assay for detection of HPV RNA and DNA targets. J Clin Virol 45(Suppl 1):S49–S54
78. Dockter J, Schroder A, Hill C, Guzenski L, Monsonego J, Giachetti C (2009) Clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. J Clin Virol 45(Suppl 1):S55–S61
79. Wu R, Belinson SE, Du H et al (2010) Human papillomavirus messenger RNA assay for cervical cancer screening: the Shenzhen Cervical Cancer Screening Trial I. Int J Gynecol Cancer 20:1411–1414
80. Monsonego J, Hudgens MG, Zerat L et al (2011) Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: the FASE study. Int J Cancer 129:691–701

81. Clarke MA, Wentzensen N, Mirabello L et al (2012) Human Papillomavirus DNA Methylation as a Potential Biomarker for Cervical Cancer. Cancer Epidemiol Biomarkers Prev 21:12 2125–2137
82. Wentzensen N, Sherman ME, Schiffman M, Wang SS (2009) Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. Gynecol Oncol 112:293–299
83. Saha B, Chaiwun B, Tsao-Wei DD et al (2007) Telomerase and markers of cellular proliferation are associated with the progression of cervical intraepithelial neoplasia lesions. Int J Gynecol Pathol 26:214–222
84. Tsoumpou I, Arbyn M, Kyrgiou M et al (2009) p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev 35:210–220
85. Schmidt D, Bergeron C, Denton KJ, Ridder R (2011) p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou cytology: results from the European equivocal or mildly abnormal Papanicolaou cytology study. Cancer Cytopathol 119:158–166
86. Ravarino A, Nemolato S, Macciocu E et al (2012) CINtec PLUS immunocytochemistry as a tool for the cytologic diagnosis of glandular lesions of the cervix uteri. Am J Clin Pathol 138:652–656
87. Samarawardana P, Singh M, Shroyer KR (2011) Dual stain immunohistochemical localization of p16INK4A and ki-67: a synergistic approach to identify clinically significant cervical mucosal lesions. Appl Immunohistochem Mol Morphol 19:514–518
88. Renshaw AA (2002) Measuring sensitivity in gynecologic cytology: a review. Cancer 96:210–217
89. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 188:1383–1392 (2003)
90. Cibas ES (2008) Laboratory management. In: Cibas ES, Ducatman BS (eds) Cytology, diagnostic principles and clinical correlates, 3rd edn. Saunders, Philadelphia, pp 495–522
91. American College of Obstetricians and Gynecologists (2012) Screening for Cervical Cancer. Practice Bulletin No. 131. Obstet Gynecol 120:1222–1238
92. Massad LS, Einstein MH, Huh WK et al. (2013) 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursor. J Low Genit Tract Dis 17(5):S1–S27
93. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ (2009) Cancer statistics, 2009. CA Cancer J Clin 59:225–249
94. Sherman ME (2000) Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod Pathol 13:295–308
95. Koornstra JJ, Mourits MJ, Sijmons RH, Leliveld AM, Hollema H, Kleibeuker JH (2009) Management of extracolonic tumours in patients with Lynch syndrome. Lancet Oncol 10:400–408
96. Sonoda Y, Barakat RR (2006) Screening and the prevention of gynecologic cancer: endometrial cancer. Best Pract Res Clin Obstet Gynaecol 20:363–377
97. Guidos BJ, Selvaggi SM (2000) Detection of endometrial adenocarcinoma with the ThinPrep Pap test. Diagn Cytopathol 23:260–265
98. Schorge JO, Hossein Saboorian M, Hynan L, Ashfaq R (2002) ThinPrep detection of cervical and endometrial adenocarcinoma: a retrospective cohort study. Cancer 96:338–343
99. Greenspan DL, Cardillo M, Davey DD, Heller DS, Moriarty AT (2006) Endometrial cells in cervical cytology: review of cytological features and clinical assessment. J Low Genit Tract Dis 10:111–122
100. de Peralta-Venturino MN, Purslow MJ, Kini SR (1995) Endometrial cells of the "lower uterine segment" (LUS) in cervical smears obtained by endocervical brushings: a source of potential diagnostic pitfall. Diagn Cytopathol 12:263–268; discussion 8–71
101. Solomon D, Davey D, Kurman R et al (2002) The Bethesda System 2001: terminology for reporting the results of cervical cytology. JAMA 287:2114–2119

102. Kipp BR, Medeiros F, Campion MB et al (2008) Direct uterine sampling with the Tao brush sampler using a liquid-based preparation method for the detection of endometrial cancer and atypical hyperplasia: a feasibility study. Cancer 114:228–235
103. Dijkhuizen FP, Mol BW, Brolmann HA, Heintz AP (2000) The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 89:1765–1772
104. Creasman W (2009) Revised FIGO staging for carcinoma of the endometrium. Int J Gynaecol Obstet 105:109
105. Fadare O, Mariappan MR, Hileeto D, Wang S, McAlpine JN, Rimm DL (2005) Upstaging based solely on positive peritoneal washing does not affect outcome in endometrial cancer. Mod Pathol 18:673–680
106. Jorns JM, Knoepp SM (2009) Occult fallopian tube carcinoma detected in routine pelvic washing specimens submitted for staging: another justification for pelvic washing cytology? Diagn Cytopathol 37:923–929
107. Lin O (2009) Challenges in the interpretation of peritoneal cytologic specimens. Arch Pathol Lab Med 133:739–742
108. Bristow RE, Purinton SC, Santillan A, Diaz-Montes TP, Gardner GJ, Giuntoli RL 2nd (2006) Cost-effectiveness of routine vaginal cytology for endometrial cancer surveillance. Gynecol Oncol 103:709–713
109. Cooper AL, Dornfeld-Finke JM, Banks HW, Davey DD, Modesitt SC (2006) Is cytologic screening an effective surveillance method for detection of vaginal recurrence of uterine cancer? Obstet Gynecol 107:71–76
110. Myers ER, Bastian LA, Havrilesky LJ et al (2006) Management of adnexal mass. Evid Rep Technol Assess (Full Rep) 130:1–145
111. Brown DL (2007) A practical approach to the ultrasound characterization of adnexal masses. Ultrasound Q 23:87–105
112. Gostout BS, Brewer MA (2006) Guidelines for referral of the patient with an adnexal mass. Clin Obstet Gynecol 49:448–458
113. Bibbo M, Wood MD, Fitzpatrick BT (2008) Peritoneal washings and ovary. In: Bibbo M, Wilbur DC (eds) Comprehensive cytopathology, 3rd edn. Elsevier, Amsterdam, pp 291–301
114. Davidson B, Risberg B, Kristensen G et al (1999) Detection of cancer cells in effusions from patients diagnosed with gynaecological malignancies. Evaluation of five epithelial markers. Virchows Arch 435:43–49
115. Selvaggi SM (2003) Diagnostic pitfalls of peritoneal washing cytology and the role of cell blocks in their diagnosis. Diagn Cytopathol 28:335–341
116. Zuna RE, Mitchell ML (1988) Cytologic findings in peritoneal washings associated with benign gynecologic disease. Acta Cytol 32:139–147
117. Lowe E, McKenna H (1989) Peritoneal washing cytology: a retrospective analysis of 175 gynaecological patients. Aust N Z J Obstet Gynaecol 29:55–61
118. Zuna RE, Mitchell ML, Mulick KA, Weijchert WM (1989) Cytohistologic correlation of peritoneal washing cytology in gynecologic disease. Acta Cytol 33:327–336
119. Zuna RE, Behrens A (1996) Peritoneal washing cytology in gynecologic cancers: long-term follow-up of 355 patients. J Natl Cancer Inst 88:980–987
120. Fadare O, Mariappan MR, Wang S, Hileeto D, McAlpine J, Rimm DL (2004) The histologic subtype of ovarian tumors affects the detection rate by pelvic washings. Cancer 102:150–156
121. Kudo R, Takashina T, Ito E et al (1990) Peritoneal washing cytology at second-look laparotomy in cisplatin-treated ovarian cancer patients. Acta Cytol 34:545–548
122. Zanetta G, Trio D, Lissoni A et al (1993) Early and short-term complications after US-guided puncture of gynecologic lesions: evaluation after 1,000 consecutive cases. Radiology 189:161–164
123. Ganjei P (1995) Fine-needle aspiration cytology of the ovary. Clin Lab Med 15:705–726
124. Davila RM (1993) Cytology of benign cystic uterine adnexal masses. Acta Cytol 37:385–390

125. Higgins RV, Matkins JF, Marroum MC (1999) Comparison of fine-needle aspiration cytologic findings of ovarian cysts with ovarian histologic findings. Am J Obstet Gynecol 180:550–553
126. Lu D, Davila RM, Pinto KR, Lu DW (2004) ThinPrep evaluation of fluid samples aspirated from cystic ovarian masses. Diagn Cytopathol 30:320–324
127. Wojcik EM, Selvaggi SM (1992) Diagnostic accuracy of fine-needle aspiration cytology in persistent or recurrent gynecologic malignancies. Diagn Cytopathol 8:322–326
128. Angstrom T, Kjellgren O, Bergman F (1972) The cytologic diagnosis of ovarian tumors by means of aspiration biopsy. Acta Cytol 16(4):336–341
129. Mulvany NJ (1996) Aspiration cytology of ovarian cysts and cystic neoplasms. A study of 235 aspirates. Acta Cytol 40:911–920
130. Mulvany N, Ostor A, Teng G (1995) Evaluation of estradiol in aspirated ovarian cystic lesions. Acta Cytol 39:663–668
131. Yee H, Greenebaum E, Lerner J, Heller D, Timor-Tritsch IE (1994) Transvaginal sonographic characterization combined with cytologic evaluation in the diagnosis of ovarian and adnexal cysts. Diagn Cytopathol 10:107–112
132. Allias F, Chanoz J, Blache G, Thivolet-Bejui F, Vancina S (2000) Value of ultrasound-guided fine-needle aspiration in the management of ovarian and paraovarian cysts. Diagn Cytopathol 22:70–80
133. Lipitz S, Seidman DS, Menczer J et al (1992) Recurrence rate after fluid aspiration from sonographically benign-appearing ovarian cysts. J Reprod Med 37:845–848
134. Zanetta G, Lissoni A, Torri V et al (1996) Role of puncture and aspiration in expectant management of simple ovarian cysts: a randomised study. BMJ 313:1110–1113
135. Fisch JD, Sher G (2004) Sclerotherapy with 5% tetracycline is a simple alternative to potentially complex surgical treatment of ovarian endometriomas before in vitro fertilization. Fertil Steril 82:437–441
136. Kafali H, Yurtseven S, Atmaca F, Ozardali I (2003) Management of non-neoplastic ovarian cysts with sclerotherapy. Int J Gynaecol Obstet 81:41–45
137. Mesogitis S, Daskalakis G, Pilalis A et al (2005) Management of ovarian cysts with aspiration and methotrexate injection. Radiology 235:668–673