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Foreword

William F. Rayburn, MD
Consulting Editor

Cancer is the second leading cause of death among women. Ideally, it is desirable to prevent or at least to detect cancer in the precancerous stage. Early detection is possible by using Papanicolaou's (Pap) test for cervical cancer, biopsies for endometrial cancer, and mammography for breast cancer. History plays a more major role in the detection of colorectal cancer, because having first-degree relatives with colon cancer; a history of colorectal, breast, endometrial, or ovarian cancer; and a history of adenomatous polyps or ulcerative colitis are identified risk factors.

For many women, obstetrician-gynecologists are physicians who provide their primary or preventive health care. Many reproductive tract malignancies are preventable. An obstetrician-gynecologist is, therefore, in an excellent position to provide select screening for reproductive tract malignancies. Evaluation of risk for cancer includes questions about high-risk habits, assessment of family history for cancer, and review of symptoms pertinent to each organ system. Counseling focuses on risk factors and early warning signs, prevention strategies, and routine or selective testing.

The obstetrician-gynecologist plays an important role in counseling patients on lifestyle factors that can reduce or increase the risk of cancer. Informed patients can make better choices by implementing certain behavior modifications. Patients should be encouraged to reduce the risk of cancer by not smoking, eating high-fiber foods, restricting fat intake, exercising daily, restricting exposure to the sun, paying attention to certain body changes, and getting regular health checkups for diagnostic evaluations (Pap test, mammography, sigmoidoscopy) and preventive therapy (vaccination).

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This issue of *Obstetrics and Gynecologic Clinics of North America*, guest edited by Carolyn Muller, MD, directs the reader to fundamental cancer prevention and screening for a better understanding of the conflicting outcomes from many studies designed to evaluate risk factors and interventions. State-of-the-art prevention strategies are presented by a distinguished group of authors who have dedicated their professional careers to the study of each of the major reproductive tract malignancies. Current clinical trials are highlighted that inform the practicing obstetrician-gynecologist about future directions into tomorrow's preventive care.

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Preface

Carolyn Y. Muller, MD
Guest Editor

In the United States this year, it is estimated that approximately 28,000 women will die from a gynecologic cancer, and another 78,290 women will be newly diagnosed. In more graphic terms, a woman is diagnosed with a gynecologic cancer every 7 minutes, and 77 women will die of their disease each day. Yet, reproductive cancers comprise some of the most preventable cancers, like cervical and uterine cancers juxtaposed to one of the most difficult cancers to prevent: ovarian cancer. Although ovarian cancers account for only 29%, or all new gynecologic cancer cases, they are responsible for nearly 55% of cancer deaths [1].

Prevention is the key to cancer-free living, with early detection the next best option for cure and long-term survival. The concepts of cancer prevention are complex and require a thorough understanding of risk assessment, cancer genetics, hereditary effect, environmental exposures (including causative agents of each type of cancer), and the identification of preinvasive lesions or precursors of the disease. Prevention strategies include behavior modification, chemoprevention, vaccination, and other more definitive interventions, such as surgery or other invasive testing. The principles of cancer screening are paramount to understand both the successes and the failures of present day screening approaches and the concepts for future advances in gynecologic cancer screening.

The purpose of this issue of the *Obstetrics and Gynecologic Clinics of North America* is to review the basics of cancer prevention and screening that will help the reader understand the often conflicting outcomes of many studies designed to evaluate risk factors and interventions. (Do hormones increase

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ovarian cancer risk? Do retinoids prevent or reverse cervical dysplasia?) In addition, each article will review the state-of-the art prevention strategies and the strengths and limitations for each of the major gynecologic cancer sites. Future directions and active ongoing research is noted also, because today's clinical trials may lead to tomorrow's standard of care practice.

The greatest impact of cancer prevention is made by the primary care providers, those in the trenches who can empower women to make necessary changes for a better chance of cancer-free living. The skill is to sort out the ''worried well'', many who have a perception of personal risk but estimated low risk of gynecologic cancers, from those who have recognizable and sometimes substantial lifetime risk of these malignancies. My expert coauthors and I have attempted to provide the tools for determining risk, the strategies to rely upon for screening and early detection, and the limitations of early detection strategies so one can avoid over treatment or a false sense of security. Better prevention could put me out of business, but for that success, I would accept early retirement!

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[1] American Cancer Society. Cancer facts and figures. Available at: http://www.cancer.org. Accessed September 15, 2007.

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Strengthening Gynecologic Cancer Prevention Studies

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Cancer prevention is the Holy Grail of medicine. Even so, despite thousands of years of exploration, modern medicine seems far away from this coveted prize. The field of cancer prevention has become more sophisticated, now involving epidemiologists, statisticians, biologists, clinicians, chemists, environmentalists, and technologists, to name just a few specialists. Prevention studies explore factors that "cause" cancer and that therefore must be avoided as well as those factors that, with intended exposure, reduce the risk of developing cancer. These studies often have contradictory outcomes: positive in some trials but negative in others. On occasion, the intervention may have a positive outcome for the primary endpoint—preventing the targeted cancer—but then turn out to have a negative effect on another target tissue. One such example is tamoxifen, which, on the one hand, helps prevent the development of breast cancer, but, on the other hand, leads to a higher risk of developing endometrial cancer. Circumstances that further complicate this field include the vast heterogeneity of modifier genetics and exposures in the population. This article delves into the basics of designing cancer-prevention trials and describes the skills needed to evaluate the many study outcomes in this field so prone to contradiction.

Efforts to prevent gynecologic malignancies face some methodological challenges common to other cancer prevention studies. Flaws in choice of study design, population to be studied, agent to be administered (or intervention to be made), and factors included in the analysis can all result in the misattribution of beneficial, adverse, or null effects, or cause important findings to be overlooked. In addition, some investigators must address distinct issues that have arisen in response to prevention-trial results from the past decade. Cancer-prevention efforts have in recent years suffered some

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well-publicized and not-so-well publicized setbacks: the failure of beta-carotene to prevent lung cancer in smokers [1,2], of vitamin E supplementation to reduce cancer incidence at a number of sites [1,3–5], and of a whole host of promising findings regarding vitamin, mineral, or full-diet interventions to be replicated in subsequent studies (reviewed in [5]). Intensive efforts to understand those trials, many of which showing that interventions actually increase risk, have reaped some insights that will benefit future efforts. The prominence of some of these findings also should not overshadow some well-established achievements [6–9] and more recent successes [10,11]. However, as the field of cancer prevention may now be facing more serious challenges to efforts to undertake clinical trials or even to obtaining funding for observational investigations, it may be timely to examine a few selected components of well-designed, carefully conducted studies. Those interested in a thorough review are referred to two excellent texts on the subject [12,13].

To carry out carefully conceived cancer-prevention studies, investigators must address some questions common to all observational investigations. Investigators may, in addition, face specific issues that have surfaced in experimental clinical trials. Both observational and experimental study designs have borne fruit for preventing gynecologic cancers. The reduced risks of ovarian and endometrial cancer among women who took oral contraceptives were first apparent in observational studies published almost 40 years ago [14–17], while experimental methods have documented the success of the Pap smear [18] as well as the recent human papillomavirus (HPV) vaccine in decreasing cervical cancer incidence or persistent HPV infection [10,11]. Findings from observational studies often provide the initial hypotheses for randomized clinical trials (RCTs). However, in some instances, nonexperimental studies are the main source of evidence for cancer-risk reduction, as randomization to some intervention is unlikely to be acceptable to many women (eg, bilateral oophorectomy among BRCA1 or BRCA2 mutation carriers).

Observational studies and gynecologic cancer prevention

Case-control and cohort studies are the two most common observational study designs. Cohorts are groups of people who differ in exposure at baseline and who are followed for subsequent illness. Cohort studies are usually large, expensive, and often require more than a decade to accumulate sufficient cases of cancer. Nonetheless, they are the design of choice to study potential cancer-reducing exposures that are best collected prospectively, such as detailed dietary information at specific ages. Case-control studies involve the comparison of previous historic exposure among people identified with a disease (cases) with that of a set of unaffected individuals (controls), who are selected to be representative of the same exposure in the population from which the cases arose. Strengths of a case-control approach include the ability to complete a study relatively quickly because exposure and disease have

already occurred; the capacity to examine exposures that have occurred over many decades or at a specific age (provided that accurate and complete data can be collected about exposures) and the opportunity to focus on extremely rare outcomes. Both observational study designs take advantage of ''natural experiments'' in which participants have chosen exposures that could not be randomly assigned (eg, genetics, childbearing patterns, obesity). However, prevention studies, in common with all observational epidemiologic studies, must seek to control sources of bias and confounding that can occur in these designs and to maintain the generalizability of the results to the targeted population.

Bias

Selection bias

In a case-control study, selection bias can arise when controls are not selected from the full population from which the cases came. Selection bias can be especially significant if controls represent populations with differential exposure to the risk factors of interest. For example, if cases for an endometrial cancer study were drawn from a particular hospital, and controls were recruited from the gynecology clinic at that same hospital, the controls would probably be more likely than the general population to have been prescribed oral contraceptives or hormone therapy. If the endometrial cancer cases represent all women diagnosed within a health plan or a defined catchment area, sampling of controls from within that same full population should ensure that women selected do not overtly differ from the source population with respect to the exposure of interest. Selection bias can also occur when study participation by eligible cases or selected controls differ by exposure. For instance, if, among participants selected for a study who happened to be aspirin users, 75% of the users that were ovarian cancer cases participated but only 60% of selected controls that were aspirin users participated, the measure of effect of aspirin use would be larger than if participation had been equal in both groups. Although it is not always possible to describe participation according to a specific exposure [19], standardized reporting of overall study participation is vital to allow the scientific community to assess the possibility that differential involvement may have influenced results [20].

Information bias

Information bias refers to systematic errors in the measurement of variables collected during a study. Recall bias, a form of information bias, may be present in case-control studies if cases diagnosed with cancer are better able than controls at correctly remembering exposures. To reduce recall bias and promote correct recall, useful tools include carefully worded questionnaires administered in a standardized manner by trained interviewers, and visual aids, such as a life-event calendars or pictures of medications [21]. In the endometrial cancer study within a health plan mentioned above,

recall bias would be diminished if all exposure information (eg, on prescription medication use) could be accessed from automated records. However, even electronic information can contain errors. For example, electronic information about prescriptions normally assumes medications are taken correctly when, in some cases, medications are skipped or taken incorrectly. Thus, an even more precise approach would involve validating the information through another source (eg, medical charts, personal interviews).

Healthy-user bias

Persons who adhere to prescribed medications or any preventive therapy may also be more likely to practice a wide range of healthy behaviors. Thus, the inclusion of such persons in a study can create a healthy user bias, giving the impression that the health of such participants is the result of therapy, when their good health stems from other healthy habits. Thus, healthy user bias can skew the results of case control or cohort studies. For example, in a cohort study using electronic records of statin use, those who renewed a statin prescription were more likely to receive a number of other preventive health services, including prostate-specific antigen testing, mammography, and influenza vaccinations, than those who didn't [22]. A related phenomenon has been termed "confounding by the health status of the user" [23]. Elderly health-plan members who received an influenza vaccine were found to have a lower risk of mortality even before flu season, suggesting that their good health and functional status had allowed them to receive the vaccine, and that any reduced mortality during flu season could not be attributed solely to the vaccine [24]. While stark differences such as this may be most visible in studies that include a wide range of health statuses, such biases occur at more subtle levels in conjunction with many other health behaviors. For example, persons who take an aspirin daily, compared to those who do not, may be much more likely to engage in daily exercise, to eat a diet rich in fruits and vegetables, and to take other measures to maintain robust health. The failure to take other health-promoting behaviors into account could result in a risk estimate that is biased in favor of finding that the use of aspirin or statin reduces disease risk. That bias may be addressed to some extent by adjustments for healthy behaviors or health status in the statistical analysis of the data, and methods to more appropriately account for such bias are evolving [22,24]. Most studies cannot completely identify, measure, and adjust for all factors that could potentially bias findings, illustrating that appropriate study design from the outset is the most essential element in eliminating bias. In early observational studies of hormone replacement therapy, confounding by the healthy-user effect may have hidden from researchers the role of such therapy in increasing breast cancer risk [25,26]. (The concentration of increased breast cancer risk among lean current users of estrogen plus progesterone also contributed.) The healthy-user effect has also been proposed as a partial explanation for why no one has been able to confirm preventive health findings in certain other experimental studies.

Confounding, including confounding by indication for therapy or medication use

Confounding occurs when both the exposure or intervention under study and the disease outcome are related to a third variable, and the estimate of the effect of that exposure is distorted by the third variable, which is termed a confounder. In a study of aspirin use and cancer prevention, a healthy diet could be the third variable that is correlated with both and that can bias the effect estimate. Potential confounders often include age, sex, and race/ethnicity, as both exposure probability and disease probability can differ by those variables. In observational studies of cancer-prevention agents, when the exposure is often a medical procedure or a medication, one form of confounding that can occur is known as confounding by indication. Confounding by indication can occur when the health condition or symptom that led to the medication or procedure is also related to the disease. Failure to take that relationship into account in the data analysis can lead to distorted estimates of relative risk. In a study of statin use in relationship to endometrial cancer risk, a diagnosis of hypertension could represent confounding by indication. That is, hypertensive individuals are more likely to be prescribed statins, and hypertension is related to an increased risk of endometrial cancer. If hypertension were not taken into account in the analysis, the data would suggest that statins increased endometrial cancer risk, whether or not they actually did. Several approaches can be used to take the presence of the confounder into consideration in statistical analyses: Including a variable representing hypertension diagnosis in a multivariate model, stratifying the analysis by hypertension (nonhypertensive/hypertensive), restricting the analysis to a subgroup (in this case, to nonhypertensive individuals), and, in case-control studies, matching on the confounder would each reduce the distortion caused by confounding [27].

Chance

Reducing the possibility of chance (random error) as an explanation for findings in cancer-prevention studies requires that careful attention be paid to the probability of type I (false positive) or type II (false negative) statistical errors. These issues are described in detail elsewhere [13] and might best be addressed in practice by a multidisciplinary study team that included individuals trained in biostatistics and epidemiology. Chance is a plausible contributor to the nonreplication of cancer prevention findings in later experimental trials; false-positive results are more likely to arise when the hypotheses examined are numerous or have low prior probability, and false-negative findings are more common when the study sample size is too small to have sufficient statistical power to detect an effect.

Generalizability

Bias, confounding, and chance challenge the internal validity of findings that address the question: Was the correct answer obtained in the study? Generalizability concerns the external validity: To what population do the findings apply? For example, the high mortality rate among women diagnosed with ovarian cancer may allow only a subset of cases to be interviewed in a case-control study. Examination of the study participation rate and the characteristics of eligible but noninterviewed women can be used to assess the generalizability of study results to all ovarian cancer cases [19,20]. Cancer-prevention studies can be subject to several specific threats to generalizability. Studies examining the effect of an intervention among carriers of high-risk cancer-predisposing mutations, such as those in BRCA and Lynch syndrome genes in gynecologic cancers, sometimes enroll prevalent cases of cancer among carriers because the mutations are so rare. Prevalent cases reflect not only exposures that contributed to the incident cancer, but also factors that influenced survival and duration since diagnosis. Thus, findings among prevalent cases may not be easily extended to incident cases. Carriers of highly penetrant mutations potentially present additional issues in applying study findings to a wider population of mutation carriers or to noncarriers [28]. Such women are more likely to be tested for mutation status (often because the highly penetrant mutation contributed to cancer development in a relative), to thus be available for inclusion in a study, to have a higher risk of cancer than other mutation carriers, and to have taken other steps to reduce risk (if available). Adjustment for some of these differences can strengthen internal validity (see above), but may not expand generalizability. If prophylactic salpingo-oophorectomy or other interventions reduce cancer incidence in these high-risk individuals, it is likely that the relative risks or odds ratios underestimate the risk reduction associated with the intervention in lower-risk women, but the actual effect is unknown.

Randomized clinical trials and gynecologic cancer prevention

By virtue of the random assignment of exposure and blinded data collection that characterize most experimental studies, comparisons made in RCTs are not subject to many of the biases that can occur in poorly designed observational studies. Women who have adopted additional healthy behaviors and those with highly penetrant cancer-predisposing mutations should be assigned equally to each arm of the study, minimizing but not always eliminating the need to adjust for confounding. Randomized trials must be large and can require many years of follow-up to obtain sufficient cancer cases. Thus, such trials are quite costly. RCTs can also be subject to issues similar to those noted above that limit the applicability of their results.

Many recent cancer-prevention randomized trials have enrolled only persons at high risk of cancer. Such a strategy serves at least two purposes: (1) Side effects may be more tolerable to such a population than to healthy, normal-risk individuals and (2) the duration of the trial may be shortened as a greater number of cancer cases will develop. The Gail risk model [29] for breast cancer and the Bach model [30] for lung cancer use exposure information to calculate a probability of disease occurrence. Individuals above a threshold are deemed ''high risk'' and eligible for prevention trials. Alternatively, some investigators have included as high risk only those diagnosed with a precancerous condition known as intraepithelial neoplasia (IEN), which has been termed ''a near obligate precursor of cancer'' [31]. The definition of IEN includes endometrial hyperplasia and cervical squamous intraepithelial lesions or cervical intraepithelial neoplasia, but no strong candidates for ovarian cancer precursor have emerged. A third definition of high risk includes those that have surgery or therapy resulting in the regression of an IEN, but who are at risk for IEN recurrence or cancer, such as some women treated for cervical intraepithelial neoplasia. Because of their toxicity, many cancer-prevention therapies that have shown efficacy are restricted to high-risk populations (eg, tamoxifen and raloxifene in breast cancer and finasteride in prostate cancer prevention), so the question of degree of benefit that might be expected in lower-risk groups has not arisen. However, results from at least one prevention trial have suggested possible heterogeneity in response to chemopreventive agents between IEN and normal tissue. In this study, previously diagnosed patients randomized to folic acid supplementation had a 1.67-fold increased risk of additional colon adenomas [32]. This increased risk was attributed to the possible dual effects of the agent. That is, based on experimental evidence [33], it is possible that among those with no precancerous tissue, the intervention may reduce risk, but among those carrying premalignant changes, folic acid may promote growth [34]. Limited evidence from animal models also suggests that interventions that may reduce risk of primary events may not be efficacious in preventing recurrences [35]. This example illustrates that even with epidemiologic and experimental data supporting the role of folic acid in primary prevention, extrapolation to a different setting may be risky. If some interventions are effective only at specific points in a multistep model of carcinogenesis, or have different effects at succeeding steps, as is biologically plausible, some of the null or adverse findings in chemoprevention trials may need to be revisited. Other issues that can limit the inferences made in RCTs for prevention include use of a single or a narrow range of doses, exposure to the intervention for generally only a few years, and limited follow-up. The latter is particularly important because prevention agents that act only on early stages of disease will not demonstrate a reduction in risk of most cancers for 10 to 15 years. In addition, if exposure during a particular age range is necessary, an RCT will rarely be able to detect it. To allow findings to be considered in the context of study quality

measures, investigators should report in publications the participation rate, drop-out or withdrawal rate, the adherence to the assigned intervention, and the drop-in rate (proportion of the nonintervention arm that adopts the exposure of interest), as well as the maintenance of double-blinding.

Modification by risk factors and biological characteristics

In the Beta Carotene and Retinol Efficacy Trial (CARET) and the Alpha Tocopherol, Beta Carotene trial, smokers randomized to a beta-carotene arm had an increased risk of lung cancer. In both settings, the risk associated with beta-carotene was higher among those who were the heaviest smokers and among those who drank more alcohol [1,2]. In CARET, the only subgroup without an elevated risk was that made up of former smokers. Initial findings from the Polyp Prevention Study Group trial indicated no effect of beta-carotene on prevention of colon adenomas [36]. However, in a later publication, analyses restricted to nonsmokers and nondrinkers demonstrated a reduced risk [37]. Thus, it is easy to visualize that when an effect occurs only in a subgroup, the trial will appear spuriously null when overall effects are reported. The results of these studies also illustrate that the effect of an intervention can be modified by other risk factors for the disease if, as suggested here, both are acting on the same or on intersecting biological pathways. (However, statistical interaction can occur without biological interaction [13].)

A reduced risk of colon adenomas among individuals who take aspirin is one of a handful of well-replicated and well-established findings in cancer chemoprevention. Germline variation in genes involved in the metabolism of aspirin—ornithine decarboxylase and uridine diphosphate glucuronosyltransferase 1A6—may modulate the effects of aspirin on risk. In five studies [38–42], those who inherited one or more variant alleles in these genes had an altered risk of adenomas following aspirin use, as compared with those who inherited wild-type alleles.

Because the number of potentially modifying factors to explore in a prevention study is not easily constrained, biologic plausibility should drive the investigation. The epidemiology of the disease and the cellular and molecular mechanisms of the intervention can direct efforts to examine risk modification. Assessment of the major risk factors for the disease in conjunction with the prevention effort addresses questions about efficacy in subgroups while possibly providing insight regarding mechanisms or biological pathways. In addition, genotypes that are known to influence the metabolism of the compound are strong candidates for effect modifiers. Biological characteristics of the tumor or of characteristic tissue changes are also plausible contenders. For example, the effect of tamoxifen and raloxifene, otherwise known as ‘‘selective estrogen receptor modulators,’’ are predominantly evident in the prevention of a distinct cancer subgroup: estrogen-receptor positive breast cancers [6,7].

Additional challenges for strengthening cancer-prevention trials

Many of the issues that have come to the forefront in response to prevention trial results from the past decade complement and reinforce those noted above. While many credible reasons for null or adverse findings have been entertained (inappropriate dose, poor choice of population, as well as focus on a single compound to the exclusion of the full "biological action package" [43]), one central concern that has emerged is the need for better selection of agents. A stronger biologic rationale, a deeper understanding of the pharmacodynamics of potential compounds, extensive pretrial assessments in animal or other experimental models, and more accurate tools to detect or predict toxicity would allow more exact identification of interventions that have a high probability of phase III clinical trial success [44].

Also, new methods are needed to identify individuals at high risk of developing cancer in the short term (within 3 to 5 years) who should be included in prevention trials. The current use of a histologic definition (eg, IEN) should give way to measures related to the molecular biology of carcinogenic progression. Gene and protein expression, loss of heterozygosity, aneuploidy, epigenetic modification, and other markers in premalignant tissue could contribute to a model that pinpoints those at highest risk [45,46]. In chemoprevention studies, toxicity is a primary concern when administering agents to individuals who are not ill. Increased assessment of germline polymorphisms in, and tissue expression of, genes that mediate metabolism and toxicity of selected compounds would allow initial trials to direct therapies to those most likely to benefit. (Gene chip arrays that assess inherited cytochrome p450 gene variants are examples of such assessment tools.)

Finally, identification of biomarkers of valid surrogate endpoints that can be assessed in prevention trials has been a long-sought prize in cancer chemoprevention research [47]. In this setting, a surrogate endpoint would be a biochemical or molecular marker of premalignant changes in a multistep carcinogenic process, and would constitute a valid surrogate if alteration in the marker was closely linked to halting the carcinogenic process and preventing frank malignancy. Use of such intermediate endpoints would result in tremendous savings in the time and costs involved in trials of chemopreventive agents. However, for such intermediates to be used as endpoints in trials, the Food and Drug Administration would have to modify the requirement that cancer incidence be the sole outcome. Most investigators accept that the progression or regression of IEN, due to its close relationship with cancer development, is a legitimate surrogate endpoint [47] and prevention of persistent HPV infection is a compelling surrogate for prevention of full cervical cancer development. However, few other strong candidates for surrogates have emerged. In organs that are difficult to sample (eg, ovaries), improvements in imaging or other technology may be necessary to evaluate alterations related to interventions. Thus, to include IEN regression in those tissues as a useful surrogate marker.

Similarly, if the definition of a high-risk individual is refined to include molecular markers in IEN or normal tissue, changes in the expression of those markers needs to be assessable to qualify as a surrogate measure of efficacy in preventing malignant transformation.

Many of these considerations are as valid for chemopreventive compounds as for other prevention agents. Exposures ranging from tumor antigen vaccines to lifestyle changes, such as increased physical activity, would benefit from more precise selection of the intervention and the population receiving it, as well as from incisive methods to determine efficacy without cancer development as an endpoint.

In light of the cumulative weight of null or adverse outcomes in prevention trials, newly proposed interventions and possibly even observational studies could face a steeper set of barriers for approval. Consideration of the lessons learned from previous studies as well as an appreciation for past prevention achievements and the remarkable success unfolding in the HPV vaccine trials will both be vital as we get to work.

References

[1] Albanes D, Heinonen OP, Taylor PR, et al. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: effects of base-line characteristics and study compliance. J Natl Cancer Inst 1996;88(21):1560–70.

[2] Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 1996;88(21):1550–9.

[3] Bjelakovic G, Nikolova D, Simonetti RG, et al. Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. Lancet 2004;364(9441): 1219–28.

[4] Cho E, Hunter DJ, Spiegelman D, et al. Intakes of vitamins A, C and E and folate and multivitamins and lung cancer: a pooled analysis of 8 prospective studies. Int J Cancer 2006; 118(4):970–8.

[5] Davies AA, Davey Smith G, Harbord R, et al. Nutritional interventions and outcome in patients with cancer or preinvasive lesions: systematic review. J Natl Cancer Inst 2006;98(14): 961–73.

[6] Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90(18):1371–88.

[7] Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA 1999;281(23):2189–97.

[8] Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med 2003;348(10):891–9.

[9] Sandler RS, Halabi S, Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003;348(10):883–90.

[10] Koutsky LA, Ault KA, Wheeler CM, et al. Proof of Principle Study Investigators. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002;347(21):1645–51.

[11] Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356(19):1928–43.

[12] Koepsell TD, Weiss NS. Epidemiologic methods. New York: Oxford University Press; 2003.
[13] Rothman K, Greenland S. Modern Epidemiology. Philadelphia: Lippincott-Raven; 1998.
[14] Newhouse ML, Pearson RM, Fullerton JM, et al. A case control study of carcinoma of the ovary. Br J Prev Soc Med 1977;31(3):148–53.
[15] Weiss NS, Sayvetz TA. Incidence of endometrial cancer in relation to the use of oral contraceptives. N Engl J Med 1980;302(10):551–4.
[16] Kaufman DW, Shapiro S, Slone D, et al. Decreased risk of endometrial cancer among oral-contraceptive users. N Engl J Med 1980;303(18):1045–7.
[17] Weiss NS, Lyon JL, Liff JM, et al. Incidence of ovarian cancer in relation to the use of oral contraceptives. Int J Cancer 1981;28(6):669–71.
[18] Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. New York: The Commonwealth Fund; 1943.
[19] Voigt LF, Koepsell TD, Daling JR. Characteristics of telephone survey respondents according to willingness to participate. Am J Epidemiol 2003;157(1):66–73.
[20] Olson SH, Voigt LF, Begg CB, et al. Reporting participation in case-control studies. Epidemiology 2002;13(2):123–6.
[21] Beresford SA, Coker AL. Pictorially assisted recall of past hormone use in case-control studies. Am J Epidemiol 1989;130(1):202–5.
[22] Brookhart MA, Patrick AR, Dormuth C, et al. Adherence to lipid-lowering therapy and the use of preventive health services: an investigation of the healthy user effect. Am J Epidemiol 2007;166(3):348–54.
[23] Jackson LA, Nelson JC, Benson P, et al. Functional status is a confounder of the association of influenza vaccine and risk of all cause mortality in seniors. Int J Epidemiol 2006;35(2): 345–52.
[24] Jackson LA, Jackson ML, Nelson JC, et al. Evidence of bias in estimates of influenza vaccine effectiveness in seniors. Int J Epidemiol 2006;35(2):337–44.
[25] Petitti DB. Coronary heart disease and estrogen replacement therapy. Can compliance bias explain the results of observational studies? Ann Epidemiol 1994;4:115–8.
[26] Matthews KA, Kuller LH, Wing RR, et al. Prior to use of estrogen replacement therapy, are users healthier than nonusers? Am J Epidemiol 1996;143(10):971–8.
[27] Psaty BM, Koepsell TD, Lin D, et al. Assessment and control for confounding by indication in observational studies. J Am Geriatr Soc 1999;47(6):749–54.
[28] Klaren HM, van't Veer LJ, van Leeuwen FE, et al. Potential for bias in studies on efficacy of prophylactic surgery for BRCA1 and BRCA2 mutation. J Natl Cancer Inst 2003;95(13): 941–7.
[29] Chen J, Pee D, Ayyagari R, et al. Projecting absolute invasive breast cancer risk in white women with a model that includes mammographic density. J Natl Cancer Inst 2006; 98(17):1215–26.
[30] Bach PB, Kattan MW, Thornquist MD, et al. Variations in lung cancer risk among smokers. J Natl Cancer Inst 2003;95(6):470–8.
[31] O'Shaughnessy JA, Kelloff GJ, Gordon GB, et al. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. Clin Cancer Res 2002;8(2):314–46.
[32] Cole BF, Baron JA, Sandler RS, et al, Polyp Prevention Study Group. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297(21): 2351–9.
[33] Song J, Medline A, Mason JB, et al. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res 2000;60(19):5434–40.
[34] Ulrich CM, Potter JD. Folate and cancer—timing is everything. JAMA 2007;297(21): 2408–9.
[35] Banach-Petrosky W, Ouyang X, Gao H, et al. Vitamin D inhibits the formation of prostatic intraepithelial neoplasia in Nkx3.1;Pten mutant mice. Clin Cancer Res 2006;12(19): 5895–901.

[36] Greenberg ER, Baron JA, Tosteson TD, et al. A clinical trial of antioxidant vitamins to prevent colorectal adenoma. Polyp Prevention Study Group. N Engl J Med 1994;331(3):141–7.
[37] Baron JA, Cole BF, Mott L, et al. Neoplastic and antineoplastic effects of beta-carotene on colorectal adenoma recurrence: results of a randomized trial. J Natl Cancer Inst 2003;95(10): 717–22.
[38] Bigler J, Whitton J, Lampe JW, et al. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. Cancer Res 2001;61(9):3566–9.
[39] Martinez ME, O'Brien TG, Fultz KE, et al. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proc Natl Acad Sci U S A 2003;100(13):7859–64.
[40] Chan AT, Tranah GJ, Giovannucci EL, et al. Genetic variants in the UGT1A6 enzyme, aspirin use, and the risk of colorectal adenoma. J Natl Cancer Inst 2005;97(6):457–60.
[41] Barry EL, Baron JA, Bhat S, et al. Ornithine decarboxylase polymorphism modification of response to aspirin treatment for colorectal adenoma prevention. J Natl Cancer Inst 2006; 98(20):1494–500.
[42] Hubner RA, Muir KR, Liu JF, et al. United Kingdom Colorectal Adenoma Prevention Consortium. Genetic variants of UGT1A6 influence risk of colorectal adenoma recurrence. Clin Cancer Res 2006;12(21):6585–9.
[43] Meyskens FL Jr, Szabo E. Diet and cancer: the disconnect between epidemiology and randomized clinical trials. Cancer Epidemiol Biomarkers Prev 2005;14(6):1366–9.
[44] Kelloff GJ, Lippman SM, Dannenberg AJ, et al. AACR Task Force on Cancer Prevention. Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer—a plan to move forward. Clin Cancer Res 2006;12(12):3661–97.
[45] Kelloff GJ, Sigman CC. Assessing intraepithelial neoplasia and drug safety in cancer-preventive drug development. Nat Rev Cancer 2007;7(7):508–18.
[46] Lee JJ, Hong WK, Hittelman WN, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. Clin Cancer Res 2000;6(5):1702–10.
[47] Kelloff GJ, O'Shaughnessy JA, Gordon GB, et al. Because some surrogate end point biomarkers measure the neoplastic process they will have high utility in the development of cancer chemopreventive agents against sporadic cancers. Cancer Epidemiol Biomarkers Prev 2003;12(7):593–6.

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Hereditary Ovarian Cancer—Assessing Risk and Prevention Strategies

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The last decade has seen significant advances in the surgical, chemotherapeutic, and biologic therapies of ovarian cancer, and patients now are living longer and better. The reality for most patients who have ovarian cancer, however, remains an initial diagnosis of metastatic disease, a surgery with subsequent chemotherapy and possible remission, recurrence, and, with growing chemoresistance, death from disease. This sequence is particularly well known in families predisposed to this deadly disease. Before the identification of the *BRCA* genes, individuals in these families had family history alone to guide their physicians in risk management, and data were scarce. Using that family history in combination with current molecular and genetic techniques, physicians now are better able to identify and counsel patients at risk for ovarian cancer and to identify individuals who do not carry the mutation in high-risk pedigrees as potentially low-risk. This article discusses the epidemiology, pathogenesis, prevention, and treatment of familial ovarian cancer syndromes.

Hereditary breast and ovarian cancer syndrome

Approximately 10% of epithelial ovarian cancers are thought to be related to a germline genetic mutation. *BRCA1* and *BRCA2* are the most commonly affected genes, accounting for approximately 90% of the mutations in hereditary ovarian cancer [1,2]. The similar phenotype conveyed by

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each of these genes in their mutant forms (that is, increased risk of breast and ovarian cancer) probably reflects their functional similarity. First identified in the mid-1990s, *BRCA1* and *BRCA2* are the tumor suppressor genes involved in the repair of double-strand DNA breaks, among other cellular signaling roles.

There are three principal means by which DNA breaks across both strands are thought to be repaired: nonhomologous end-joining, direct conversion, and single-strand annealing. *BRCA1* and *BRCA2* have been demonstrated to co-localize with *RAD51*, itself critical for DNA repair, at sites of DNA damage [3]. *BRCA1* currently is thought to function upstream of both the direct conversion and the single-strand annealing pathways and may influence selection of a repair mechanism. *BRCA1* also is involved in cell-cycle checkpoint control. *BRCA2* complexes directly with *RAD51* and controls its function in homologous recombination [3]. Given their importance in the DNA repair machinery in breast and ovarian epithelial cells, it currently is unclear why *BRCA1* and *BRCA2* mutations do not dramatically increase the risk of other solid-organ malignancies.

In its homozygous state, a *BRCA1* or *BRCA2* mutation is embryonically lethal. Therefore all patients who have *BRCA* mutations are, by definition, heterozygotes, or carriers. The incomplete penetrance seen with regard to the development of the malignancies associated with hereditary breast and ovarian cancer syndrome (HBOCS) is a result of various gene–environment and gene–gene interactions resulting in the inactivation or mutation of the sole remaining wild-type allele in a particular cell. Understanding this situation resolves the apparent paradox that *BRCA* mutations behave in an autosomal dominant fashion with incomplete penetrance in an individual, whereas on an individual cellular level they are autosomal recessive mutations.

Although identified initially on the basis of breast cancer risk, a *BRCA1* mutation carries with it a lifetime risk of epithelial ovarian cancer of 54% (estimates range from 20% to 60%); with a *BRCA2* mutation the risk of ovarian cancer is about half, 23% (estimates range from 10% to 40%) [4–7]. As is true in most familial cancer syndromes, *BRCA1*-associated ovarian cancers tend to occur at a younger age than their sporadic counterparts, with a median age at diagnosis in the mid-forties. By contrast, *BRCA2*-associated ovarian cancers have a median age of onset of 63 years and can occur as late as the ninth decade of life [8]. This observation is crucial in assessing family pedigrees, because an ovarian cancer in a close relative at any age may be significant if clustered in a family with other breast or ovarian cancers.

BRCA genetic testing

Despite increasingly sophisticated molecular and genetic analysis techniques, a thorough family history remains the cornerstone of the diagnosis

of individuals who have HBOCS. Inherited *BRCA* mutations are relatively rare, affecting approximately 1 in 500 individuals in the general population. The likelihood of a particular individual carrying a mutation increases with a high-risk family history. This statement, however, begs the question as to what, exactly, defines a high-risk family history. Although recommendations vary somewhat, it generally is accepted that patients who have two or more first- or second-degree relatives who have ovarian cancer, early-onset breast cancer (age $<$ 50 years), bilateral breast cancer, or male breast cancer may benefit from genetic testing. There also is some evidence that *BRCA2* mutations modestly increase the risk of pancreatic, prostate, and other malignancies [9]. Although these cancers are not formally considered part of HBOCS, they may influence a clinician's decision to offer genetic counseling services. There are several predictive models (BRCAPRO, Myriad II, and modified Couch) that the clinician can use to assess the risk of gene mutation and the lifetime probability of breast or ovarian cancer objectively, but each has its own specific limitations (Table 1). Small family size or a male-dominated pedigree can confound efforts at accurate risk assessment. In a recent investigation, Weitzel and colleagues [10] reported on 306 women who had breast cancer diagnosed before 50 years of age but who did not have an affected first- or second-degree relative (essentially a "negative" family history). For those women who had small families, the risk of testing mutation positive was 13.7%, versus 5.2% for women who had an adequate family structure. The BRCAPRO, Myriad II, and modified Couch models underpredicted mutation frequency in patients who had limited family structures and therefore should be used with caution in counseling these individuals. Risk-prediction models are currently in development that will overcome the limitations of existing tools and include both family history and individual risk factors, such as hormone use and obesity, as well as such clinical measurements such as mammographic breast density to generate a more comprehensive calculation of cancer risk for a given individual [11]. In addition to family history, patient ethnicity can play a significant role in the risk stratification of a given individual for a *BRCA* mutation. Although the prevalence of a germline *BRCA1* or *BRCA2* mutation in the general population is 1 in 500, several ethnic groups carry founder mutations at a much higher rate. The most notable of these is the Ashkenazi Jewish population, in which the prevalence of one of three founder mutations (*BRCA1* codon 185 deletion AG, *BRCA1* codon 5374 insert C, and *BRCA2* codon 6174 deletion T) is approximately 1 in 40 [12]. Therefore most authorities recommend a lower threshold for genetic testing in this population.

Mutations in *BRCA1* or *BRCA2* often cause deletions or insertions resulting in a shortened, inactive protein product. Less often, point mutations leading to amino acid substitution at a critical region for protein function can be deleterious as well, although many substitutions are

Table 1
A comparison of multiple risk assessment models

Inputs	Gail et al	Claus et al	Couch et al	BRCAPRO	Myriad
Age	Yes	Yes	No	No	No
First-degree relatives with breast cancer?	Yes	Yes	Yes	Yes	Yes
Second-degree relatives with breast cancer?	No	Yes	Yes	Yes	Yes
Relatives with ovarian cancer?	No	No	Yes	Yes	Yes
Other cancers (pancreas, prostate)?	No	No	No	No	No
Age of affected relatives	No	Yes	Yes	Yes	Yes
Age of menarche	Yes	No	No	No	No
Age at first live birth	Yes	No	No	No	No
Number of breast biopsies	Yes	No	No	No	No
Atypical hyperplasia	Yes	No	No	No	No
Race	Yes	No	No	No	No
Outputs					
Breast cancer risk	Yes	Yes	Yes[a]	Yes[a]	Yes[a]
Ovarian cancer risk	No	No	Yes[a]	Yes[a]	Yes[a]
Risk of carrying *BRCA1* mutation	No	No	Yes	Yes	Yes
Risk of carrying *BRCA2* mutation	No	No	No	Yes	Yes
Strengths	Long track record, accounts for personal history	Paternal relatives represented	Original model for *BRCA* risk assessment	Bayesian formula allows more exact, personalized risk assessment	Simple tabular format, easy clinical use
Weaknesses	Maternal family history only, based on largely white population	No personal history	*BRCA1* only, no longer often used	Computer model, somewhat time-consuming.	Reliant on physician-submitted histories to match with mutation likelihood

[a] Breast and ovarian cancer risks in the Couch, BRCAPRO, and Myriad models are calculated by a product of the likelihood of carrying a particular mutation and the penetrance of that particular mutation for breast or ovarian cancer.

merely innocent polymorphisms or polymorphisms of uncertain clinical significance. Evolving data suggest that intronic mutations may affect RNA splicing [13], leading to deletions of adjacent exons' contributions to mRNA. Genetic testing can provide some indication of patient risk but is not always definitive. Direct nucleotide sequencing can fail to recognize clinically important genomic reorganizations or epigenetic alterations. Genetic testing is most useful in the confirmation or exclusion of a single, particular, known deleterious mutation within a family. In the Ashkenazi Jewish population, in particular, genetic testing may consist solely of hybridization for the three main founder mutations. Thus, in evaluating whether a familial mutation is present, the most important information can be gained from assessing the *BRCA* status of an affected proband (a relative with cancer). When affected family members have not themselves been tested, the evaluation of a high-risk patient involves full-length sequencing of *BRCA1* and *BRCA2*, which costs around $3500. In the case of a polymorphism, the clinical significance frequently is unknown. In these cases, Myriad Genetic Laboratories (Salt Lake City, Utah), as the holder of the United States patents for *BRCA1* and *BRCA2* testing, serves as a national reference laboratory. A "negative" test is most reassuring if it excludes a specific mutation documented elsewhere in the pedigree, although a negative gene test does not preclude a sporadic ovarian cancer diagnosis later in life. Given the prevalence of the three founder mutations in Ashkenazi Jews, all three founder mutations should be screened for in patients who have Ashkenazi heritage from both parents, even if a proband mutation is known in one family lineage.

Many patients and physicians share concerns that genetic data may be used by prospective employers or insurance companies to disqualify the patients for health insurance or life insurance coverage. This concern has led to some reluctance on the part of some patients to have testing performed and also has led to some reluctance by physicians to document a patient's mutation status in the medical record. Given the protections afforded by the Health Insurance Portability and Accountability Act of 1996 regarding genetic discrimination and the now widespread acceptance and coverage of *BRCA* testing by health insurance carriers, it is advisable to record mutation status in the medical record. This record is particularly important for patients who desire prophylactic surgery, because the testing results provide the surgical indication. There are no reported cases of attempted discrimination on the basis of *BRCA* status to date. Given the potential complexity of the issues surrounding genetic testing for *BRCA* mutations, it is important to involve genetic counselors or other individuals who have specialized knowledge in this area. Despite the potential drawbacks to testing and the anxiety a positive result can generate, patients who have undergone genetic screening and are found to be carriers in general retain a positive attitude toward genetic screening [14].

Management of *BRCA*-related ovarian cancer risk

In individuals found to carry a *BRCA* mutation, three principal management strategies are available: intensified screening, chemoprevention, and prophylactic surgery. Although none of these modalities is perfect, it is possible to alter an individual patient's risk substantially. Perhaps to an even greater degree than with most other health concerns, it is imperative for the patient to understand the risks, benefits, and limitations of each strategy.

Intensified screening

In large, unselected groups of patients, screening for ovarian cancer by serum CA-125 levels and/or transvaginal sonography has proven to be of little to no benefit. This lack of benefit results, in large part, from the poor sensitivity and specificity of these tests. In patients who have a germline *BRCA* mutation, it can be hypothesized that screening tests might prove more relevant. Large, well-designed studies analyzing the effectiveness of screening in women carrying *BRCA* mutations are difficult to perform, however, because of the small numbers of carriers available and willing to participate. In one of the best studies to date on this issue [15], Scheuer and colleagues report on 62 confirmed *BRCA1* or *BRCA2* carriers who elected to undergo surveillance through twice-annual transvaginal sonography and CA-125 levels. In a mean of 17 months of follow-up, 22 of 62 patients had at least one abnormality prompting further testing. Twelve of these patients had normalization of their CA-125 levels with serial measurements; 10 were taken to surgery for exploration. Of these, five had ovarian or primary peritoneal cancers, all of which were stage I-II. Two patients who had normal screening tests who subsequently chose risk-reducing bilateral salpingo-oophorectomy (BSO) had ovarian malignancies on the removed ovaries at the time of the procedure. This experience led the authors to conclude that the overall sensitivity for screening for CA-125 levels and ultrasound on a semiannual basis was 71%; the specificity reached 91%. Other reports, however, have failed to support screening as an effective modality for the diagnosis of early-stage ovarian cancer in *BRCA* mutation carriers. Liede and colleagues [16] described 33 mutation carriers followed over a 10-year period. In this interval, seven patients developed ovarian or primary peritoneal cancers. Only one patient was stage I, and only three patients were diagnosed by screening, for a sensitivity of 43%. Similarly discouraging results were recently reported by a Dutch group in which screening failed to identify any early-stage malignancies but did identify six women who had stage III/IV ovarian cancers [17].

For screening to be of value, it must, by definition, detect asymptomatic disease at a more treatable stage. Although data are somewhat conflicting, National Comprehensive Cancer Network guidelines currently recommend concurrent transvaginal ultrasound plus CA-125 screening every 6 months

starting at age 35 years or 5 to 10 years earlier than the earliest age of first diagnosis of ovarian cancer in the family, and preferably on day 1 through 10 of the cycle for premenopausal women [18]. Approaches involving serum proteomic panels are in development but are not yet clinically useful. In counseling patients electing to forego prophylactic surgery, it is imperative that they understand the limitations of currently available screening modalities. Although frequently regarded as a fairly silent disease symptomatically, recent data suggest that ovarian cancer does have a spectrum of recognizable symptoms [19]. Symptoms that were associated significantly with ovarian cancer were pelvic/abdominal pain, urinary urgency/frequency, bloating, and early satiety when they were present for less than 1 year and occurred more than 12 days per month. Patients who have *BRCA* mutations should be made aware of these symptoms and should seek prompt consultation with a physician if they occur.

Chemoprevention

Although the goal of screening is for intervention early in the course of an already established disease, primary prevention of the disease is of even more potential benefit. There are several recognized protective factors for ovarian cancer in general: late menarche, early menopause, multiparity, tubal ligation, and oral contraceptive pill (OCP) use, to name a few. Although the application of these protective factors to *BRCA* mutation lineages is not as firmly established epidemiologically, the appeal of ovarian cancer prevention by simply taking oral contraceptives is undeniable. Retrospective studies have investigated this issue. In 1998, Narod and colleagues [20] reported on 207 *BRCA* carriers who had ovarian cancer and 161 sisters who were cancer free, matched for various reproductive factors including OCP use. In this population, the odds ratio for developing ovarian cancer was 0.5 for women who had ever used OCPs and decreased to 0.4 for women who had used OCPs for more than 6 years. A 2001 study by Modan and colleagues [21] seemed to refute this finding, however. This group found that multiparity, and not OCP use, was the key protective factor for ovarian cancer risk. One of the criticisms of this work, however, is that the high rate of multiparity and the relatively low use of contraceptive pills limited this study's ability to address the risk attributable to OCP use. In what is by far the largest study of its kind to date, McLaughlin and colleagues [22] support the conclusions of Narod and colleagues [20]. In an epidemiologic survey of more than 3000 *BRCA* mutation carriers, the risk of ovarian cancer was reduced for *BRCA1* and *BRCA2* carriers, with odds ratios for developing ovarian cancer of 0.56 and 0.39, respectively, for women who had used OCPs at any point.

Although the data supporting OCP use as a chemopreventative strategy for ovarian cancer in *BRCA* carriers are fairly convincing, prospective studies have not confirmed these retrospective observations. In counseling

patients about the use of OCPs, practitioners should be careful to weigh the risk of ovarian cancer risk against the risk of breast cancer. Similar-cohort studies have documented a slightly increased risk of early breast cancer in *BRCA* carriers who had ever used OCPs (odds ratio, 1.2) compared with women who had never used OCPs [23], and thus patients must be counseled appropriately.

Prophylactic surgery

Prophylactic BSO is widely considered the most effective strategy for reducing the risk of ovarian cancer in *BRCA* carriers. In general, it is a relatively low-risk surgical procedure that often can be performed laparoscopically. Patient acceptance is high [24], and it may affect patient body image less adversely than prophylactic mastectomy. Despite these generalities, there are many important considerations in the planning and execution of prophylactic BSO for the reduction of risk in *BRCA* mutation carriers.

Although generally accepted, the benefit of prophylactic salpingo-oophorectomy on overall patient survival has not been proven for *BRCA* carriers in a prospective manner. Significant retrospective data provide evidence of efficacy, however. A study that examined the effect of prophylactic BSO revealed a 75% lower rate of breast and ovarian cancer over several years of follow-up [25]. A separate study on 551 *BRCA1/2* carriers from various registries also was reported in 2002 [26]. Among 259 women who had undergone prophylactic oophorectomy, 6 women (2.3%) were found to have stage I ovarian cancer at the time of the procedure, and 2 women (0.8%) subsequently developed serous peritoneal carcinoma. Among the controls, 58 women (19.9%) developed ovarian cancer after a mean follow-up of 8.8 years. With the exclusion of the 6 women whose cancer was diagnosed at surgery, prophylactic oophorectomy reduced the risk of ovarian, tubal, and peritoneal epithelial cancer by 96%.

The timing of prophylactic surgery needs to be individualized for each patient. Many women are torn between the conflicting goals of cancer prevention and childbearing. Although epithelial ovarian cancers have been reported in *BRCA* carriers in their twenties, the risk of hereditary ovarian cancer does not rise sharply until the late thirties for *BRCA1* carriers [27] and the late fifties for women who have *BRCA2* mutations [4]. This knowledge has led to the current practice of recommending prophylactic BSO at the completion of childbearing, with a preference for acting by age 35 years, especially in *BRCA1* carriers. In mutation carriers undergoing oophorectomy before 35 years of age, a 60% reduction in breast cancer incidence can be seen [26], solidifying this age as a potential target for patient decision making. Negative effects of this aggressive risk-reduction strategy include surgical menopause, with the attendant increased risk of cardiovascular disease, vasomotor symptoms, and bone loss. Hormone replacement therapy in

these *BRCA* carriers is an area of some controversy, given the theoretic increase in breast cancer risk this treatment may confer. In a study of 155 women undergoing prophylactic BSO, however, the risk of breast cancer in a median 3.6 years of follow-up was identical between those who took hormone replacement therapy and those who did not [28]. In this regard, it seems that short-term use of hormone replacement therapy following prophylactic BSO is a safe treatment alternative for many patients and could substantially improve quality of life.

The method of prophylactic BSO and the pathologic handling and examination of the removed tissues are of critical importance to the proper management of the *BRCA* carrier (Box 1). The adnexae are relatively easy to remove completely. Attention should be paid to transecting the ovarian blood vessels at least 2 cm proximal from the ovary so that ovarian remnants are not left behind. If there are adhesions between the adnexa and adjacent structures, careful dissection should be performed to ensure complete removal of the ovaries and fallopian tubes. Early-stage fallopian tube cancers have been found in *BRCA* carriers undergoing prophylactic BSO [29]. Malignant cells also have been found in pelvic peritoneal washings from women undergoing prophylactic BSO, and in some of these cases a primary cancer in the ovary or fallopian tube cannot be identified [30]. In view of these data, it seems reasonable to recommend that cytologic washings of the pelvis be obtained when performing prophylactic BSO. The pathologist should be informed of the indication for prophylactic BSO, and multiple sections of the ovaries and fallopian tubes should be examined to exclude the presence of occult carcinoma [31,32]. There is some evidence suggesting

Box 1. Appropriate steps in a risk-reducing bilateral salpingo-oophorectomy

1. Carefully survey all abdominal organ and peritoneal surfaces.
2. Perform abdomino-pelvic wash with saline and send for cytologic evaluation.
3. Biopsy any suspicious nodules and send them for immediate frozen pathologic evaluation.
4. Transect the ovarian vessels at least 2 cm proximal to the ovary.
5. Excise the entire ovary and fallopian tube, transecting the tube as close as possible to its insertion into the uterine cornu.
6. Remove the specimens intact and communicate the nature of the surgery explicitly with the consulting pathologist so that appropriate processing of the specimens occurs.
7. If at any time in these steps a malignancy is encountered, immediate consultation with a gynecologic oncologist is ideal.

that the distal fallopian tube and tubal fimbria may be the most common sites of cancer development in *BRCA* mutation carriers [33].

Peritoneal serous carcinoma indistinguishable histologically or macroscopically from ovarian cancer has been described in rare instances following prophylactic BSO [34,35], but the origin of these cancers is unclear. Careful examination of prophylactic BSO specimens has led to the identification of occult cancers in as many as 12% of cases, but much less commonly in most series [31,32,36]. These findings add support to the theory that primary peritoneal cancers that occur years after BSO may represent recurrences of ovarian or tubal cancers. In this regard, case reports have been published in which retrospective examination of the ovaries and fallopian tubes has revealed occult cancers that were not recognized originally [37]. In addition, it is possible that some serous cancers that occur after prophylactic BSO may arise from benign peritoneal glandular inclusions (endosalpingiosis) rather than from the peritoneum. Most *BRCA*-associated ovarian cancers are invasive serous lesions [2,38]. Although there are conflicting reports about whether *BRCA* mutations increase the risk of serous cancers of the uterus [39,40], there is strong evidence to support inclusion of serous fallopian tube cancers in this syndrome [37,41,42]. In the presence of abdominal carcinomatosis, it often is difficult to determine with certainty whether the cancer arose in the fallopian tube or ovary. Some fallopian tube cancers may be misclassified as ovarian, leading to an underestimation of the incidence of fallopian tube cancer.

Recommendations for hysterectomy as part of risk-reducing surgery in *BRCA* mutation carriers remain controversial. Many patients elect to have the uterus removed when undergoing prophylactic BSO because they have completed their family or have other gynecologic indications for hysterectomy. Furthermore, the possible future exposure to tamoxifen, which increases endometrial cancer risk two- to threefold, in the context of breast cancer prevention or adjuvant treatment, also argues for concomitant hysterectomy. In young patients who may decide to pursue postoperative hormone replacement therapy, a potentially improved side-effect profile associated with estrogen replacement alone versus estrogen and progesterone therapy also may argue for hysterectomy. When hysterectomy is performed laparoscopically, there is only modest prolongation of postoperative hospital discharge or recovery. Some patients may elect not to have a hysterectomy, and this choice is reasonable, because there is no clear evidence that hysterectomy reduces cancer mortality in *BRCA* carriers. When hysterectomy is not performed, the fallopian tubes should be removed as completely as possible, however. The interstitial portion of the tube may potentially be left in situ, but the significance of doing so is uncertain, because thus far there are no case reports of fallopian tube cancer developing in such remnants.

Ovarian cancers in individuals who have germline *BRCA* mutations seem to behave somewhat differently than sporadic ovarian cancers. In particular,

although the distribution of stage, grade, and histology are similar, mutation carriers seem to have a better prognosis with regard to disease-free and overall survival than their counterparts who have sporadic ovarian cancer [43,44]. This difference has been hypothesized to result from enhanced platinum sensitivity attributable to impaired DNA repair in the tumor cells [44,45].

Hereditary non-polyposis colorectal cancer syndrome–associated ovarian cancer

Thus far, this review of hereditary ovarian cancer has centered on the *BRCA* genes. Although this focus reflects the relative importance of these mutations in hereditary ovarian cancer, other mutations also increase the risk of ovarian cancer. The DNA mismatch-repair genes *MLH1*, *MSH2*, and *MSH6* account for approximately 5% of hereditary ovarian malignancies. In a review of 80 patients who had ovarian cancer and who had a germline mutation in a mismatch-repair gene, a family history of an affected first-degree relative, or a history of concurrent colon or endometrial cancer in themselves or their offspring, several key differences were noted between hereditary non-polyposis colorectal cancer (HNPCC)-related ovarian cancer and sporadic ovarian cancer. In this group, HNPCC-related ovarian cancers occurred at a relatively young age (mean age, 42.7 years), were almost exclusively epithelial, were frequently well or moderately differentiated, and were more often associated with a concurrent endometrial cancer; 85% were International Federation of Gynecology and Obstetrics stage I or II [46].

The DNA mismatch-repair genes (*MLH1*, *MSH2*, and *MSH6*, among others) are critical entities in the normal function of the cell. In the absence of intact DNA mismatch repair, DNA strands develop microsatellite instability, leading to rapid accumulation of mutations in critical genes of proliferation and cell-cycle control. The diagnosis of HNPCC can be made by family history, as defined by the revised Amsterdam criteria. A full description of the clinical criteria is presented elsewhere in this issue. Genetic testing is advisable in all individuals meeting the Amsterdam criteria and in those who have a documented mutation in a close relative.

Given the lower relative frequency of HNPCC-associated ovarian cancer, there are comparatively few data evaluating screening and treatment strategies to reduce the incidence of ovarian cancer in mutation carriers. In a report by Rijcken and colleagues [47], screening with transvaginal sonography and serum CA-125 measurements for ovarian and endometrial cancer in a small cohort of women who had either *MMR* gene mutations or met the Amsterdam criteria failed to identify any individuals who had endometrial or ovarian cancer. This study was underpowered to ascertain the impact of screening, however; there were no incident cases of ovarian cancer, and there was only one case of endometrial cancer (diagnosed

because of symptoms between screening studies). Given the ambiguous data among *BRCA* cohorts in which the incidence of ovarian cancer is much higher, screening for ovarian cancer in asymptomatic HNPCC carriers may offer little benefit and may result in a number of unnecessary surgeries. Despite these limitations, current recommendations for the gynecologic screening of HNPCC carriers include annual transvaginal sonographic evaluation of the ovaries [48]. In addition, clinicians should be aware of the increased risk of ovarian cancer in this population and should have a low threshold for imaging in patients who develop abdominal or pelvic pain, bloating, or a change in bowel or bladder habits, because these symptoms may correlate with an undiagnosed ovarian cancer [19].

As with *BRCA* mutations, the most effective strategy to reduce the incidence of ovarian cancer in women who have *MMR* gene mutations is prophylactic oophorectomy. In the largest and best-controlled study of its kind [49], investigators from three cancer registries pooled patients who had demonstrated germline *MLH1*, *MSH2*, or *MSH6* mutations and compared outcomes between 47 patients who underwent bilateral oophorectomy surgery and 223 age-matched controls who had not. In a median of 10.6 years of follow-up, 12 controls had developed ovarian cancer, versus none of the oophorectomy patients. None of the oophorectomy patients developed a primary peritoneal malignancy. Given a lifetime risk of endometrial cancer of up to 60% [50], the prophylactic operation of choice should be a total hysterectomy with BSO. Given the relatively young age of onset of HNPCC-related gynecologic malignancies, this operation should be performed soon after the completion of childbearing. Prophylactic hysterectomy and BSO also should be considered by surgeons planning colon cancer surgery in a gene carrier. Although there are no data with regard to the effects of estrogen replacement in carriers of the *MMR* gene mutation, it can be considered in appropriately counseled patients. It is imperative that gynecologists manage these patients in parallel with practitioners who have experience in surveillance for colorectal cancers.

Summary

Hereditary ovarian cancers are almost entirely attributable to mutations in *BRCA1/2* or the genes of DNA mismatch repair. Identifying individuals at risk requires a complete family history and evidence-guided genetic testing. Screening of women at increased risk for ovarian cancer can be considered in those not wishing prophylactic surgery and typically should include a twice-annual pelvic examination, serum CA-125 measurement, and transvaginal sonography. Patients must understand, however, that these measures have not been conclusively proven to improve early detection or long-term survival. In all mutation carriers who have completed or do not desire childbearing, prophylactic BSO must be strongly considered.

References

[1] Rubin SCM, Blackwood MA, Bandera CM, et al. *BRCA1*, *BRCA2*, and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: relationship to family history and implications for genetic testing. Am J Obstet Gynecol 1998; 178:670–5.

[2] Reedy M, Gallion H, Fowler JM, et al. Contribution of *BRCA1* and *BRCA2* to familial ovarian cancer: a gynecologic oncology group study. Gynecol Oncol 2002;85:255–9.

[3] Gudmundsdottir K, Ashworth A. The roles of *BRCA1* and *BRCA2* and associated proteins in the maintenance of genomic stability. Oncogene 2006;25(43):5864–74.

[4] King M-C, Marks JH, Mandell JB, for The New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. Science 2003; 302:643–6.

[5] Risch HA, McLaughlin JR, Cole DEC, et al. Prevalence and penetrance of germline *BRCA1* and *BRCA2* mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001;68:700–10.

[6] Whittemore AS, Gong G, Itnyre J. Prevalence and contribution of *BRCA1* mutations in breast cancer and ovarian cancer: results from three U.S. population-based case- control studies of ovarian cancer. Am J Hum Genet 1997;60:496–504.

[7] Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. N Engl J Med 1997;336:1401–8.

[8] Moslehi R, Chu W, Karlan B, et al. *BRCA1* and *BRCA2* mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am J Hum Genet 2000;66:1259–72.

[9] The Breast Cancer Linkage Consortium. Cancer risks in *BRCA2* mutation carriers. J Natl Cancer Inst 1999;91:1310–6.

[10] Weitzel JN, Lagos VI, Cullinane CA, et al. Limited family structure and *BRCA* gene mutation status in single cases of breast cancer. JAMA 2007;297(23):2587–95.

[11] Freedman AN, Seminara D, Gail MH, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. J Natl Cancer Inst 2005;97(10):715–23.

[12] Szabo CI, King MC. Population genetics of *BRCA1* and *BRCA2*. Am J Hum Genet 1997;60: 1013–20.

[13] Payne SR, Newman B, King MC. Complex germline rearrangement of *BRCA1* associated with breast and ovarian cancer. Genes Chromosomes Cancer 2000;29(1):58–62.

[14] Lynch HT, Snyder C, Lynch JF, et al. Patient responses to the disclosure of *BRCA* mutation tests in hereditary breast-ovarian cancer families. Cancer Genet Cytogenet 2006;165(2):91–7.

[15] Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in *BRCA* mutation carriers. J Clin Oncol 2002;20(5):1260–8.

[16] Liede A, Karlan BY, Baldwin RL, et al. Cancer incidence in a population of Jewish women at risk of ovarian cancer. J Clin Oncol 2002;20(6):1570–7.

[17] Gaarenstroom KN, van der Hiel B, Tollenaar RA, et al. Efficacy of screening women at high risk of hereditary ovarian cancer: results of an 11-year cohort study. Int J Gynecol Cancer 2006;16(Suppl 1):54–9.

[18] National Comprehensive Cancer Network clinical practice guidelines in oncology, genetic/familial high risk assessment: breast and ovarian. V.I. 2007. Available at: www.nccn.org. Accessed September 1, 2007.

[19] Goff BA, Mandel LS, Melancon CH, et al. Frequency of symptoms of ovarian cancer in women presenting to primary care clinics. JAMA 2004;291(22):2705–12.

[20] Narod SA, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary Ovarian Cancer Clinical Study Group. N Engl J Med 1998;339(7):424–8.

[21] Modan B, Hartge P, Hirsh-Yechezkel G, et al, National Israel Ovarian Cancer Study Group. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a *BRCA1* or *BRCA2* mutation. N Engl J Med 2001;345(4):235–40.

[22] McLaughlin JR, Risch HA, Lubinski J, et al, Hereditary Ovarian Cancer Clinical Study Group. Reproductive risk factors for ovarian cancer in carriers of *BRCA1* or *BRCA2* mutations: a case-control study. Lancet Oncol 2007;8(1):26–34.
[23] Narod SA, Dube MP, Klijn J, et al. Oral contraceptives and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. J Natl Cancer Inst 2002;94(23):1773–9.
[24] Lynch HT, Lemon SJ, Durham C, et al. A descriptive study of *BRCA1* testing and reactions to disclosure of test results. Cancer 1997;79:2219–28.
[25] Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a *BRCA1* or *BRCA2* mutation. N Engl J Med 2002;346:1609–15.
[26] Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of *BRCA1* or *BRCA2* mutations. N Engl J Med 2002;346:1616–22.
[27] Ford D, Easton DF, Bishop DT, et al. Risks of cancer in *BRCA1*-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343:692–5.
[28] Rebbeck TR, Friebel T, Wagner T, et al, PROSE Study Group. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in *BRCA1* and *BRCA2* mutation carriers: the PROSE Study Group. J Clin Oncol 2005;23(31):7804–10, Epub 2005 Oct 11.
[29] Paley PJ, Swisher EM, Garcia RL, et al. Occult cancer of the fallopian tube in *BRCA-1* germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. Gynecol Oncol 2001;80:176–80.
[30] Colgan TJ, Boerner SL, Murphy J, et al. Peritoneal lavage cytology: an assessment of its value during prophylactic oophorectomy. Gynecol Oncol 2002;85:397–403.
[31] Colgan TJ, Murphy J, Cole DE, et al. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with *BRCA* germline mutation status. Am J Surg Pathol 2001;25:1283–9.
[32] Powell CB, Kenley E, Chen L-M, et al. Risk-reducing salpingo-oophorectomy in *BRCA* mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005;23:127–32.
[33] Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006;30: 230–6.
[34] Finch A, Beiner M, Lubinski J, et al, Hereditary Ovarian Cancer Clinical Study Group. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a *BRCA1* or *BRCA2* mutation. JAMA 2006;296:185–92.
[35] Piver MS, Jishi MF, Tsukada Y, et al. Primary peritoneal carcinoma after prophylactic oophorectomy in women with a family history of ovarian cancer. A report of the Gilda Radner Familial Ovarian Cancer Registry. Cancer 1993;71:2751–5.
[36] Lu KH, Garber JE, Cramer DW, et al. Occult ovarian tumors in women with *BRCA1* or *BRCA2* mutations undergoing prophylactic oophorectomy. J Clin Oncol 2000;18: 2728–32.
[37] Chen KT, Schooley JL, Flam MS. Peritoneal carcinomatosis after prophylactic oophorectomy in familial ovarian cancer syndrome. Obstet Gynecol 1985;66:93S–104S.
[38] Stratton JF, Gayther SA, Russell P, et al. Contribution of *BRCA1* mutations to ovarian cancer. N Engl J Med 1997;336:1125–30.
[39] Lavie O, Hornreich G, Ben Arie A, et al. *BRCA1* germline mutations in women with uterine serous papillary carcinoma. Obstet Gynecol 2000;96:28–32.
[40] Levine DA, Lin O, Barakat RR, et al. Risk of endometrial carcinoma associated with *BRCA* mutation. Gynecol Oncol 2001;80:395–8.
[41] Zweemer RP, van Diest PJ, Verheijen RH, et al. Molecular evidence linking primary cancer of the fallopian tube to *BRCA1* germline mutations. Gynecol Oncol 2000;76:45–50.
[42] Aziz S, Kuperstein G, Rosen B, et al. A genetic epidemiological study of carcinoma of the fallopian tube. Gynecol Obstet 2001;80:341–5.

[43] Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of *BRCA*-linked and sporadic ovarian cancer. JAMA 2000;283(17):2260–5.
[44] Cass I, Baldwin RL, Varkey T, et al. Improved survival in women with *BRCA*-associated ovarian carcinoma. Cancer 2003;97(9):2187–95.
[45] Cortez D, Wang Y, Qin J, et al. Requirement of ATM dependent phosphorylation of *BRCA1* in the DNA damage response to double-strand breaks. Science 1999;286:1162–6.
[46] Watson P, Butzow R, Lynch HT, et al, International Collaborative Group on HNPCC. The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2001;82(2):223–8.
[47] Rijcken FE, Mourits MJ, Kleibeuker JH, et al. Gynecologic screening in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2003;91(1):74–80.
[48] Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006; 296(12):1507–17.
[49] Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354(3):261–9.
[50] Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214–8.

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Strategies for Ovarian Cancer Prevention

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One out of every 55 women the United States (1.8%) will develop ovarian cancer during her lifetime in [1]. Approximately 20,000 cases are diagnosed annually, and 15,000 ovarian cancer deaths occur each year [2]. Only a slight decrease in the mortality attributable to ovarian cancer has been achieved in the past 30 years. Most patients are found to have advanced disease at the time of diagnosis, in part because the symptoms of early disease are often mild, mostly nonspecific, or occasionally absent. The survival rate of women who have advanced-stage disease is 10% to 30% at 5 years, as opposed to more than 90% of women who have stage I disease, rendering ovarian cancer the leading cause of death from gynecologic malignancies in the United States [3]. Much attention has focused on prevention and early detection of disease, with particular interest in screening modalities for high-risk patient subsets. This article discusses the efficacy of available screening modalities and reviews current risk-reduction strategies and their effectiveness for preventing ovarian cancer prevention.

The high-risk patient

Any attempt at disease prevention first must identify those patients at highest risk of disease development. Epidemiologic cohort studies have noted a significant inverse association between parity and ovarian cancer risk [4–6]. Increasing duration of breastfeeding likewise has been associated with a reduction in cancer risk [7]. Risk factors associated with the development of epithelial ovarian cancer include advancing age, North American/European ethnicity, a personal history of breast, endometrial, or colon cancer, and a family history of ovarian cancer. In addition, patients who have certain genetic mutations comprise a particularly high-risk category for

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developing ovarian cancer over their lifetime. Mutations in the breast and ovarian cancer-susceptibility genes *BRCA1* and *BRCA2* confer an increased lifetime risk of ovarian cancer. These mutations also increase the risk of breast and pancreatic cancer in both male and female carriers; the risk of prostate cancer is increased in males who have a *BRCA2* mutation [8,9]. Pooled pedigree data from 22 studies involving 8139 index cases indicate an average cumulative risk of ovarian cancer of 39% by age 70 years in *BRCA1* mutation carriers, as compared with 11% in *BRCA2* mutation carriers [10]. Studies of highly selected kindreds (as opposed to population-based studies) have suggested an even higher risk of 30% to 60% by the age of 70 years in *BRCA1* mutation carriers and of 27% in *BRCA2* carriers [11–13]. The baseline prevalence of *BRCA* mutations is 1 in 300 in the United States [14,15], whereas the mutation prevalence among Ashkenazi Jews approaches 2% [16]. Conversely, a woman diagnosed with ovarian cancer has a 10% chance of harboring a *BRCA* mutation, with odds increasing to one in three among Ashkenazi Jewish women who have ovarian cancer [17–19].

BRCA mutations do not account for the entire breadth of hereditary ovarian cancer syndromes. Site-specific ovarian cancer syndrome is found in families affected only by ovarian but not breast cancer, although there is some question whether this syndrome is distinct from *BRCA* mutation. Nearly half of all of the pedigrees seemingly consistent with a *BRCA* pedigree test negative for mutations despite full-gene sequencing. Hereditary nonpolyposis colorectal cancer, previously referred to as the ''Lynch II syndrome,'' involves a constellation of early-onset colorectal, endometrial, ovarian, urothelial, and select upper gastrointestinal malignancies. Endometrial cancer is much more common than ovarian cancer in affected women, frequently presenting before colorectal cancer. Taken as a whole, approximately 10% of epithelial ovarian cancer is explained by these three hereditary predispositions [20]. A full discussion of the hereditary syndromes and screening specific to the mutation carrier is given elsewhere in this issue.

A common clinical scenario is the patient who has either a personal history of breast cancer, especially premenopausal breast cancer, or a strong family history of ovarian and/or breast cancer, who, in the absence of genetic screening, requests education about preventative measures for ovarian cancer. The odds of finding a deleterious *BRCA* mutation within an individual or family can be extrapolated from pedigree data, according to various clinical situations, and is best done in conjunction with a certified cancer genetic counselor [17–19,21]. These odds vary by type of cancer (ie, breast versus ovarian) and increase with the number of family members affected and the age at diagnosis [22]. A general premise in genetic testing is to target first the members of a family personally affected by cancer. This strategy increases both the yield of the test and the likelihood that testing will be covered by insurance. If an affected family member tests positive for a mutation, other family members can be screened for only that specific mutation, decreasing the cost of their testing. When family members have

already died, genetic testing of index cases is not possible. The decision to recommend genetic testing to patients requires extensive counseling regarding the implications of testing and management strategies for the presence or absence of a deleterious mutation. It is important to reiterate that hereditary cancer syndromes are rare, so the majority of patients who have a family history suggestive of a familial syndrome will test negative for known genetic mutations [23]. Should a mutation be uncovered, targeted screening or risk-reduction surgery can be offered; this scenario is discussed fully elsewhere in this issue.

Chemoprevention

Oral contraceptive pills

The majority of concerned women will find themselves at average risk for ovarian cancer but will still inquire about prevention measures. Longstanding efforts in ovarian cancer prevention have focused on the use of medications to reduce the risk of disease. Chemoprevention trials, although rendered difficult by necessary length, expense, and lack of animal models, have yielded promising results in this area [24,25].

Oral contraceptive pills (OCP) have been the most widely studied chemopreventive agent in ovarian cancer. A growing volume of data indicates that ovarian cancer risk can be reduced by 50% after 5 years of OCP use and by 30% in the setting of ever-use [26]. An analysis of 20 studies over 2 decades confirmed this finding, noting a decrease in cancer risk with each increasing year of OCP use up to 5 years [27]. This protective effect was found to be equivalent in nulliparous and parous women [27]. OCP use of greater than 10 years' duration has been shown to reduce risk in women who have endometriosis, a condition with a propensity (odds ratio, 1.32) for malignant transformation [28]. Multiple studies have demonstrated that the protective effect of OCPs persists beyond discontinuation of pill use, for at least 10 years and possibly as long as 20 years [25,27,29,30].

Postulated mechanisms of OCP risk reduction in ovarian cancer include inhibition of ovulation and progesterone-induced cellular apoptosis [24,31]. A number of studies have examined the composition and potency of OCP formulations with regard to protective effect, generally confirming that low-dose OCP pills confer a level of ovarian cancer risk-reduction equivalent to that of older high-dose formulations [32–39]. These results indicate that the protective effect of OCP is not dose dependent. Chemoprevention with OCPs also has demonstrated a positive effect of this intervention in *BRCA* mutation carriers, as discussed fully elsewhere in this issue.

Other chemopreventive agents for ovarian cancer

Through the investigation of disease pathogenesis and epidemiology, several other agents have emerged with possible chemopreventive action in

ovarian cancer. Retinoids, vitamin D, cyclo-oxygenase inhibitors, and peroxisome proliferator activated receptor-gamma ligands have shown promise in early investigations of disease prevention, but further studies are needed [24]. The Gynecologic Oncology Group is actively studying the effect of fenretidine (N-[4-hydroxyphenyl]retinamide) as a chemoprevention agent in women at genetic risk for ovarian cancer. This is a randomized exploratory study aimed at determining impact on histopathologic markers for precursor lesions as well as biomarkers of cellular proliferation and apoptosis. Identifying surrogate markers of drug effect is an important step in measuring the impact of the intervention. The data from this and other similar trials will be critical in the design of future chemoprevention trials in ovarian cancer.

Screening

When epithelial ovarian cancer is diagnosed at an early stage, 5-year survival is approximately 85% to 95%. Most women who have ovarian cancer, however, are diagnosed at stage III, when disease already has spread outside the ovary to the upper abdomen. These patients have a 5-year survival of 15% to 30% [40]. Given the poor prognosis associated with advanced-stage ovarian cancer, research efforts have focused on the attempt to detect disease at an earlier and more curable stage. The use of serum cancer antigen 125 (CA-125) levels and pelvic ultrasound has been suggested for screening. Any effort to identify a safe and cost-effective screening test for ovarian cancer is hampered by the disease's low prevalence in the population. Even a test with perfect sensitivity (the ability to detect individuals who have disease) and nearly perfect specificity (the ability to identify correctly individuals who do not have disease) will yield an extremely low positive predictive value, given the relative rarity of ovarian cancer [41]. Thus a large number of false-positive screening tests will be encountered, potentially giving rise to unnecessary surgeries and subsequent issues of morbidity and cost.

Rectovaginal pelvic examination

Historically, physical examination including bimanual pelvic examination comprised the only means of diagnosing ovarian cancer. As a screening tool for early-stage disease, physical examination alone lacks both sensitivity and specificity [42,43]. A review of the literature found that ovarian malignancies detected on the basis of pelvic examination alone are usually of an advanced stage and thus associated with a poor prognosis [44]. Likewise, the Papanicolaou's (Pap) smear, which was designed to screen for cervical dysplasia, has a sensitivity for detecting ovarian malignancies of only 10% to 39% [44,45]. Women must understand that the Pap smear is not an adequate tool to screen against ovarian cancer, a common misconception in the lay public. These components of an annual gynecologic examination thus comprise an insufficient screening mechanism for the detection of ovarian cancer.

Cancer antigen 125 tumor marker

The CA-125 tumor marker, a serum glycoprotein antigen detected by radioimmunoassay, is elevated in 80% of epithelial ovarian cancers. Only half of all patients who have stage I ovarian cancer have elevated CA-125 levels, however, yielding low test sensitivity for early-stage disease [46]. Additionally, levels are known to be elevated in the setting of benign ovarian pathology [47] and in other benign gynecologic conditions including endometriosis, pelvic inflammatory disease, and leiomyomatous disease [48,49]. Further, any condition associated with free peritoneal fluid or inflammatory changes in the abdomen, such as cirrhosis, congestive heart failure, and inflammatory bowel disease, can elevate CA-125 levels [47,50,51]. There is evidence that CA-125 levels are elevated in 1% of normal healthy women and can fluctuate during the course of the normal menstrual cycle [52]. Significant CA-125 heterogeneity among healthy women at high risk for ovarian cancer has been observed also [53]. A variety of malignancies other than ovarian cancer, including breast, colon, lung, and endometrial cancer, also can give rise to elevated CA-125 levels [54].

Given the greater lack of specificity for the detection of ovarian cancer associated with an elevated CA-125 level in the premenopausal population, most screening studies using CA-125 values have focused on postmenopausal women. Two studies examined the risk of subsequent cancer associated with elevated CA-125 levels in asymptomatic postmenopausal women. In the first, 22,000 women were screened with annual CA-125 levels, with referral for ultrasound in the event of abnormal levels and surgical investigation for abnormal ultrasound findings. Forty-nine index cancers developed in the group over almost 7 years. The relative risks of developing cancer within 1 and 5 years were found to be 35.9 and 14.3, respectively, for a CA-125 level greater than or equal to 30 U/mL [55]. In the same group, a subset of women who had elevated CA-125 levels were matched to women who had normal serum tumor marker values and were followed for the development of cancer. Study subjects were found to be at significantly increased risk of gynecologic malignancy (odds ratio, 30.09) but were not more likely to develop breast or other nongynecologic cancers. The authors reported a specificity of 98.6% for a single CA-125 assay in detecting ovarian cancer. Sensitivity estimates ranged from 53% to 89% [56].

The performance of serial CA-125 determinations also has been the focus of active research [57–60]. A retrospective study examined serial CA-125 levels in women who ultimately developed ovarian neoplasia (including borderline and malignant tumors) and matched controls. The distribution of CA-125 levels was found to be significantly different between the groups. Fifty percent of the study subjects exhibited levels greater than 30 U/mL within the 18 months preceding diagnosis, compared with 7% of controls. When the authors defined a positive CA-125 test as one that initially was elevated and subsequently doubled within 6 months, the specificity of the

screen improved to greater than 99%, although sensitivity subsequently decreased [57]. Similar results were reported in a prospective study of women ultimately developing ovarian cancer following serial CA-125 determinations. Compared with matched controls, the women who developed cancer exhibited higher median CA-125 levels before disease diagnosis (35.4 U/mL versus 9.0 U/mL over the initial 3 years of the study), yielding a specificity of 100% but a sensitivity of only 57% for the screen [58].

Large screening studies have failed to uphold the efficacy of CA-125 as a screening test for ovarian cancer. A prospective study of more than 5000 women in Sweden used initial CA-125 levels to screen for ovarian cancer in healthy pre- and postmenopausal women. Women who had elevated levels and matched controls were followed with pelvic examination, transvaginal ultrasound, and serial CA-125 determinations. Six women in the study group ultimately were found to have ovarian cancer at laparotomy (two each with stages IA, IIB, and IIIC). Three control subjects, all less than 50 years of age, were also diagnosed with cancer. The authors concluded that the specificity of a CA-125 level greater than or equal to 35 U/mL for ovarian cancer was 98.5% for women age 50 years or older and was 94.5% for women younger than 50 years. Sensitivity estimates ranged widely in this study (67%–100%), given the inability to identify the true onset of these tumors [61].

Combined tumor markers

The limited ability of CA-125 levels to screen effectively for early-stage ovarian cancer has led to studies of combinations of tumor markers in an effort to improve screening performance. The combination of serum tumor markers CA-125, CA 72-4, and macrophage colony-stimulating factor (M-CSF) cutoff values together increased the sensitivity in detecting early stage ovarian cancer to 70%, compared with 45% with CA-125 alone, while preserving a screening specificity rate of 98% [62]. A second study examined the combination of CA-125, OVX1 (a tumor marker for mucinous cells), and M-CSF in women who had malignant and benign epithelial ovarian tumors, matched against healthy controls. Combination marker analysis was found to increase the sensitivity for detecting a malignant ovarian tumor from 80% to 85% and of detecting a stage I malignancy from 66% to 76%. Seventeen percent of healthy women had a false- positive screen using combination markers, however, as opposed to only 4% of healthy women screened with only CA-125 [63].

A number of other studies have reported promising results of combination serum marker screening, but prospective trials are needed to substantiate these findings. Two studies examined the addition of serum markers CA 15-3 and TAG 72 to CA-125 levels, with reported increases in specificity for differentiating malignant from benign ovarian disease [64,65]. Additional support has been lent to the status of M-CSF as a marker for ovarian cancer [66]. A combination of the serum proteins leptin, insulin-like growth factor, prolactin, and osteopontin also has been found to screen sensitively for

ovarian, breast, and uterine cancers, although the specificity of the combination assay for any one disease is unknown [67]. Finally, several studies have examined elevations of serum lipid lysophosphatidic acid in ovarian cancer, with initially promising results [68,69]. More work regarding concordant tumor marker screening in ovarian cancer is ongoing.

Transvaginal ultrasonography

A second focus of screening efforts has centered on the use of transabdominal and transvaginal ultrasonography to diagnose ovarian neoplasms. Transvaginal ultrasonography remains the preferred diagnostic approach, given the improved visualization of adnexal structures. Recent attention has also focused on the addition of color flow Doppler to standard transvaginal sonography, with promising results [70–74]. A study aimed at examining changes in intraovarian vasculature and blood flow impedance found that ovaries with morphologically normal or benign pathologic findings exhibited no evidence of neovascularization and demonstrated normal pulsatile indices, whereas seven patients who had malignant ovarian masses had markedly abnormal findings in both Doppler categories [70]. Subsequent trials have incorporated such augmented sonographic modalities in their screening protocols.

The largest prospective screening trial of annual transvaginal ultrasound in asymptomatic women was completed in 2005. More than 25,000 women over age 50 years, or over age 25 years with a family history of ovarian cancer, underwent annual transvaginal sonography as a screening method for ovarian cancer. A persistent increase in ovarian volume was seen in 1.4% of patients on sonogram, leading to surgical exploration. Within this subset, 35 invasive ovarian malignancies, nine serous borderline tumors, and seven metastatic tumors to the ovary were diagnosed (28 patients had stage I disease, 8 had stage II, and 8 had stage III). Nine women developed cancer within 12 months of a normal ultrasound. There were 10 deaths in the study population, including 7 within the annually screened population. The authors calculated sensitivity of 85%, specificity of 98.7%, and a positive predictive value of 14% for ultrasound screening. The authors concluded that annual ultrasound screening was associated with both a lesser disease stage at diagnosis and a decrease in observed case-specific ovarian cancer mortality but was not a sufficient screening method in women with normal ovarian volume [75]. The results of this study reflect a prevalence bias and indicate that a number of malignancies were already present at the time of study entry. Data regarding cases of ovarian cancer diagnosed subsequent to the initial screen therefore will be of great interest.

Similarly, a second study sought to examine the ability of abdominal ultrasonography to screen effectively for ovarian cancer in asymptomatic women. Three annual screening ultrasounds in 5479 women led to the diagnosis of five cases of primary ovarian cancer (0.9% prevalence; all with stage I disease, three with borderline tumors). The authors reported an apparent

sensitivity of 100% and a specificity of 97.7% over the study period, but the positive predictive value was less than 2%, and the false-positive rate was 2.3%. The authors subsequently published revised screening criteria incorporating ovarian volume change at the time of rescan as a criterion for a positive screen, with improvement in the false-positive rate from 2.3% to 1.6% [76]. In the study, 65 exploratory laparotomies were performed for each case of ovarian cancer diagnosed.

The National Ovarian Cancer Early Detection Program evaluated the effectiveness of ultrasound in screening high-risk women, including 4526 women who had a *BRCA* mutation, a personal history of breast cancer, or a family history of ovarian, breast, or other cancer syndromes. More than 12,000 sonograms were performed to detect 12 cases of ovarian, fallopian, primary peritoneal, and endometrial cancer. All cancers identified in the study were stage III, indicating a lack of ability to diagnose early-stage disease in a high-risk population. All malignancies detected were in asymptomatic women who had had normal pelvic examinations and ultrasounds 6 and 12 months before disease diagnosis [77].

In a study of 2500 self-referred pre- and postmenopausal asymptomatic women who had a family history of ovarian cancer, patients underwent transvaginal ultrasound screening with blood flow imaging in an effort to screen for early ovarian cancer. Persistent ovarian abnormalities or increasing ovarian volume were seen in 2.5%, prompting surgical exploration. Eleven women had primary ovarian cancer diagnosed at the time of surgery, seven of which were stage I. One patient developed cancer within 12 months of a negative screen. Eight additional interval cancers were detected in follow-up over the next 9 years, all of which presented with stage III disease. The authors concluded that ultrasound screening in the study population was 92% sensitive and 97.8% specific. CA-125 values also were examined retrospectively in the cancer cohort; using a CA-125 cutoff level of 35 U/mL would have resulted in an early cancer detection rate of only 33%, although the overall number of women undergoing ultrasonography would have been reduced significantly [26].

Taken as a whole, these study results indicate that ultrasound technologies continue to evolve, but current sonographic screening algorithms have not demonstrated acceptable levels of sensitivity and specificity for early-stage disease.

Combined screening modalities

Given the limited ability of unimodal screening methods, considerable attention has been focused on the ability of combined modalities to improve the efficacy of ovarian cancer screening [20,78–80]. To address the question of efficacy, large clinical trials with adequate power to determine the impact of screening algorithms on mortality have been launched in the United States and the United Kingdom.

A randomized pilot trial examined the efficacy of annual CA-125 determinations, followed by ultrasonography and surgery as indicated by the

screening algorithm, in 22,000 postmenopausal women. Among study subjects, six cases of ovarian cancer were diagnosed on initial entry screen, with 23 false-positive screens (positive predictive value of 20.7%). During the 7-year follow-up, 10 additional cases of cancer were detected in the screening group, as opposed to 20 cases in the control group. Mortality rates from index cancers did not differ between the two groups over the study period, but there was a trend toward improved survival in the annually screened group [81].

The United Kingdom Collaborative Trial of Ovarian Cancer Screening has reported preliminary data on a prospective study of 13,582 (projected n = 200,000) postmenopausal women randomly allocated to control versus screening groups. Patients randomly allocated to screening have been classified as having normal, intermediate, or elevated risk, according to baseline CA-125 levels. Individuals at normal risk returned to annual screening. Patients at intermediate risk were subsequently reclassified into normal or elevated risk groups, based on repeat CA-125 determinations. All women found to be at elevated risk because of abnormal CA-125 levels were referred for transvaginal ultrasound. The first report of the study, published in 2005, sought to evaluate prevalence screening within the trial. Of 144 women undergoing transvaginal sonography, 16 underwent surgery based on persistently abnormal ultrasound findings. Findings included three women who had invasive epithelial ovarian cancer (two stage I and one stage II), one woman who had a borderline tumor, one woman who had metastatic breast cancer to the ovaries, and 11 women who had benign pathology. The authors thus reported a specificity of 99.8% and a positive predictive value of 19% in the ability of the algorithm to screen for invasive epithelial ovarian cancer in postmenopausal women [82].

The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial is a third clinical trial currently seeking to address both the efficacy of CA-125 and transvaginal ultrasound as a means of multimodal ovarian cancer screening and their ultimate ability to reduce mortality from the disease. A cohort of 39,115 women between the ages of 55 and 74 years currently are undergoing annual CA-125 determinations for 5 years and annual transvaginal ultrasonography for 3 years. Initial prevalence results of the trial have identified abnormal CA-125 levels in 1.4% of the study group (n = 402) and abnormal ultrasound findings in 4.7% (n = 1338). Twenty-nine gynecologic neoplasms have been diagnosed so far, including 9 borderline tumors and 20 frankly invasive ones (26 stage III/IV ovarian, two stage III fallopian tube, and one stage III primary peritoneal). Positive predictive values for invasive ovarian cancer of 1.0% for abnormal ultrasound, 3.7% for abnormal CA-125, and 23.5% for combined modalities have been reported [83,84]. Thirty-four women in the trial had both an abnormal CA-125 level and ultrasound, accounting for only 8 of the 29 tumors found. Preliminary results of the screening protocol subsequent to the diagnosis of the baseline prevalence cases have reported a positive predictive value of 1.5% among average-risk women (ie, 36 tumors found in 28,460 women

over three annual screening examinations). Surgical evaluation in 570 women detected 29 tumors [79]. The trial is scheduled for completion in 2008.

Studies of multimodal screening in high-risk patient populations have yielded less than promising results. The combination of pelvic examination, CA-125 levels, and transvaginal ultrasound was used over 4 years to screen 312 women who were *BRCA* carriers or members of families that had hereditary breast/ovarian cancer syndrome. At initial screen three cancers (stages IC, IIIB, and IV, respectively) were diagnosed, and one interval cancer (stage IV) was detected subsequently, yielding a sensitivity and positive predictive value of 40% and a specificity of 99%. Five occult carcinomas also were detected in 152 women undergoing prophylactic bilateral salpingo-oophorectomy (BSO). Three of these cases represented early-stage disease diagnosed after normal tumor marker and ultrasound screening [85].

Other similar studies of multimodal screening in high-risk populations have reported low positive predictive values of a positive combined screen for ovarian cancer [79,86], the inability of combined screening to detect disease at an early stage [87], or the frank inability of combined screening to detect subsequently diagnosed malignancies of the ovary, fallopian tube, and peritoneum [88,89].

The current data thus suggest that combination tumor marker/ultrasound methodology may improve the specificity of screening for ovarian cancer, but issues of limited sensitivity and low positive predictive values and the inherent issues of unnecessary surgical risk and patient anxiety persist, particularly in women at average risk of disease.

Proteomics

The search for an ovarian cancer screening modality with improved specificity and sensitivity has led to the examination of serum biomarker patterns using new proteomic technologies. Surface-enhanced laser desorption time-of-flight and other technologies have led to the identification of specific serum protein values that seem to behave differently in malignant as opposed to benign ovarian disease, reflecting pathologic changes within the ovary [81]. Studies have reported sensitivities between 90% and 96% and specificities of 93.5% to 100% in discriminating ovarian cancer cases from healthy controls [85,86].

In a case-control study across five centers, three potential biomarkers were identified using proteomic analysis in subjects who had invasive epithelial ovarian cancer and were compared with values in healthy women and women who had benign ovarian disease. The addition of the combined biomarker panel to CA-125 levels resulted in an increased sensitivity of 74% in the detection of early-stage ovarian cancer, compared with 65% sensitivity for CA-125 alone. Specificity was equivalent in the two groups, at 97%. The combined panel exhibited a higher specificity for disease than CA-125 alone, when held at a fixed sensitivity [80].

Studies of proteomic screening have been challenged by allegations of chance and bias. In 2002, mass spectroscopy was used to generate a cluster pattern of proteomic spectra within a group of patients who had ovarian cancer. These spectra then were able to identify correctly all 50 cases of ovarian cancer within a masked subject set (including 18 cases of stage I disease). The authors reported a sensitivity of 100%, specificity of 95%, and a positive predictive value of 94% [90]. Other authors subsequently questioned both the biologic plausibility and reproducibility of the study's method, claiming that the method performed no better than chance in correctly discriminating cancer from normal subjects [91]. An exploration of this controversy is beyond the scope of this discussion and is well-addressed elsewhere [92], but it is paramount to highlight the need for rigorous validation of method and well-designed clinical trials addressing issues of bias, in an effort to explore further the promising arena of molecular marker biotechnology.

Surgery

An evolving pool of data currently is establishing the effectiveness of prophylactic surgery for reducing cancer risk in high-risk patients. Numerous studies support the benefit of risk-reducing BSO in *BRCA1/2* and HNPCC mutation carriers, related to decreased incidences of both gynecologic (ovarian, fallopian tube) and breast cancers. The role of prophylactic oophorectomy (eg, at the time of hysterectomy), especially in the low-risk pre- or perimenopausal woman, is controversial, and issues of long-term effects of oophorectomy on many other systems and functions should be weighed carefully when counseling women [93–113,141,142].

Bilateral salpingo-oophorectomy

A number of studies have reported reduced risk of ovarian cancer associated with risk-reducing BSO in high-risk patients [114–121]. Although studies generally have been limited by small numbers, lack of definitive genetic testing, and their retrospective nature, several larger and prospective studies have further supported the benefit of risk-reducing BSO in these women. Risk-reducing BSO has been shown to give adequate but incomplete protection in *BRCA* mutation carriers, who have the highest lifetime risk of developing ovarian cancer [122]. The more complex issue is the role of prophylactic oophorectomy in women at baseline risk of developing ovarian cancer. Issues of removing normal ovaries at the time of concomitant gynecologic or other surgery raises a complex risk–benefit assessment that should be individualized. The American College of Obstetricians and Gynecologists 1999 practice bulletin acknowledges that prophylactic oophorectomy is appropriate for ''high-risk'' women but recognizes that the impact of hormone-replacement therapy (HRT), with a full discussion regarding its risks and benefits, and the impact of surgery on quality-of-life issues such as body image, personal feelings, and sexuality must be

evaluated [123]. In addition, it must be recognized that abnormal changes in retained ovaries at the time of hysterectomy are seen in approximately 50% of women throughout their lifetime, with 5% requiring subsequent surgical intervention for symptoms related to the retained ovaries. One also must account for the stress, worry, and cost of interval testing when a cystic adnexa is found after hysterectomy [123].

In premenopausal women, risk-reducing BSO also leads to a surgical menopause, a scenario that has significant complications. Premature menopause can be associated with both significant symptomatic complaints and an increased risk of morbidity related to osteoporosis and cardiovascular disease, effects that may be particularly pronounced in young women [124–126]. Vasomotor symptoms of menopause may be alleviated by HRT in young high-risk women, but at least one study found that symptoms were not improved to a level comparable to premenopausal women [127]. There is also a concern among *BRCA* mutation carriers regarding the slight increase in subsequent breast cancer associated with HRT, a concern that some high-risk women find prohibitive to hormone use. At least one study of short-term (less than 5 years) HRT following risk-reducing BSO in *BRCA* carriers, however, found that HRT use did not diminish the protective effect of risk-reducing BSO on the resultant risk of breast cancer [128]. Finally, the importance of progestin as part of an HRT formulation is stressed, in an effort to protect the uterine endometrium from estrogen-induced neoplastic change. Recent data also show an overall increase in all-cause mortality in premenopausal women who undergo oophorectomy without subsequent hormone replacement. This increased mortality is not seen if women receive low-dose HRT until the age of 50 years [97].

The decision to undergo risk-reducing BSO is complex and must be individualized. Factors associated with the decision for prophylactic BSO include age over 40 years, parity, and a personal history of breast cancer [129]. Other studies have noted inciting factors including a family history of ovarian cancer and anxiety, even in individuals testing negative for *BRCA* mutations [130]. Counseling to cover all of the risks/benefits and effects for each individual woman takes a large amount of time, often over multiple office visits, and uses many resources that, in turn, will help women feel satisfied about their choice.

Other gynecologic procedures

Existing evidence supports a decrease in the incidence of ovarian cancer following other gynecologic procedures, including bilateral tubal ligation and hysterectomy with ovarian preservation [131–137]. Mechanisms of risk reduction may be related to impairment of ovarian function (with subsequent anovulation) or decreased passage of inflammatory substances through the fallopian tube into the peritoneal cavity. The exact protective means are unclear [5,131].

Tubal ligation has been associated with a reduction in the risk of ovarian cancer by one third to one half [132,134,136], an effect that has been found to persist up to 20 years or more after surgery [133]. Studies in *BRCA* carriers likewise have confirmed the reduction in the risk of ovarian cancer associated with tubal ligation (odds ratio, 0.39), even after adjustment for OCP use, parity, and a history of breast cancer [136].

Summary and recommendations

There is no current scientific evidence to support the efficacy of either unimodal or combined screening in women at average risk of ovarian cancer in the population. Organizations including the NIH [16], the US Preventative Services Task Force [137], and the American College of Obstetricians and Gynecologists [138] have published guidelines recommending against routine ovarian cancer screening. The search for a sensitive, specific, and cost-effective screening strategy for average-risk women continues to be a focus of ongoing research efforts. Certainly, all women should continue to undergo annual rectovaginal examination as one component of routine medical care. The importance of taking a careful family history also is highlighted to exclude any overt evidence of a hereditary ovarian cancer syndrome.

Likewise, no data exist to support intensive screening in women who have one isolated relative who has ovarian cancer (although lifetime risk increases to 5% in this setting). Such a woman should be counseled on the basis of her individual risk factors for disease (parity, history of oral contraceptive use, and so forth) and risks for adverse effects of screening (degree of potential surgical risk, issues of anxiety and cost, and so forth). A woman should be counseled carefully regarding the limitations of routine screening in such a setting, and the decision regarding screening in these patients must be an individual one to be made by the patient and her physician. A reasonable screening approach in such a setting would be annual CA-125 determinations, followed by transvaginal ultrasound in the event of CA-125 level greater than 30 U/ml (versus annual tumor marker and ultrasound testing, although even less well supported by the literature). The exception to this "isolated-relative" general rule would be the Ashkenazi-Jewish patient who has a first-degree relative who has ovarian cancer at any age (or one relative who has breast cancer at age less than 50), because such an individual has a significantly higher chance of carrying a genetic mutation (and should be referred for genetic testing) and thus developing ovarian cancer.

Women who have two or more first-degree relatives who have ovarian cancer have a 7% lifetime risk of developing ovarian cancer, and they should be referred for genetic testing. Should testing fail to identify a genetic mutation, no data exist to support routine screening in such women, and screening recommendations should be based on patient and physician preference.

For high-risk women who have known genetic mutations (BRCA or HNPCC carriers), the lifetime risk of ovarian cancer can be as high as 40% (BRCA) and 12% (HNPCC) [139]. Although no studies have proven a mortality reduction from screening algorithms, the recommendation exists for (at least) annual rectovaginal exam, CA-125 levels, and transvaginal ultrasound [140]. Some experts have recommended twice yearly ovarian cancer screening with TVUS and CA-125 levels beginning at age 35, in those patients who have not undergone prophylactic bilateral salpingo-oophorectomy (BSO) [140,141]. Best evidence supports a recommendation for risk-reducing BSO in *BRCA* mutation carriers, a measure effective in reducing lifetime risk of ovarian cancer. Women known to have BRCA mutations should be referred to a gynecologic oncologist for further evaluation and follow-up. The benefit of risk-reducing BSO seems to be highest when surgery is performed at the completion of childbearing and by the age of 35 to 40 years, when the risk of *BRCA1*-associated cancer begins to rise more dramatically. Despite BSO, *BRCA* carriers remain at small risk for primary peritoneal cancer and should be followed closely with annual pelvic examinations. The use of prophylactic oophorectomy in women at intermediate or low risk of ovarian cancer should be individualized, and a full risk–benefit profile, including the immediate and long-term effects of oophorectomy on quality of life and noncancer mortality, should be generated.

References

[1] Ries LAG, Hankey BF, Miller BA, et al. Cancer statistics review: 1973–1988, vol. 91. Bethesda (MD): National Cancer Institute, NIH Publication; 1991. p. 2789.
[2] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–30.
[3] Hogg R, Friedlander M. Biology of epithelial ovarian cancer: implications for screening women at high genetic risk. J Clin Oncol 2004;22(7):1315–27.
[4] Hankinson SE, Colditz GA, Hunter DJ, et al. A prospective study of reproductive factors and risk of epithelial ovarian cancer. Cancer 1995;76(2):284–90.
[5] Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of twelve U.S case-control studies II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol 1992;136(10):1212–20.
[6] John EM, Whittemore AS, Harris R, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of seven U.S. case-control studies. Epithelial ovarian cancer in black women. Collaborative Ovarian Cancer Group. J Natl Cancer Inst 1993;85(2):142–7.
[7] McLaughlin JR, Risch HA, Lubinski J, et al. Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. Lancet Oncol 2007;8(1): 26–34.
[8] Brose MS, Rebbeck TR, Calzone KA, et al. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst 2002;94(18):1365–72.
[9] The Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 1999;91:1310–6.
[10] Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72(5):1117–30.
[11] Easton DF, Ford D, Bishop T, et al. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Am J Hum Genet 1995;56(1):265–71.

[12] Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 1998;62(3):676–89.
[13] Ford D, Easton DF, Bishop DT, et al. Risks of cancer in BRCA1-mutation carriers. Lancet 1994;343(8889):692–5.
[14] Ford D, Easton DF, Peto J. Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 1995;57(6):1457–62.
[15] Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst 2006;98(23):1694–706.
[16] Khoury-Collado F, Bombard AT. Hereditary breast and ovarian cancer: what the primary care physician should know. Obstet Gynecol Surv 2004;59(7):537–42.
[17] Frank TS, Manley SA, Olopade OI, et al. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J Clin Oncol 1998; 16(7):2417–25.
[18] Greene MH. Genetics of breast cancer. Mayo Clin Proc 1997;72(1):54–65.
[19] Shattuck-Eidens D, McClure M, Simard J, et al. A collaborative study of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene: implications for presymptomatic testing and screening. JAMA 1995;273(7):535–41.
[20] Piver MS. Hereditary ovarian cancer: lessons learned from the first twenty years of the Gilda Radner Familial Ovarian Cancer Registry. Gynecol Oncol 2002;85:9–17.
[21] Kerlikowske K, Brown JS, Grady DG, et al. Should women with familial ovarian cancer undergo prophylactic oophorectomy? Obstet Gynecol 1992;80(4):700–7.
[22] Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol 2002;20(6):1480–90.
[23] Rubin SC, Blackwood MA, Bandera C, et al. BRCA1, BRCA2, and hereditary nonpolyposis colorectal gene mutations in an unselected ovarian cancer population: Relationship to family history and implications for genetic testing. Am J Obstet Gynecol 1998;178(4): 670–7.
[24] Zorn KK, Gardner G, Birrer MJ. Epithelial ovarian cancer. In: Kelloff GJ, Hawk ET, Sigman CC, editors. Cancer chemoprevention: strategies for cancer chemoprevention, vol. 2. Totowa (NJ): Humana; 2005.
[25] Bosetti C, Negri E, Trichopoulos D, et al. Long-term effects of oral contraceptives on ovarian cancer risk. Int J Cancer 2002;102(3):262–5.
[26] Tailor A, Bourne TH, Campbell S, et al. Results from an ultrasound-based familial ovarian cancer screening clinic: a 10-year observational study. Ultrasound Obstet Gynecol 2003; 21(4):378–85.
[27] Hankinson SE, Colditz GA, Hunter DJ, et al. A quantitative assessment of oral contraceptive use and risk of ovarian cancer. Obstet Gynecol 1992;80(4):708–14.
[28] Modugno F, Ness RB, Allen GO. Oral contraceptive use, reproductive history, and risk of epithelial ovarian cancer in women with and without endometriosis. Am J Obstet Gynecol 2004;191(3):733–4.
[29] Riman T, Persson I, Nilsson S. Hormonal aspects of epithelial ovarian cancer: review of epidemiologic evidence. Clin Endocrinol 1998;49:695–707.
[30] Siskind V, Green A, Bain C, et al. Beyond ovulation: oral contraceptives and epithelial ovarian cancer. Epidemiology 2000;11:106–10.
[31] Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst 1998;90:1774–86.
[32] Ness RB, Grisso JA, Klapper J, et al. Risk of ovarian cancer in relation to estrogen and progestin dose and use characteristics of oral contraceptives. SHARE Study Group. Steroid Hormones and Reproductions. Am J Epidemiol 2000;152(3):233–41.
[33] Sanderson M, Williams MA, Weiss NS, et al. Oral contraceptives and epithelial ovarian cancer. Does dose matter? J Reprod Med 2000;45(9):720–6.

[34] Lurie G, Thompson P, McDuffie KE, et al. Association of estrogen and progestin potency of oral contraceptives with ovarian carcinoma risk. Obstet Gynecol 2007;109(3):597–607.
[35] Whittemore AS, Balise RR, Pharoah PD, et al. Oral contraceptive use and ovarian cancer risk among carriers of BRCA1 or BRCA2 mutations. Br J Cancer 2004;91(11):1911–5.
[36] Narod SA, Sun P, Risch HA. Ovarian cancer, oral contraceptives, and BRCA mutations. N Engl J Med 2001;345:1706–7.
[37] Walker GR, Schlesselman JJ, Ness RB. Family history of cancer, oral contraceptive use, and ovarian cancer risk. Am J Obstet Gynecol 2002;186(1):8–14.
[38] Modan B, Hartge P, Hirsch-Yechezkel G, et al. Parity, oral contraceptives, and the risk of ovarian cancer among BRCA1 or BRCA2 mutation. N Engl J Med 2001;345(4):235–40.
[39] Narod SA, Dube MP, Klijn J, et al. Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2002;94(23):1773–9.
[40] Friedlander ML. Prognostic factors in ovarian cancer. Semin Oncol 1998;25:305–14.
[41] Gordis L. Assessing the validity and reliability of diagnostic and screening tests. In: Epidemiology. 2nd edition. Philadelphia: W.B. Saunders; 2000. p. 63–81.
[42] van Nagell JR Jr, DePriest PD, Puls LE, et al. Ovarian cancer screening in asymptomatic postmenopausal women by transvaginal sonography. Cancer 1991;68:458–62.
[43] Andolf E, Jorgensen C, Astedet B. Ultrasound examination for detection of ovarian carcinoma in risk groups. Obstet Gynecol 1990;75:106–9.
[44] Smith LH, Oi RH. Detection of malignant ovarian neoplasms: a review of the literature. I. Detection of the patient at risk; clinical, radiological and cytological detection. Obstet Gynecol Surv 1984;39(6):313–28.
[45] Shapiro SP, Nunez C. Psammoma bodies in the cervicovaginal smear in association with a papillary tumor of the peritoneum. Obstet Gynecol 1983;61(1):130–4.
[46] Carlson KJ, Skates SJ, Singer DE. Screening for ovarian cancer. Ann Intern Med 1994;121: 124–32.
[47] Gallup DG, Talledo OE. Management of the adnexal mass in the 1990s. South Med J 1997; 90(10):972–81.
[48] Mol BW, Bayram N, Lijmer JG, et al. The performance of CA-125 measurement in the detection of endometriosis: a meta-analysis. Fertil Steril 1998;70(6):1101–8.
[49] Topalak O, Saygili U, Soyturk M, et al. Serum, pleural effusion, and ascites CA-125 levels in ovarian cancer and nonovarian benign and malignant diseases: a comparative study. Gynecol Oncol 2002;85(1):108–13.
[50] Zuckerman E, Lanir A, Sabo E, et al. Cancer antigen 125: a sensitive marker of ascites in patients with liver cirrhosis. Am J Gastroenterol 1999;94(6):1613–8.
[51] Devarbhavi H, Kaese D, Williams AW, et al. Cancer antigen 125 in patients with chronic liver disease. Mayo Clin Proc 2002;77(6):538–41.
[52] Bast RC, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309(15):883–7.
[53] Crump C, McIntosh MW, Urban N, et al. Ovarian cancer tumor marker behavior in asymptomatic healthy women: implications for screening. Cancer Epidemiol Biomarkers Prev 2000;9(10):1107–11.
[54] Sjovall K, Nilsson B, Einhorn N. The significance of serum CA125 elevation in malignant and nonmalignant diseases. Gynecol Oncol 2002;85:175–8.
[55] Jacobs IJ, Skates S, Davies AP, et al. Risk of diagnosis of ovarian cancer after raised serum CA125 concentrations: a prospective cohort study. BMJ 1996;313(7069):1355–8.
[56] Jeyarajah AR, Ind TE, Skates S, et al. Serum CA125 elevation and risk of clinical detection of cancer in asymptomatic postmenopausal women. Cancer 1999;85(9):2068–72.
[57] Zurawski VR Jr, Orjaseter H, Anderson A, et al. Elevated CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. Int J Cancer 1988; 42(5):677–80.
[58] Helzlsouer KJ, Bush TL, Alberg AJ, et al. Prospective study of serum CA-125 levels as markers of ovarian cancer. JAMA 1993;269(9):1123–6.

[59] Skates SJ, Menon U, MacDonald N, et al. Calculation of the risk of ovarian cancer from serial CA-125 values for preclinical detection in postmenopausal women. J Clin Oncol 2003; 21(Suppl 10):206–10.
[60] Zurawski VR Jr, Sjovall K, Schoenfeld DA, et al. Prospective evaluation of CA 125 levels in a normal population, phase I: the specificities of single and serial determinations in testing for ovarian cancer. Gynecol Oncol 1990;36(3):299–305.
[61] Einhorn N, Sjovall K, Knapp RC, et al. Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer. Obstet Gynecol 1992;80(1):14–8.
[62] Skates SJ, Horisk N, Yu Y, et al. Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA15-3, CA72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions. J Clin Oncol 2004;22(20):4059–66.
[63] van Haaften-Day C, Shen Y, Xu F, et al. OVX1, macrophage-colony stimulating factor, and CA125-II as tumor markers for epithelial ovarian carcinoma: a critical appraisal. Cancer 2001;92(11):2837–44.
[64] Bast RC Jr, Knauf S, Epenetos A, et al. Coordinate elevation of serum markers in ovarian cancer but not in benign disease. Cancer 1991;68(8):1758–63.
[65] Jacobs IJ, Oram DH, Bast RC Jr. Strategies for improving the specificity of screening for ovarian cancer with tumor-associated antigens CA 125, CA 15-3, and TAG 72.3. Obstet Gynecol 1992;80(3 Pt 1):396–9.
[66] Suzuki M, Ohwada M, Aida I, et al. Macrophage colony-stimulating factor as a tumor marker for epithelial ovarian cancer. Obstet Gynecol 1993;82(6):946–50.
[67] Mor G, Visintin I, Lai Y, et al. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci U S A 2005;102(21):7677–82.
[68] Xu Y, Shen Z, Wiper DW, et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. JAMA 1998;280(8):719–23.
[69] Shen Z, Wu M, Elson P, et al. Fatty acid composition of lysophosphatidic acid and lysophosphatidylinositol in plasma from patients with ovarian cancer and other gynecological diseases. Gynecol Oncol 2001;83:25–30.
[70] Bourne T, Campbell S, Steer C, et al. Transvaginal color flow imaging: a possible new screening technique for ovarian cancer. BMJ 1989;299(6712):1367–70.
[71] Kurjak A, Prka M, Arenas JM, et al. Three-dimensional ultrasonography and power Doppler in ovarian cancer screening of asymptomatic peri- and postmenopausal women. Croat Med J 2005;46(5):757–64.
[72] Vuento MH, Pirhonen JP, Makinen JI, et al. Evaluation of ovarian findings in asymptomatic postmenopausal women with color Doppler ultrasound. Cancer 1995;76(7): 1214–8.
[73] Weiner Z, Thaler I, Beck D, et al. Differentiating malignant from benign ovarian tumors with transvaginal color flow imaging. Obstet Gynecol 1992;79(2):159–62.
[74] Weiner Z, Beck D, Shteiner M, et al. Screening for ovarian cancer in women with breast cancer with transvaginal sonography and color flow imaging. J Ultrasound Med 1993; 12(7):387–93.
[75] Van Nagell JR Jr, DePriest PD, Ueland FR, et al. Ovarian cancer screening with annual transvaginal sonography: findings of 25,000 women screened. Cancer 2007;109(9):1887–96.
[76] Campbell S, Royston P, Bhan V, et al. Novel screening strategies for early ovarian cancer by transabdominal ultrasonography. Br J Obstet Gynaecol 1990;97(4):304–11.
[77] Fishman DA, Cohen L, Blank SV, et al. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. Am J Obstet Gynecol 2005;192(4):1214–21.
[78] Karlan BY, Raffel LJ, Crvenkovic G, et al. A multidisciplinary approach to the early detection of ovarian carcinoma: rationale, protocol design, and early results. Am J Obstet Gynecol 1993;169(3):494–501.
[79] Lacey JV Jr, Greene MH, Buys SS, et al. Ovarian cancer screening in women with a family history of breast or ovarian cancer. Obstet Gynecol 2006;108(5):1176–84.

[80] Menon U, Talaat A, Rosenthal AN, et al. Performance of ultrasound as a second line test to serum CA125 in ovarian cancer screening. BJOG 2000;107(2):165–9.
[81] Jacobs IJ, Skates SJ, MacDonald N, et al. Screening for ovarian cancer: a pilot randomized controlled trial. Lancet 1999;353(9160):1207–10.
[82] Menon U, Skates SJ, Lewis S, et al. Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. J Clin Oncol 2005;23(31):7919–26.
[83] Buys SS, Partridge E, Greene MH, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: findings from the initial screen of a randomized trial. Am J Obstet Gynecol 2005;193(5):1630–9.
[84] Porok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal And Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000;21(Suppl 6): 273S–309S.
[85] Olivier RI, Lubsen-Brandsma MA, Verhoef S, et al. CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer. Gynecol Oncol 2006;100(1):20–6.
[86] Bosse K, Rhiem K, Wappenschnmidt B, et al. Screening for ovarian cancer by transvaginal ultrasound and serum CA125 measurement in women with a familial predisposition: a prospective cohort study. Gynecol Oncol 2006;103(3):1077–82.
[87] Gaarenstroom KN, van der Hiel B, Tollenaar RA, et al. Efficacy of screening women at high risk of hereditary ovarian cancer: results of an 11-year cohort study. Int J Gynecol Cancer 2006;16(Suppl 1):54–9.
[88] Karlan BY, Baldwin RL, Lopez-luevanos E, et al. Peritoneal serous papillary carcinoma, a phenotypic variant of familial ovarian cancer: implications for ovarian cancer screening. Am J Obstet Gynecol 1999;180(4):917–28.
[89] Meeuwissen PA, Seynaeve C, Brekelmans CT, et al. Outcome of surveillance and prophylactic salpingo-oophorectomy in asymptomatic women at high risk for ovarian cancer. Gynecol Oncol 2005;97(2):476–82.
[90] Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet 2002;359(9306):572–7.
[91] Baggerly KA, Morris JS, Edmonson SR, et al. Signal in noise: evaluating reported reproducibility of serum proteomic tests for ovarian cancer. J Natl Cancer Inst 2005; 97(4):307–9.
[92] Ransohoff DF. Lessons from controversy: ovarian cancer screening and serum proteomics. J Natl Cancer Inst 2005;97(4):316–9.
[93] Rocca WA, Grossardt BR, de Andrade M, et al. Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. Lancet Oncol 2006;7(10):821–8.
[94] Domchek SM, Friebel TM, Neuhausen SL, et al. Mortality after bilateral salpingo-oophorectomy in *BRCA1* and *BRCA2* mutation carriers: a prospective cohort study. Lancet Oncol 2006;7(3):223–9.
[95] Levine DA, Argenta PA, Yee CJ, et al. Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. J Clin Oncol 2003;21(22):4222–7.
[96] Carcangiu ML, Peissel B, Pasini B, et al. Incidental carcinomas in prophylactic specimens in BRCA1 and BRCA2 germ-line mutation carriers, with emphasis on fallopian tube lesions: report of 6 cases and review of literature. Am J Surg Pathol 2006;30(10):1222–30.
[97] Finch A, Shaw P, Rosen B, et al. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol 2006; 100(1):58–64.
[98] Mcewen AR, McConnell DT, Kenwright DN, et al. Occult cancer of the fallopian tube in a BRCA2 germline mutation carrier at prophylactic salpingo-oophorectomy. Gynecol Oncol 2004;92(3):992–4.
[99] Lamb JD, Garcia RL, Goff BA, et al. Predictors of occult neoplasia in women undergoing risk-reducing salpingo-oophorectomy. Am J Obstet Gynecol 2006;194(6):1702–9.

[100] Paley PJ, Swisher EM, Garcia RL, et al. Occult cancer of the fallopian tube in BRCA-1 germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. Gynecol Oncol 2001;80(2):176–80.
[101] Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006;30(2): 230–6.
[102] Olivier RI, Lubsen-Brandsma LA, van Boven H, et al. Additional salpingectomy after previous prophylactic oophorectomy in high-risk women: sense or nonsense? Gynecol Oncol 2005;96(2):439–43.
[103] Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol 2005;23(1):127–32.
[104] Weber AM, Hewett WJ, Gajewski WH, et al. Serous carcinoma of the peritoneum after oophorectomy. Obstet Gynecol 1992;80(3 Pt 1):558–60.
[105] Chen KT, Schooley JL, Flam MS. Peritoneal carcinomatosis after prophylactic oophorectomy in familial ovarian cancer syndrome. Obstet Gynecol 1985;66(Suppl 3):93S–4S.
[106] Casey MJ, Bewtra C. Peritoneal carcinoma in women with genetic susceptibility: implications for Jewish populations. Fam Cancer 2004;3(3–4):265–81.
[107] Dowdy SC, Stefanek M, Hartmann LC. Surgical risk reduction: prophylactic salpingo-oophorectomy and prophylactic mastectomy. Am J Obstet Gynecol 2004;191(4):1113–23.
[108] Hornreich G, Beller U, Lavie O, et al. Is uterine serous papillary carcinoma a BRCA1-related disease? Case report and review of the literature. Gynecol Oncol 1999;75(2):300–4.
[109] Lavie O, Hornreich G, Ben-Arie A, et al. BRCA germline mutations in Jewish women with uterine serous papillary carcinoma. Gynecol Oncol 2004;92(2):521–4.
[110] Goshen R, Chu W, Elit L, et al. Is uterine papillary serous adenocarcinoma a manifestation of the hereditary breast-ovarian cancer syndrome? Gynecol Oncol 2004;79(3):477–81.
[111] Beiner ME, Finch A, Rosen B, et al. The risk of endometrial cancer in women with *BRCA1* and *BRCA2* mutations: a prospective study. Gynecol Oncol 2007;104(1):7–10.
[112] Schmeler K, Lynch H, Chen L, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in Lynch syndrome. NEJM 2006;354(3):261–9.
[113] Chen L, Yang K, Little S, et al. Gynecologic cancer prevention in Lynch dyndrome/hereditary nonpolyposis colorectal cancer families. Obstet Gynecol 2007;110(1):18–25.
[114] Olivier RI, van Beurden M, Lubsen MA, et al. Clinical outcome of prophylactic oophorectomy in BRCA1/BRCA2 mutation carriers and events during follow-up. Br J Cancer 2004; 90(8):1492–7.
[115] Struewing JP, Watson P, Easton DF, et al. Prophylactic oophorectomy in inherited breast/ovarian cancer families. J Natl Cancer Inst Monogr 1995;17:33–5.
[116] Rutter JL, Wacholder S, Chetrit A, et al. Gynecologic surgeries and risk of ovarian cancer in women with BRCA1 and BRCA2 Ashkenazi founder mutations: and Israeli population-based case-control study. J Natl Cancer Inst 2003;95(14):1072–8.
[117] Olopade OI, Artioli G. Efficacy of risk-reducing salpingo-oophorectomy in women with BRCA-1 and BRCA-2 mutations. Breast J 2004;10(Suppl 1):S5–9.
[118] Piver MS, Jishi MF, Tsukada Y, et al. Primary peritoneal carcinoma after prophylactic oophorectomy in women with a family history of ovarian cancer. A report of the Gilda Radner Familial Ovarian Cancer Registry. Cancer 1993;71(9):2751–5.
[119] Rebbeck TR, Lynch HT, Neuhausen SL, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002;346(21):1616–22.
[120] Kauf ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med 2002;346(21):1609–15.
[121] Rosen B, Kwon J, Fung Kee Fung M, et al. Systematic review of management options for women with a hereditary predisposition to ovarian cancer. Gynecol Oncol 2004;93(2): 280–6.

[122] Finch A, Beiner M, Lubinski J, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a *BRCA1* or *BRCA2* mutation. JAMA 2006;296(2):185–92.
[123] ACOG Practice Bulletin: Prophylactic oophorectomy. Int J Gynecol Obstet 1999;67:193–9.
[124] Madalinska JB, Hollenstein J, Bleiker E, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. J Clin Oncol 2005;23(28):6890–8.
[125] Colditz GA, Willet WC, Stampfer MJ, et al. Menopause and the risk of coronary heart disease in women. N Engl J Med 1987;316(18):1105–10.
[126] National Institutes of Health State-of-the-Science Conference Statement: management of menopause-related symptoms. Ann Intern Med 2005;142(12):1003–13.
[127] Madalinska JB, van Beurden M, Bleiker EM, et al. The impact of hormone replacement therapy on menopausal symptoms in younger high-risk women after prophylactic salpingo-oophorectomy. J Clin Oncol 2006;24(22):3576–82.
[128] Rebbeck TR, Friebel T, Wagner T, et al. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol 2005;23(31):7772–4.
[129] Schmeler KM, Sun CC, Bodurka DC, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. Obstet Gynecol 2006; 108(3 Pt 1):515–20.
[130] Schwartz MD, Kaufman E, Peshkin BN, et al. Bilateral prophylactic oophorectomy and ovarian cancer screening following BRCA1/BRCA2 mutation testing. J Clin Oncol 2003; 21(21):4034–41.
[131] Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999;91(17):1459–67.
[132] Ness RB, Grisso JA, Vergona R, et al. Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer. Epidemiology 2001;12(3):307–12.
[133] Ness RB, Grisso JA, Cottreau C, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology 2000;11(2):111–7.
[134] Daly M, Obrams GI. Epidemiology and risk assessment for ovarian cancer. Semin Oncol 1998;25(3):255–64.
[135] Hankinson SE, Hunter DJ, Colditz GA, et al. Tubal ligation, hysterectomy, and risk of ovarian cancer. JAMA 1993;270(23):2813–8.
[136] Narod SA, Sun P, Ghadirian P, et al. Tubal ligation and risk of ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. Lancet 2001;357(9267):1467–70.
[137] Society of Gynecologic Oncologists Clinical Practice Committee. Statement on prophylactic salpingo-oophorectomy. Gynecol Oncol 2005;98:179–81.
[138] U.S. Preventative Services Task Force. Screening for ovarian cancer: recommendation statement. Ann Fam Med 2004;2(3):260–2.
[139] ACOG Committee on gynecologic practice, in association with the Society of Gynecologic Oncology. The role of the generalist obstetrician-gynecologist in the early detection of ovarian cancer. Gynecol Oncol 2002;87(3):237–9.
[140] Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214–8.
[141] National Comprehensive Cancer Network (NCCN) Guidelines for hereditary breast and/or ovarian cancer. Available at: www.nccn.com/physician_gls/index.html. Accessed October 30, 2007.
[142] Scheuer L, Kauff N, Robson M, et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. J Clin Oncol 2002;20(5):1260–8.

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Ovarian Cancer Hormonal and Environmental Risk Effect

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Ovarian cancer is the deadliest gynecologic cancer, claiming more lives than all other gynecologic cancers. An estimated 22,430 new cases are expected in the United States in 2007, with an estimated 15,280 deaths [1]. The incidence of ovarian cancer is highest in Westernized industrialized countries, particularly in Europe, Canada, and North America [2]. The epithelial subtype of ovarian cancer accounts for 90% of all ovarian cancer deaths [3]. Much research has been devoted to investigating the relationship between the risk of epithelial ovarian cancer and the use of oral contraceptives in premenopausal women, as well as the risk associated with hormone replacement therapy (HRT) use in postmenopausal women.

To understand the influence hormonal and environmental factors have on the risk of ovarian cancer, it is important to remember the established risk factors and postulated mechanisms that lead to the development of ovarian cancer.

Several risk factors have been identified as increasing the risk of epithelial ovarian cancer, including low parity, infertility, early age of menarche, and late age of menopause [3]. Postulated mechanisms in the etiology of epithelial ovarian cancer relate to the known risk factors and include

1. The incessant ovulation theory, whereby, with repeated damage and trauma to the ovarian epithelium during each ovulatory cycle, there is an increased potential for genetic mutation and ovarian neoplasm during the repair process [4]
2. The pituitary gonadotropin hypothesis, which postulates that high levels of gonadotropins increase stimulation of estrogen, which can cause ovarian epithelial cells to become entrapped in inclusion cysts and undergo malignant change [2,5]

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3. The androgen/progesterone hypothesis, which suggests that androgens may stimulate ovarian cancer formation, whereas progestins are protective [2,5,6]
4. The inflammation hypothesis, which proposes that factors that predispose to inflammation, such as endometriosis, pelvic inflammatory disease, perineal talc use, and hyperthyroidism, may stimulate ovarian cancer formation [2,7]
5. The ovarian stromal hypothesis, which states that there may be a failure of apoptosis of granulosa and theca cells after ovulation; these cells continue to produce steroid hormones, thereby stimulating the formation of cancer [2,8]

This article looks further into the different hypotheses and focuses on hormonal and environmental risk factors, as well the chemoprevention of epithelial ovarian cancer.

General risk factors

Many identified risk factors for ovarian cancer are associated with menstrual and reproductive factors, such as early age of menarche and late age of menopause. The increased risk of developing epithelial ovarian cancer from a reproductive standpoint has been well studied. The highest risk of ovarian cancer has been seen in nulliparous women, whereas multiparous women have a decreased risk. Nulliparous women tend to have more ovulatory cycles than multiparous women and, as theorized by the incessant ovulation theory, are more likely to have potential damage to the ovarian surface epithelium [7,9,10]. It has been shown that with each full ovulation year a woman experiences, there is a 6% increase in risk of ovarian cancer. This finding is especially true in the 20- to 29-year age group, in which the risk is highest, with a 20% increase in risk with each ovulatory year a woman experiences [11]. These data suggest that suppression of ovulation might decrease the risk of ovarian cancer, although the exact mechanism of ovarian carcinogenesis is still unclear.

Infertility also has been suggested as a risk factor for ovarian cancer, but the treatment for infertility and its role in carcinogenesis remains controversial [12]. Many older studies have found increased risks of ovarian cancer in women experiencing infertility, even when ovulation-induction agents were not used [13]. Some theories as to the increased risk for the development of ovarian cancer in infertile patients hypothesize that high circulating levels of gonadotropins may stimulate DNA synthesis and proliferation of ovarian cancer cell lines, perhaps by increasing protein kinase C activity within the tumors [14,15]. Another theory considers the role of growth factors such as insulin-like growth factor (IGF)-1, transforming growth factor,

and tumor necrosis factor. IGF concentrations are elevated in anovulatory patients, and this factor has been shown to stimulate tumor progression [12,16]. The findings on ovarian cancer risk and its association with fertility drug treatment are not definitive, but studies have not shown a direct correlation [17]. The theory explaining the relationship between ovulation-induction agents and their possible risk in increasing ovarian cancer is based on the incessant ovulation hypothesis: that increasing ovulation by methods of infertility treatment can cause numerous oocytes to maturate and ovulate during one cycle. This increased ovulation can increase the mechanical trauma on the ovary, making way for genetic mutation and cellular neoplasia [12]. The data on whether infertility treatment conveys an elevated risk of ovarian cancer are still unclear, but on balance infertility treatment seems not to be associated with risk of ovarian cancer.

Numerous studies link endometriosis, a well-established cause of infertility, with an increased risk of ovarian cancer, especially clear cell and endometrioid subtypes (39.2% and 21.2%, respectively) [18,19]. Ness [18] theorizes that endometriotic implants grow through a mechanism of positive reinforcement when the levels of local estradiol and inflammatory mediators are increased, thus promoting angiogenesis, extracellular disintegration, cell proliferation, and abnormal apoptosis. This process may lead to growth and invasion, promoting the formation of ovarian cancer. Endometriosis as a cause of infertility may be another possible explanation for the increased risk of ovarian cancer seen in infertile patients [17].

Hereditary risk factors are the most strongly identified and account for approximately 5% to 10% of all ovarian malignancies [3]. Most of these mutations are in the *BRCA1* and *BRCA2* genes. The risk of ovarian cancer is approximately 40% by age 70 years in carriers of the *BRCA1* mutation and is approximately 10% in carriers of the *BRCA2* mutation [20]. Women who have hereditary non-polyposis colorectal cancer syndrome have about a 12% lifetime risk of developing ovarian cancer [21]. Women who have a genetic predisposition to ovarian cancer should be identified early, if possible, so that preventive measures can be implemented to decrease their risk of developing cancer. Extensive information on these hereditary syndromes is available in subsequent articles in this issue.

Sexual preference also may be a risk factor. A study examining the distribution of risk factors for the development of ovarian cancer in lesbian and heterosexual women found that lesbian women may have a greater risk of developing ovarian cancer than heterosexual women. The risk may be increased, in part, because a large proportion of lesbian women lack protective factors such pregnancy, breast feeding, miscarriages, abortions, and use of oral contraceptives [22]. This finding is clinically important because it suggests that health care providers need to be comfortable asking patients about their sexual orientation. It is essential that women in these high-risk groups be identified so they can be offered preventive measures to decrease their risk of developing ovarian cancer.

General protective factors

Several well-studied protective factors, often in direct contrast to known risk factors, have been described. Women who have been pregnant at least once have an overall decreased risk of ovarian cancer; multiparity has been observed, time and again, to be a strong protective factor. This protective effect increases with an increasing number of pregnancies, accounting for a reduction of about 12% with each additional birth [23,24]. Four or more births provide a 40% reduction in risk. Similarly, there is an inverse relationship seen between the risk of ovarian cancer and the number of abortions and with multiple births such as with twins and triplets [25,26]. Theories examining the protective effect of pregnancy are based mainly on suppression of ovulation, but the tenfold increase of elevated progesterone levels during the 8 to 9 months of gestation also may confer protection [6].

Progesterone has been implicated as a very important protective factor against ovarian cancer. Loss of the progesterone receptor is seen frequently in ovarian cancer, implicating the progesterone receptor gene as a possible tumor suppressor gene [27]. In addition, the high levels of progesterone that are seen during pregnancy or during use of oral contraceptives have been theorized to induce cell-cycle arrest or cause apoptosis in ovarian surface epithelium cells [5].

Age at last birth has been strongly associated with a reduced risk. Women with a last birth after age 30 to 35 years have a 58% decreased risk of ovarian cancer compared with nulliparous women, and this reduction was independent of total parity and use of hormonal contraceptives, with a specific decreased risk of endometrioid and clear cell tumors [28,29]. One theory to explain the protective effect of later age at last pregnancy on ovarian cancer, the exfoliate theory, is based on the suspicion that older women are more likely than younger women to have accumulated transformed surface epithelial ovarian cells, and progestins that are present during pregnancy may induce apoptosis of these cells. The elimination of the increased number of transformed cells might reduce the risk of tumor formation later in life in women with later age of parity and last birth [27,29].

Tubal ligation also has been documented to decrease the risk of developing epithelial ovarian cancer, especially endometrioid tumors [30]. Tubal ligation has been theorized to decrease this risk by reducing utero-ovarian blood flow and altering local hormonal and growth factor levels. It also might interrupt the upward migration of inflammatory factors and carcinogens so they never reach the ovaries [31,32]. Not surprising, hysterectomy also has been shown to reduce this risk. Hysterectomy also may confer its protective effect by altering ovarian blood flow and possibly by providing a better opportunity for examining the ovaries [33]. Breastfeeding lowers the risk of ovarian cancer, especially of the nonmucinous subtypes [30,34]. The protective effect of breastfeeding has been shown to correlate with duration, because a significant decrease in risk is seen in women who had

breastfed for 18 or more months. Lactation probably reduces the risk of ovarian cancer by suppressing ovulation and by decreasing gonadotropin levels [34]. In counseling women who are considering lactation, the potential for its protective effect on the ovaries should be mentioned [2].

Environmental risk factors

A number of environmental risk factors have been shown to increase the risk of ovarian cancer. The use of talcum powder, perhaps the first risk factor reported, has been shown in previous studies to increase the risk of ovarian tumors, especially serous tumors. Talc is structurally similar to asbestos, and studies have suggested that there are histologic similarities between serous adenocarcinomas and the mesotheliomas seen in asbestos exposure. These facts may explain findings of increased risk of serous tumors in talc powder users [35]. Animal studies have demonstrated that talc migrates from the vagina through the peritoneal cavity to the ovaries. Talc then may stimulate the entrapment of the ovarian surface epithelium, causing a reaction similar to the reaction that occurs during ovulation, thus increasing the risk in a similar way to the incessant ovulation theory. It also has been suggested that talc may stimulate the formation of granulomas, which are associated with persistent acute inflammatory responses [35,36]. Despite these reports, other studies have failed to demonstrate a significantly increased risk for the development of ovarian cancer even with prolonged talcum powder use [37,38].

Cigarette smoking may be a risk factor for ovarian cancer, although its role is controversial, because a few studies have failed to find a significant correlation between smoking and ovarian cancer [39]. Studies that have shown smoking to increase the risk point to an increased risk of mucinous tumors [23,40]. In one study smoking doubled the risk of developing mucinous ovarian cancer, but smoking cessation reversed the risk to that of never having smoked within 20 to 30 years of smoking cessation [41]. This finding suggests that smoking may be a modifiable risk factor that can be used in the primary prevention of ovarian cancer.

The data on obesity and ovarian cancer are inconclusive [2]. A recent systematic review of the literature on obesity and ovarian cancer did find a positive association between obesity (defined as a body mass index over 30) and ovarian cancer, with a small increased risk for those who were overweight. There was no evidence in this study that obesity/overweight increased the risk of any specific subtype of ovarian cancer [42]. Other studies, however, that have examined the histologic subtypes that are increased in obesity have noted an increase in benign serous tumors and tumors of the endometrioid subtype [43,44]. Both recent obesity and obesity in young adulthood have been associated with increased risk of benign serous ovarian tumors [43]. The increased risk of ovarian cancer may result from the changes in

synthesis and bioavailability of endogenous sex steroids seen in obese women, changes that are believed to be involved in the etiology of ovarian cancer [6].

Studies also have shown that physical activity protects against ovarian cancer, independent of body mass index [45]. Also, increased height (taller than 165 cm) correlates positively with a risk of ovarian cancer [46]. Theories behind this finding have suggested that genetic factors, calorie restriction in early life, and increased exposure to sex and growth hormones may somehow play a role.

Dietary factors may play a role in ovarian carcinogenesis, but there is much conflicting evidence. Studies evaluating the intake of red meat have shown an increased risk of ovarian cancer with an odds ration of 1.53 for highest versus lowest quintile of intake [47]. A study examining the dietary intake in Japan after World War II showed that the age-standardized death rate for ovarian cancer increased fourfold, and it was hypothesized that this increase might be caused by changing lifestyles after 1945 as the Japanese moved toward a more Westernized diet with increased consumption of milk, meat, and eggs [48]. This hypothesis may be reasonable, because foods high in fat and starch have been associated with increased risk of ovarian cancer, whereas foods high in fiber, carotene, and vitamins seem to be protective [49,50].

The data on the influence of alcohol intake on the risk of ovarian cancer have been inconsistent, with several studies showing no association between risk of ovarian cancer and total alcohol intake [51,52]. One study demonstrated that current heavy consumption of alcohol (24 g or more per day) might be a risk factor for mucinous epithelial ovarian cancer [53]. Caffeine intake has been studied also, and its effects on the risk of ovarian cancer also are inconsistent. One study by Kuper and colleagues [54] demonstrated that coffee and caffeine intake might increase the risk for ovarian cancer among premenopausal women. The suggested mechanism is that caffeine may interfere with the repair of damaged DNA or may raise intracellular levels of cyclic AMP and mimic the effect of gonadotropins. Other studies, however, have demonstrated no association between caffeine and risk of ovarian cancer [55]. One study actually showed a risk reduction, concluding that consumption of four or more cups of coffee per day was associated with a decreased risk of invasive epithelial ovarian cancer that was significant only for serous, endometrioid, and clear cell histologic subtypes [56].

The effect of food and nutrition intake is controversial. Dietary intake of vitamins A, C, D, and E has, for the most part, shown some reduced risk of ovarian cancer [57–59]. Fruits and vegetables also have been shown to reduce the risk of ovarian cancer [45,57], but some recent studies have cast doubt on this finding, concluding that fruit and vegetable consumption has no important association with the risk of ovarian cancer [60,61]. The consumption of tea, specifically green and black tea, has been shown to reduce the risk of epithelial ovarian cancer in a dose–response manner. Each

additional cup of tea consumed per day was associated with an 18% reduction in risk of ovarian cancer [62]. Some suggested mechanisms for the protective effect of green tea include antioxidant activity, changes in cell signaling pathways, induction of apoptosis, and the possibility of the modulation of endogenous hormones [45]. The data on dietary intake seem to be inconclusive for the most part, and so the overall risks and benefits of consumption of these foods should be taken into account.

Hormonal replacement therapy and risk of ovarian cancer

HRT use has been on the decline since the publication of the Women's Health Initiative (WHI) study showing an increased risk of breast cancer, stroke and cardiovascular diseases in the estrogen-plus-HRT arm of the trial [63,64]. The WHI randomized trial examining the effects of estrogen plus progestin on gynecologic cancers also found an increased risk of invasive ovarian cancer with a hazard ratio of 1.58 [65]. The Norwegian Women and Cancer study found an increased risk of breast cancer with HRT but found no increased risk of ovarian cancer [66]. Numerous other studies, however, have had similar findings to the WHI, also suggesting an increased risk of epithelial ovarian cancer with long-term use of HRT [67,68]. The recent Million Women Study from the United Kingdom examining the risk of ovarian cancer associated with the use of HRT found that women who currently use HRT are at an increased risk for incident and fatal ovarian cancer, with relative risks of 1.20 and 1.23, respectively, associated with HRT use for at least 5 years [69]. The Nurses' Health Study found that use of unopposed estrogen for longer than 5 years did indeed increase the risk of developing epithelial ovarian cancer, especially serous cancer, but the addition of progestin to estrogen was not associated with an increased risk, a finding in contrast to the WHI [69,70]. The time association between the duration of use of HRT and risk of development of ovarian cancer seems to be between 5 and 10 years and may last up to 29 years after HRT use has stopped [69,71,72]. The study also suggested that the risk was reduced to that of never-users once use of HRT ceased. Past users of HRT were not found to be at an increased risk for this malignancy [69,70].

Oral contraception

Oral contraception seems to have profound benefits well beyond contraception. Numerous studies now prove that oral contraceptives have significant protective effects against epithelial ovarian cancer [73–75]. Women using oral contraceptives had a risk reduction of at least 30% to 40%, with increasing risk reduction with longer duration of use. Women using oral contraceptives for more than 5 years were found to have a stronger reduction than those who used it for less than 5 years, although a significant

protective effect was seen after only 1 year of use [76]. This protective effect continued 15 to 20 years after oral contraception use ceased and is independent of any specific type of oral contraceptive formulation [74,76]. Researchers have looked at various oral contraceptive preparations of estrogen and progestins and the difference in risk of ovarian cancer with low- and high-dose formulations. One study reported that the use of low-dose formulations afforded the same risk reduction as high-dose formulations [77]. A more recent study has examined the association of oral contraceptive potency with the risk of epithelial ovarian cancer and found that users of the low-potency formulations had the highest risk reduction. Another study that examined the estrogen and progestin potency in oral contraceptives and their risk on ovarian cancer, however, found that the formulations with high-progestin potency seem to be associated with a greater reduction in ovarian cancer risk than those with low-progestin potency [75,78]. The low-potency regimen used was 0.035 mg ethinyl estradiol with 0.3 mg megestrol. Use of norethindrone (0.5 mg or less) by itself also carried a reduced risk of ovarian cancer, compared with the 10-mg formulation, that was independent of any specific epithelial ovarian histologic subtype [73].

Mechanisms of risk reduction may involve the inhibition of ovulation or the strong role that progesterone plays in the induction of apoptosis of ovarian surface epithelium cells [5]. Nonetheless, the protective effect of oral contraception was consistent across all factors of parity, menopausal status, age, and hereditary factors [76].

Women who have endometriosis have been found to be at increased risk for ovarian cancer. Oral contraceptives are the primary treatment for endometriosis, and the long-term use of oral contraceptive (for more than 10 years) has been found to offer protection against ovarian cancer in this high-risk population [79]. In the same study found multiparity and having a tubal ligation or hysterectomy to be associated with a similar risk reduction in women who had endometriosis. Women who have a family history of ovarian cancer also have been found to realize benefits in risk reduction from oral contraceptive use. A study looking at high-risk women found that the long-term use of oral contraceptives (about 4–8 years) may reduce the risk of ovarian cancer substantially, from approximately 4 women per 100 in women who did not use oral contraceptives to only 2 women per 100 in women who did use oral contraceptives [80].

HRT is used by postmenopausal women and may increase the risk of ovarian cancer, whereas oral contraceptives seem to protect young women from developing ovarian cancer. Theories behind these findings suggest that estrogen may inhibit early events in ovarian cancer, possibly through inhibition of incessant ovulation, but in postmenopausal women estrogen may accelerate the growth of an already existing tumor. These differences may also result from the different types of compounds and doses used [81]. In considering all the recent data on HRT and the increased risk of

breast and ovarian cancer with the use of combined estrogen and progestin, the benefits and risks of HRT should be weighed carefully, as should the duration of treatment. In contrast, oral contraceptives clearly have a profound role in reducing the risk of ovarian cancer, and clinicians should be mindful of this fact in their younger female patients.

Chemoprevention of ovarian cancer

Prevention of ovarian cancer is the ultimate goal. Cancer chemoprevention is the administration of chemical agents to prevent or delay the development of cancer in healthy people [82]. Aside from lifestyle factors and their impact on the risk of epithelial ovarian cancer, chemopreventive agents have been studied in preventing the progression of precancerous epithelial cells to overt cancer. Oral contraceptives seem to have the greatest impact on decreasing the risk of developing epithelial ovarian cancer, as reported in numerous studies [73–76]. Other agents are now being studied as well. N-(4-hydroxyphenyl) retinamide (4-HPR) and fenretinide, a vitamin A analogue, have been correlated with a lower incidence of ovarian carcinoma, with a possible protective effect in women who carry the *BRCA* mutation [83,84]. Retinoids can affect human ovarian cancer cell growth by inhibiting proliferation and inducing apoptosis, in part by increasing mitochondrial permeability [82]. 4-HPR also increases the expression of *p53*, which is another mechanism thought to be important in cancer prevention. This protective effect seems to disappear after stopping treatment, however [84]. The combination of 4-HPR and oral contraceptives has been shown to have a greater effect on the expression of retinoid receptors in ovarian biopsies from Rhesus macaques than the use of either agent alone [85].

Studies examining analgesic drug use have found an inverse relationship with risk of ovarian cancer [86]. More recent studies have shown a decrease in risk with acetaminophen use but not with aspirin use [87]. Acetaminophen use seems to have a dose effect, with regular use associated with a 30% risk reduction compared with non-use [88]. One proposed theory is that acetaminophen may have an antigonadotropic effect [89]. Other theories on its mechanism of action suggest that it may induce specific reproductive atrophy, that it may cause reduction of glutathione pools that play an important role in sterilizing premalignant ovarian lesions, or that it may inhibit the activity of macrophage migration inhibitory factor that is necessary for ovulation [88]. The risks of liver and chronic renal failure with long-term acetaminophen use may make this recommendation impractical for widespread use in a low-risk population, however [88].

Cactus pear also has been shown to serve potentially as a chemopreventive agent. Cactus pear contains pectin, carotenes, betalains, ascorbic acid, quercetina, and quercetin derivatives, all of which have antioxidant activity. In one study, cells exposed to cactus pear extracts had an increase in

apoptosis and growth inhibition, and tumor growth in nude mice was suppressed significantly [90]. The use of cactus pear in the chemoprevention of cancer would be ideal, because it is a natural product and seems to be safe and well tolerated.

Studies on the potential use of vaccines aimed at antigens in the chemoprevention of ovarian cancer are currently underway. Breast and ovarian cancers express certain mucins that potentially could serve as targets in the use of vaccines against these cancers [91]. One particular mucin being studied is mucin 1 (MUC1). MUC1 is a member of the mucin family of glycoproteins, including CA-125, that can be overexpressed in ovarian cancer [92]. Anti-MUC1 antibodies may develop in ovarian cancers that overexpress MUC1 and have been correlated with a more favorable prognosis. Anti-MUC1 immunity that is generated by other mechanisms, such as tubal sterilization, breast mastitis, and oral contraceptive use, also may lower the risk of MUC1-related ovarian cancers by inducing formation of anti-MUC1 antibodies through immune recognition by inflammatory or hormonal processes [92]. As seen in previous studies, early age at first birth, longer menstrual cycle duration ($\geq$ 30 days), and oral contraceptive use has been shown to decrease the risk of ovarian cancer with a mechanism of action explained primarily by the incessant ovulation theory. A newly proposed mechanism for this decreased risk links the incessant ovulation theory to the formation of anti-MUC1 antibodies, proposing that incessant ovulation is associated with a lower likelihood of anti-MUC1 antibodies and an increased risk of ovarian cancer [92]. The potential of using MUC1 as a target antigen in the creation of a vaccine against ovarian cancer and potentially breast and endometrial cancer would be most useful.

Summary

Epithelial ovarian cancer is a devastating disease whose exact pathogenesis is still unclear. Despite the several hypotheses regarding the etiology of ovarian cancer, more research needs to be done to address specifically the roles of hormones, inflammation, and immunology as potential causes. Of equal importance is the emphasis on the chemoprevention of ovarian cancer. The optimal strategy in chemoprevention available at this time seems to be the use of the oral contraceptives in premenopausal women. Data are conflicting as to a specific formulation that gives the greatest risk reduction. Therefore, it seems that patient tolerability to a particular formulation should be a desired goal, because these patients already have placed themselves in a lower risk category by deciding to take oral contraceptives. There is hope that in the near future there will be a role for other chemopreventive agents, such as a simple vaccine. At present, the benefit of decreasing the risk of epithelial ovarian cancer with the use of oral contraception should be discussed when counseling women about birth control options.

A thorough discussion on the risks and benefits of prolonged HRT use in postmenopausal women should take place between the clinician and patient. The patient should be informed of her potential increased risks of ovarian cancer, breast cancer, stroke, and cardiovascular disease and should decide whether the benefits outweigh the potential risks.

References

[1] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007;57(1):43–66.
[2] Hanna L, Adams M. Prevention of ovarian cancer. Best Pract Res Clin Obstet Gynaecol 2006;20(2):339–62, Epub 2005 Dec 20.
[3] Berek JS, Hacker NF. Practical gynecologic oncology. 4th edition. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 444.
[4] Fathalla MF. Incessant ovulation—a factor in ovarian neoplasia? Lancet 1971;2:163.
[5] Zheng H, Kavanagh JJ, Hu W, et al. Hormonal therapy in ovarian cancer. Int J Gynecol Cancer 2007;17(2):325–38 [review].
[6] Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. J Natl Cancer Inst 1998;90:1774–86.
[7] Ness RB, Grisso JA, Cottreau C, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology 2000;11:111.
[8] Cramer DW, Barbieri RL, Fraer AR, et al. Determinants of early follicular phase gonadotrophin and estradiol concentrations in women of late reproductive age. Hum Reprod 2002; 17(1):221–7.
[9] Kvale G, Heuch I, Nilssen S, et al. Parity, age at first childbirth, and risk of ovarian cancer: a prospective study. Int J Cancer 1988;42:246–51.
[10] Titus-Ernstoff L, Perez K, Cramer DW, et al. Menstrual and reproductive factors in relation to ovarian cancer risk. Br J Cancer 2001;84:714.
[11] Purdie DM, Bain CJ, Siskind V, et al. Ovulation and risk of epithelial ovarian cancer. Int J Cancer 2003;104:228–32.
[12] Meirow D, Schenker JG. The link between female infertility and cancer: epidemiology and possible aetiologies. Hum Reprod Update 1996;2(1):63–75.
[13] Rossing MA, Daling JR, Weiss NS, et al. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994;331:771–6.
[14] Stadel BV. The etiology and prevention of ovarian cancer. Am J Obstet Gynecol 1975;123: 772–4.
[15] Simon WE, Holzel F. Hormone sensitivity of gynecological tumor cells in tissue cultures. J Cancer Res Clin Oncol 1979;94:307–23.
[16] Conover CA, Lee PDK, Kanaley JA, et al. Insulin regulation of insulin-like growth factor binding protein-1 in obese and non obese humans. J Clin Endocrinol Metab 1992;74:1355–9.
[17] Mahdavi A, Pejovic T, Nezhat F. Induction of ovulation and ovarian cancer: a critical review of the literature. Fertil Steril 2006;85(4):819–26.
[18] Ness RB. Endometriosis and ovarian cancer: thoughts on shared pathophysiology. Am J Obstet Gynecol 2003;189(1):280–94.
[19] Yoshikawa H, Jimbo H, Okada S, et al. Prevalence of endometriosis in ovarian cancer. Gynecol Obstet Invest 2000;50(Suppl 1):11–7.
[20] Lakhani SR, Manek S, Penault-Llorca F, et al. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. Clin Cancer Res 2004;10(7):2473–81.
[21] Lu KH, Goodman MT, Wu AH, et al. Gynaecologic cancer as a 'sentinal cancer' for women with hereditary nonpolyposis colorectal cancer syndrome. Obstet Gynecol 2005;105:569–74.
[22] Dibble SL, Roberts SA, Robertson PA, et al. Risk factors for ovarian cancer: lesbian and heterosexual women. Oncol Nurs Forum 2002;29(1):E1–7.

[23] Soegaard M, Jensen A, Hogdall E, et al. Different risk factor profiles for mucinous and non-mucinous ovarian cancer: results from the Danish MALOVA study. Cancer Epidemiol Biomarkers Prev 2007;16(6):1160–6.
[24] Modan B, Hartge P, Hirsh-Yechezkel G, et al. Parity, oral contraceptive, and the risk of ovarian cancer among carriers and noncarriers of a BRCA 1 or BRCA 2 mutation. N Engl J Med 2001;345:235–40.
[25] Negri E, Franceschi S, Tzonou A, et al. Pooled analysis of 3 European case-control studies: reproductive factors and risk of epithelial ovarian cancer. Int J Cancer 1991;49(1):50–6.
[26] Whiteman DC, Murphy MF, Cook LS, et al. Multiple births and risk of epithelial ovarian cancer. J Natl Cancer Inst 2000;92(14):1172–7.
[27] Ho SM. Estrogen, progesterone and epithelial ovarian cancer. Reprod Biol Endocrinol 2003; 1:73.
[28] Pike MC, Pearce CL, Peters R, et al. Hormonal factors and the risk of invasive ovarian cancer: a population-based case-control study. Fertil Steril 2004;82:186.
[29] Whiteman DC, Siskind V, Purdie DM, et al. Timing of pregnancy and the risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2003;12:42.
[30] Tung KH, Goodman MT, Wu AH, et al. Reproductive factors and epithelial ovarian cancer risk by histologic type: a multiethnic case-control study. Am J Epidemiol 2003;158(7): 629–38.
[31] McGuire V, Felberg A, Mills M, et al. Relation of contraceptive and reproductive history to ovarian cancer risk in carriers and noncarriers of BRCA1 gene mutations. Am J Epidemiol 2004;160(7):613–8.
[32] Ness RB, Grisso JA, Vergona R, et al. Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer. Epidemiology 2001;12(3):307–12.
[33] Parazzini F, Negri E, La Vecchia C, et al. Hysterectomy, oophorectomy, and subsequent ovarian cancer risk. Obstet Gynecol 1993;81(3):363–6.
[34] Danforth KN, Tworoger SS, Hecht JL, et al. Breastfeeding and risk of ovarian cancer in two prospective cohorts. Cancer Causes Control 2007;18(5):517–23, Epub 2007 Apr 21.
[35] Mills PK, Riordan DG, Cress RD, et al. Perineal talc exposure and epithelial ovarian cancer risk in the Central Valley of California. Int J Cancer 2004;112(3):458–64.
[36] Cramer DW, Liberman RF, Titus-Ernstoff L, et al. Genital talc exposure and risk of ovarian cancer. Int J Cancer 1999;81(3):351–6.
[37] Wong C, Hempling RE, Piver MS, et al. Perineal talc exposure and subsequent epithelial ovarian cancer: a case control study. Obstet Gynecol 1999;94(1):160–1.
[38] Gertig DM, Hunter DJ, Cramer DW, et al. Prospective study of talc use and ovarian cancer. J Natl Cancer Inst 2000;92(3):249–52.
[39] Riman T, Dickman PW, Nilsson S, et al. Some life-style factors and the risk of invasive epithelial ovarian cancer in Swedish women. Eur J Epidemiol 2004;19:1011–9.
[40] Marchbanks PA, Wilson H, Bastos E, et al. Cigarette smoking and epithelial ovarian cancer by histologic type. Obstet Gynecol 2000;95(2):255–60.
[41] Jordan SJ, Whiteman DC, Purdie DM, et al. Does smoking increase risk of ovarian cancer? A systematic review. Gynecol Oncol 2006;103(3):1122–9.
[42] Olsen CM, Green AC, Whiteman DC, et al. Obesity and the risk of epithelial ovarian cancer: a systematic review and meta-analysis. Eur J Cancer 2007;43(4):690–709.
[43] Jordan SJ, Green AD, Whiteman DC, et al. Risk factors for benign serous and mucinous epithelial ovarian tumors. Obstet Gynecol 2007;109(3):647–54.
[44] Szamborski J, Czerwinski W, Gadomska H, et al. Case control study of high-risk factors in ovarian carcinomas. Gynecol Oncol 1981;11(1):8–16.
[45] Rieck G, Fiander A. The effect of lifestyle factors on gynaecological cancer. Best Pract Res Clin Obstet Gynaecol 2006;20(2):227–51.
[46] Schouten LJ, Goldbohm RA, van den Brandt PA. Height, weight, weight change, and ovarian cancer risk in the Netherlands cohort study on diet and cancer. Am J Epidemiol 2003;157: 424–33.

[47] Bosetti C, Negri E, Franceschi S, et al. Diet and ovarian cancer risk: a case-control study in Italy. Int J Cancer 2001;93:911–5.
[48] Li XM, Ganmaa D, Sato A. The experience of Japan as a clue to the etiology of breast and ovarian cancers: relationship between death from both malignancies and dietary practices. Med Hypotheses 2003;60:268–75.
[49] Zhang M, Lee AH, Binns CW. Reproductive and dietary risk factors for epithelial ovarian cancer in China. Gynecol Oncol 2004;92:320–6.
[50] Bidoli E, La Vecchia C, Montella M, et al. Nutrient intake and ovarian cancer: an Italian case-control study. Cancer Causes Control 2002;13:255–61.
[51] Chang ET, Canchola AJ, Lee VS, et al. Wine and other alcohol consumption and risk of ovarian cancer in the California Teachers Study cohort. Cancer Causes Control 2007; 18(1):91–103.
[52] Peterson NB, Trentham-Dietz A, Newcomb PA. Alcohol consumption and ovarian cancer risk in a population-based case-control study. Int J Cancer 2006;119(10):2423–7.
[53] Modugno F, Ness RB, Allen GO. Alcohol consumption and the risk of mucinous and non-mucinous epithelial ovarian cancer. Obstet Gynecol 2003;102(6):1336–43.
[54] Kuper H, Titus-Ernstoff L, Harlow BL. Population based study of coffee, alcohol and tobacco use and risk of ovarian cancer. Int J Cancer 2000;88(2):313–8.
[55] Larsson SC, Wolk A. Coffee consumption is not associated with ovarian cancer incidence. Cancer Epidemiol Biomarkers Prev 2005;14(9):2273–4.
[56] Jordan SJ, Purdie DM, Green AC, et al. Coffee, tea, and caffeine and risk of epithelial ovarian cancer. Cancer Causes and Control 2004;15:359–65.
[57] McCann SE, Moysich KB, Mettlin C. Intakes of selected nutrients and food groups and risk of ovarian cancer. Nutr Cancer 2001;39:19–28.
[58] Fleischauer AT, Olson SH, Mignone L, et al. Dietary antioxidants, supplements, and risk of epithelial ovarian cancer. Nutr Cancer 2001;40:92–8.
[59] Salazar-Martinez E, Lazcano-Ponce EC, Gonzalez Lira-Lira G, et al. Nutritional determinants of epithelial ovarian cancer risk: a case-control study in Mexico. Oncology 2002;63:151–7.
[60] Mommers M, Schonten LJ, Goldbohm RA, et al. Consumption of vegetables and fruits and risk of ovarian cancer. Cancer 2005;104(7):1512–9.
[61] Koushik A, Hunter DJ, Spiegelman D, et al. Fruits and vegetables and ovarian cancer risk in a pooled analysis of 12 cohort studies. Cancer Epidemiol Biomarkers Prev 2005;14(9): 2160–7.
[62] Larsson SC, Wolk A. Tea consumption and ovarian cancer risk in a population-based cohort. Arch Intern Med 2005;165(22):2683–6.
[63] Guay MP, Dragomir A, Pilon D, et al. Changes in pattern of use, clinical characteristics and persistence rate of hormone replacement therapy among postmenopausal women after the WHI publication. Pharmacoepidemiol Drug Saf 2007;16(1):17–27.
[64] Nelson HD, Humphrey LL, Nygren P, et al. Postmenopausal hormone replacement therapy: scientific review. JAMA 2002;288(7):872–81.
[65] Anderson GL, Judd HL, Kaunitz AM, et al. Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. JAMA 2003;290(13):1739–48.
[66] Bakken K, Alsaker E, Eggen AE, et al. Hormone replacement therapy and incidence of hormone-dependent cancers in the Norwegian Women and Cancer study. Int J Cancer 2004; 112(1):130–4.
[67] Mills PK, Riordan DG, Cress RD. Hormone replacement therapy and invasive and borderline epithelial ovarian cancer risk. Cancer Detect Prev 2005;29(2):124–32.
[68] Negri E, Tzonou A, Beral V, et al. Hormonal therapy for menopause and ovarian cancer in a collaborative re-analysis of European studies. Int J Cancer 1999;80(6):848–51.
[69] Beral V, Bull D, Green J, et al, Million Women Study Collaborators. Ovarian cancer and hormone replacement therapy in the Million Women Study. Lancet 2007;369(9574): 1703–10.

[70] Danforth KD, Tworoger SS, Hecht JL, et al. A prospective study of postmenopausal hormone use and ovarian cancer risk. Br J Cancer 2007;96(1):151–6.
[71] Lacey JV, Brinton LA, Leitzmann MF, et al. Menopausal hormone therapy and ovarian cancer risk in the National Institutes of Health-AARP diet and health study cohort. J Natl Cancer Inst 2006;98(19):1397–405.
[72] Rodriguez C, Patel AV, Calle EE, et al. Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. JAMA 2001;285(11):1460–5.
[73] The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. The reduction in risk of ovarian cancer associated with oral-contraceptive use. N Engl J Med 1987;316(11):650–5.
[74] La Vecchia C. Oral contraceptives and ovarian cancer: an update, 1998-2004. Eur J Cancer Prev 2006;15(2):117–24.
[75] Lurie G, Thompson P, McDuffie KE, et al. Association of estrogen and progestin potency of oral contraceptives with ovarian carcinoma risk. Obstet Gynecol 2007;109(3):597–607.
[76] Bosetti C, Negri E, Trichopoulos D, et al. Long-term effects of oral contraceptives on ovarian cancer risk. Int J Cancer 2002;102(3):262–5.
[77] Ness RB, Grisso JA, Klapper J. Risk of ovarian cancer in relation to estrogen and progestin dose and use characteristics of oral contraceptives. SHARE Study Group. Steroid Hormones and Reproductions. Am J Epidemiol 2000;152(3):233–41.
[78] Schildkraut JM, Calingaert B, Marchbanks PA, et al. Impact of progestin and estrogen potency in oral contraceptives on ovarian cancer risk. J Natl Cancer Inst 2002;94(1):32–8.
[79] Modugno F, Ness RB, Allen GO, et al. Oral contraceptive use, reproductive history, and risk of epithelial ovarian cancer in women with and without endometriosis. Am J Obstet Gynecol 2004;191(3):733–40.
[80] Walker GR, Schlesselman JJ, Ness RB. Family history of cancer, oral contraception use, and ovarian cancer risk. Am J Obstet Gynecol 2002;186(1):8–14.
[81] Narod SA. Ovarian cancer and HRT in the Million Women Study. Lancet 2007;369(9574): 1667–8.
[82] Brewer M, Wharton JT, Wang J, et al. In vitro model of normal, immortalized ovarian surface epithelial and ovarian cancer cells for chemoprevention of ovarian cancer. Gynecol Oncol 2005;98(2):182–92.
[83] De Palo G, Veronesi U, Camerini T, et al. Can fenretinide protect women against ovarian cancer? J Natl Cancer Inst 1995;87:146–7.
[84] De Palo G, Mariani L, Camerini T, et al. Effect of fenretinide on ovarian carcinoma occurrence. Gynecol Oncol 2002;86(1):24–7.
[85] Brewer M, Ranger-Moore J, Satterfield W, et al. Combination of 4-HPR and oral contraceptive in monkey model of chemoprevention of ovarian cancer. Front Biosci 2007;12:2260–8.
[86] Schildkraut JM, Moorman PG, Halabi S, et al. Analgesic drug use and risk of ovarian cancer. Epidemiology 2006;17(1):104–7.
[87] Moyisch KB, Mettlin C, Piver MS, et al. Regular use of analgesic drugs and ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10(8):903–6.
[88] Bonovas S, Filioussi K, Sitaras NM. Paracetamol use and risk of ovarian cancer: a meta-analysis. Br J Clin Pharmacol 2006;62(1):113–21.
[89] Cramer DW, Harlow BL, Titus-Ernstoff L, et al. Over-the-counter analgesics and risk of ovarian cancer. Lancet 1998;351:104–7.
[90] Zou DM, Brewer M, Garcia F, et al. Cactus pear: a natural product in cancer chemoprevention. Nutr J 2005;4:25.
[91] Bast RC, Brewer M, Zou C, et al. Prevention and early detection of ovarian cancer: mission impossible? Recent Results Cancer Res 2007;174:91–100 [review].
[92] Terry KL, Titus-Ernstoff L, McKolanis JR, et al. Incessant ovulation, mucin 1 immunity, and risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev 2007;16(1):30–5.

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Endometrial Cancer Associated with Defective DNA Mismatch Repair

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Defective DNA mismatch repair is one of the most common and best-characterized genetic defects detected in endometrial cancer, occurring in approximately 20% to 25% of all cases [1]. Defective DNA mismatch repair in endometrial cancer can be either inherited or acquired (sporadic). For women who have inherited defective DNA mismatch repair, known as "Lynch syndrome" or "hereditary nonpolyposis colorectal cancer" (HNPCC), the onset of endometrial cancer usually occurs at a younger age. This article describes the clinical and pathologic significance of acquired defective DNA mismatch repair and inherited defective DNA mismatch repair in endometrial cancer. Although there are fewer direct clinical implications for patients who have endometrial cancer and who have acquired defective DNA mismatch repair, there are significant clinical implications for patients who have Lynch syndrome, and there are a variety of opportunities for cancer prevention for those at-risk individuals. This article also discusses clinical recommendations for patients who have Lynch syndrome.

Identification of defective DNA mismatch repair

DNA mismatch-repair proteins fix mistakes that commonly occur during DNA replication. This system of DNA mismatch repair was described initially in prokaryotes and subsequently was found to be highly conserved across species. In humans a defective DNA mismatch-repair system was identified as the underlying cause of Lynch syndrome, an inherited cancer-susceptibility syndrome characterized by early-onset colon cancer and endometrial

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cancer. Before the identification of the involved genes, the diagnosis of Lynch syndrome was based on clinical criteria, the Amsterdam criteria (Box 1) [2]. The specific genes responsible for Lynch syndrome are *hMLH1, hMSH2, hMSH6*, or *hPMS2*. Germline mutations in *hMLH1* and *hMSH2* account for more than 90% of cases of Lynch syndrome. Individuals who have Lynch syndrome have inherited one allele of a mismatch-repair gene that is nonfunctional because of mutation. Subsequent somatic loss of function of the corresponding normal allele results in defective DNA mismatch repair. These genetic defects in the DNA mismatch-repair system result in microsatellite instability (MSI) and the absence of the respective protein expression in the tumor. This functional loss is manifested clinically by a substantially increased risk of colon and endometrial cancer and by increased risk of ovarian, small bowel, stomach, renal pelvis, and ureteral cancers as well.

In Lynch syndrome, the gene mutation is inherited in an autosomal dominant fashion, and each child has a 50% risk of inheriting the mutation. Not all individuals who have a germline Lynch syndrome mutation will have cancer, however (incomplete penetrance). Other unidentified genetic and environmental factors probably play a role and are under active investigation. Overall, Lynch syndrome accounts for less than 5% of all colon cancers and less than 5% of endometrial cancers. It is important, however, to identify this genetic mutation in patients diagnosed with cancer because they are at very high risk for developing second cancers. In addition, identification of the specific genetic defect in an individual who has a colon or endometrial cancer allows their relatives to undergo predictive genetic testing and opportunities for screening and prevention. Identifying Lynch syndrome in a woman who has endometrial cancer can have a significant impact on the long-term survival of the patient and her family.

Defects in the DNA mismatch-repair system also can be acquired or sporadic. In sporadic colon and endometrial adenocarcinomas, loss of *hMLH1* protein expression occurs because of the aberrant methylation of the *hMLH1* gene promoter, an epigenetic modification [3]. Such methylation is a common mechanism of reducing gene expression and is not hereditary.

Box 1. Amsterdam II criteria

The patient must meet all of the following criteria:

- Three of more relatives with a histologically verified HNPCC-associated cancer or cancer of the endometrium, colon, ovary, small bowel, ureter, or renal pelvis, one of whom is a first-degree relative of the other two; familial adenomatous polyposis should be excluded
- HNPCC-associated cancer involving at least two generations
- One or more HNPCC-associated cancer cases diagnosed before the age of 50 years

Molecular studies of human tumors can identify the presence of a mismatch-repair defect. In addition, these tools can help distinguish acquired mismatch repair (sporadic cancer) from inherited mismatch repair (Lynch syndrome). Microsatellite instability (MSI)analysis, a polymerase chain reaction (PCR)-based assay, identifies tumors with defective mismatch repair. Microsatellites are regions of the DNA in which there are single, di-, tri- or quadra-nucleotide repeats (for example, CACACA). An MSI assay compares an individual's tumor DNA with normal DNA. When the number of nucleotides in these repeat sequences in tumor differs from those in normal tissue, this finding is indicative of an abnormally functioning DNA mismatch-repair system. The National Institutes of Health has identified a panel of six microsatellite regions of DNA—BAT 25, BAT 26, BAT40, D5S346, D2S123, and D17S250—that help identify MSI [4]. By convention, if allelic shift is detected in one of the six microsatellites, the tumor is designated as microsatellite instability-low (MSI-L). The clinical significance, if any, of MSI-L tumors is not currently known. If a tumor has allelic shift in two or more of the six microsatellites, the tumor is designated as microsatellite instability-high (MSI-H). This finding indicates a defect in the mismatch-repair system. MSI analysis can be performed on formalin-fixed paraffin imbedded tissue. An example of this analysis is shown in Fig. 1.

Defective DNA mismatch repair in tumors also can be identified by immunohistochemical analysis of each of the DNA mismatch-repair proteins. Loss of expression of any of these mismatch-repair proteins in the tumor indicates a functional defect in mismatch repair. Fig. 2 gives an example of immunohistochemical analysis. This technique is more readily available in

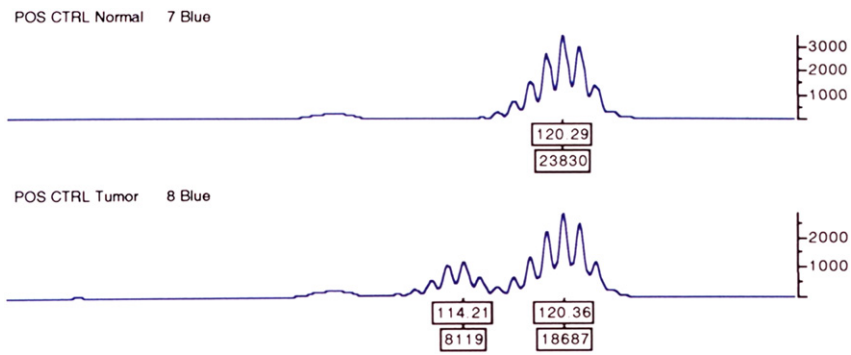

Fig. 1. Chromatogram of BAT 26 microsatellite instability analysis. DNA was extracted from formalin-fixed, paraffin-embedded sections of an endometrial carcinoma. DNA from microscopically confirmed normal ovary was used as normal tissue control. Allelic shift is present when the tumor DNA has more peaks on the chromatogram than the normal DNA. In this case, the tumor DNA has at least four more peaks than the normal DNA. Thus, for BAT 26 there is allelic shift. Allelic shift in at least two of the six markers analyzed is indicative of microsatellite instability-high.

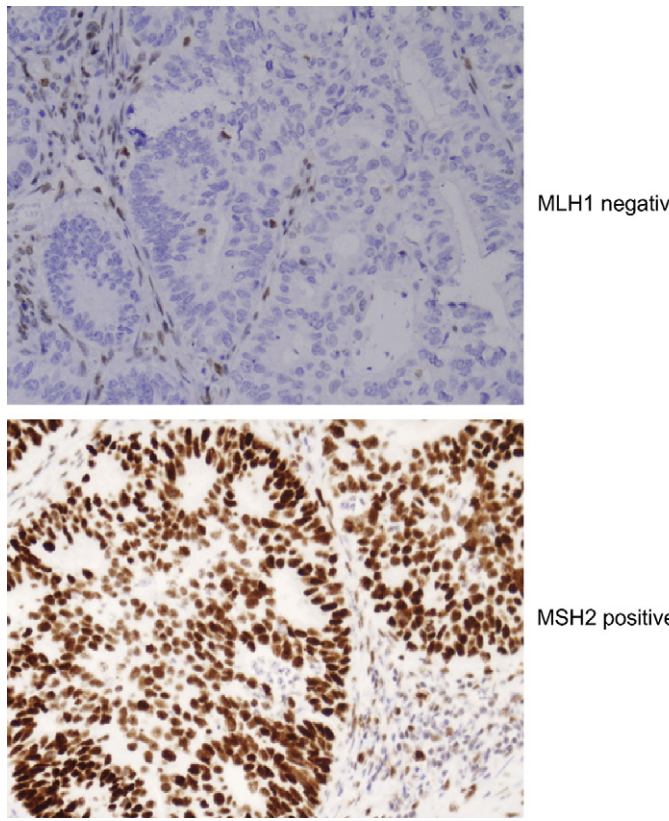

Fig. 2. Immunohistochemistry for *MLH1* (*top*) and *MSH2* (*bottom*). Immunohistochemistry was performed using formalin-fixed, paraffin-embedded sections of an endometrial carcinoma. Tumor cell nuclei are strongly positive for *MSH2* (*dark brown staining*). Tumor cell nuclei do not stain for *MLH1*, however. Note that adjacent stromal cells do stain positive for *MLH1*. Adjacent stromal cells, inflammatory cells, and normal endometrium can serve as useful internal positive controls for immunohistochemistry analyses.

most clinical pathology laboratories, whereas MSI analysis is a PCR-based assay that may be available only in larger laboratories.

Immunohistochemical and MSI analyses can be performed on the tumor tissue of a patient clinically suspected of having Lynch syndrome (see Box 1). If there is loss of immunohistochemical expression of one of the DNA mismatch-repair proteins and MSI, directed germline testing of a peripheral blood sample is indicated, and full sequencing of the appropriate mismatch-repair gene can be performed. Germline DNA testing is important, because it can identify the exact mutation in the affected DNA mismatch-repair gene, which then can be used to identify other mutation carriers in a family quickly and inexpensively. If a family member is found to have such a mutation, intensified cancer screening or prevention strategies can be initiated.

Acquired defective DNA mismatch repair

There is a subset of tumors that have MSI-H and loss of immunohistochemical expression of *MLH1* caused by epigenetic silencing caused by methylation of the *hMLH1* promoter. The methylation of the *hMLH1* promoter can be identified by DNA amplification using PCR primers that are specific for methylated and unmethylated versions of *hMLH1*. This finding is a strong indication that the patient has a sporadic and not an inherited defect in DNA mismatch repair.

A number of studies have found that MSI caused by methylation of the *hMLH1* promoter occurs in approximately 20% of all endometrial cancers [1]. When examined by histologic subtype, acquired defective DNA mismatch repair secondary to *hMLH1* methylation occurs primarily in endometrioid adenocarcinomas (type I) of the endometrium; *hMLH1* methylation is uncommon in nonendometrioid tumors. Unlike Lynch syndrome, the age of diagnosis for women who have endometrial cancer associated with sporadic, acquired defective DNA mismatch repair is the same as for women who do not have defective DNA mismatch repair.

There is an abundance of literature examining MSI in colon cancer. MSI-H colon cancer is associated with a better clinical outcome than microsatellite-stable colon cancers [5]. In addition, MSI-H colon cancers tend to be unresponsive to 5-fluorouracil–based chemotherapy regimens, the primary chemotherapy agent for colon cancer [1,6–14]. A number of studies have examined the clinical outcomes of MSI-H endometrial cancer. In one of the largest studies, Black and colleagues [1] examined 473 patients who had endometrial cancer. Ninety-three (20%) of the 473 were MSI-H. As compared with the microsatellite-stable tumors, MSI-H tumors were predominantly endometrioid (94% versus 23%), had a higher proportion with myometrial invasion, and were more advanced in stage. Overall, the patients who had MSI-H tumors had a better disease-free and disease-specific survival. A recent large study by Zighelboim and colleagues [15] examined the issue of improved survival and MSI status in a more detailed fashion by including only endometrioid endometrial cancers. MSI was identified in 147 cases (33%) and was associated with higher-grade tumors. No difference in overall or disease-free survival was found between the patients who had MSI-H tumors or microsatellite-stable tumors, however.

Inherited defective DNA mismatch repair and risk of endometrial cancer

Individuals who have Lynch syndrome have inherited one nonfunctional allele in a mismatch-repair gene. Loss of the corresponding allele results in defective mismatch repair in the target tissues. This molecular defect is manifested clinically by a substantially increased risk of colon and endometrial cancer. The estimates of lifetime endometrial cancer risk for individuals who have a germline *hMLH1* and *hMSH2* mutation are between 40%

and 60% (Fig. 3) [16,17]. In fact, for mutation carriers, these two studies found that the risk of endometrial cancer is higher than the risk of colon cancer. Aarnio and colleagues [16] reported a 60% lifetime risk for endometrial cancer in women who had Lynch syndrome, compared with a 54% lifetime risk for colon cancer. Dunlop and colleagues [17] reported a 42% lifetime risk of endometrial cancer and a 30% lifetime risk of colon cancer in mutation-positive women. When examining the germline mutations separately, Vasen and colleagues [18] reported a 35% to 40% risk of endometrial cancer in women who had *hMSH2* mutations and a 25% risk of developing endometrial cancer in women who had *hMLH1* mutations. They also reported that the risk of developing colon cancer in women who had either *hMLH1* or *hMSH2* germline mutations was 50% to 60%. Green and colleagues [19] examined a large *hMSH2* kindred in Newfoundland and found that, for women, the cumulative risk of endometrial cancer by age 70 years was 79%, and the cumulative risk of colon cancer was 64%. Data from all these studies were obtained from Lynch syndrome families that had documented *hMLH1* and *hMSH2* germline mutations. The reported risks of endometrial cancer in these studies are higher than the previously reported risk of 20%, which was based on families that fulfilled Amsterdam criteria but had not undergone genetic testing [20]. Clearly, women who have Lynch syndrome have a significant risk for endometrial cancer, and that risk may, in fact, exceed their risk of colon cancer.

Wijnen and colleagues [21] reported an excess of endometrial cancers in female carriers of *hMSH6* germline mutations. Truncating *hMSH6* mutations were identified in 10 of 214 Lynch syndrome kindred in whom an *hMLH1* or *hMSH2* mutation had not been identified. The authors report

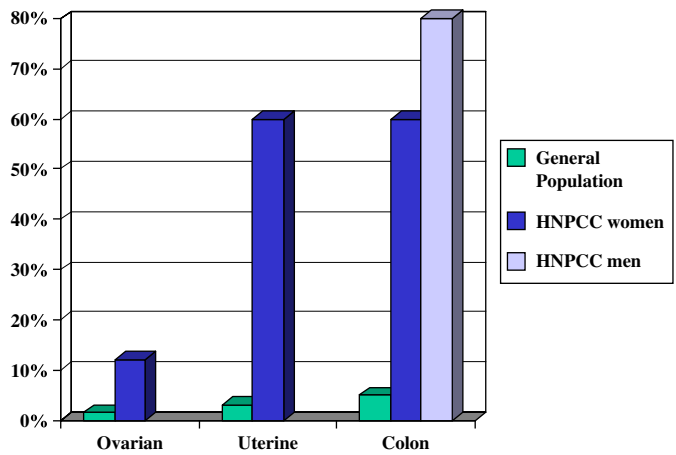

Fig. 3. Lifetime risk for colon, endometrial and ovarian cancer in men and women who have Lynch syndrome compared with general-population risk. HNPCC, hereditary nonpolyposis colorectal cancer.

that the frequency of endometrial cancer and hyperplasia was 73% in their cohort of female *hMSH6* mutation carriers, compared with 29% in *hMSH2* mutation carriers and 31% in *hMLH1* mutation carriers. Recently, Hendriks and colleagues [22] examined a large number of individuals from 20 families that had *hMSH6* mutations. They reported that women who had *hMSH6* mutations had a 71% cumulative risk of endometrial cancer by age 70 years, substantially higher than their risk for colon cancer. In addition, they found that the mean age of onset of endometrial cancer in these women was 55 years, with a sharp increase in risk after age 50 years.

Ovarian cancer in Lynch syndrome has been poorly described and is not well understood. The risk of ovarian cancer in women who have a defect in DNA mismatch repair has been reported to be 12% [16]. Vasen and colleagues [18] reported that the risk of ovarian cancer with an *hMSH2* mutation was approximately 10%, whereas the risk of developing ovarian cancer with an *hMLH1* mutation was lower, 3%. Green and colleagues [19] reported a 36% risk of ovarian cancer in a large kindred that had an *hMSH2* mutation. Other cancer risks for individuals who have Lynch syndrome include cancers of the small bowel, stomach, ureter, renal pelvis, and brain. As with ovarian cancer, very little is known about these tumors in Lynch syndrome.

Identifying individuals who have Lynch syndrome

Identifying individuals who have Lynch syndrome has important clinical implications. First, patients who have a germline mutation in one of the mismatch-repair genes have a substantial lifetime risk of developing a second primary cancer. Second, identifying the specific gene mutation in a woman who has endometrial cancer and Lynch syndrome allows her family members to undergo predictive genetic testing. Historically, gastrointestinal surgeons, medical oncologists, and gastroenterologists have been responsible for identifying individuals at risk for Lynch syndrome. The gynecologic community has played a less significant role in identifying individuals who have Lynch syndrome. Therefore, published criteria used to assist clinicians in identifying individuals with Lynch syndrome are focused primarily on colon cancer. The revised Bethesda criteria include criteria relating to family history, age of onset of cancer, synchronous and metachronous cancers, and specific histopathologic features of colon cancer (Box 2) [4]. In contrast, there have been no well-defined guidelines for identifying individuals who have endometrial cancer as potentially having Lynch syndrome.

The authors recently examined a large series of women from families that had Lynch syndrome who had both colorectal and either endometrial or ovarian cancer in their lifetime. Of the 117 women, 16 had a colorectal cancer and either endometrial or ovarian cancer diagnosed simultaneously. Of the remaining 101 women, 52 women (51%) were first diagnosed with endometrial or ovarian cancer. Forty-nine women (49%) had a colorectal cancer

Box 2. The revised Bethesda guidelines for testing colorectal tumors for MSI

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous, metachronous colorectal or other HNPCC-associated tumors, regardless of age (HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain [usually glioblastoma as seen in Turcot syndrome] tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.)
3. Colorectal cancer with the MSI-H histology (presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern) diagnosed in a patient who is less than 60 years of age (There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3; participants voted to retain the criterion of less than 60 years of age in the guidelines.)
4. Colorectal cancer diagnosed in one or more first-degree relatives who had an HNPCC-related tumor, with one of the cancers being diagnosed before the age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives who had HNPCC-related tumors, regardless of age.

diagnosed first [23]. In identifying the patient who has endometrial cancer as part of the Lynch syndrome, clinicians may institute screening for colon cancer and potentially prevent the development of a second cancer. Developing criteria to assist gynecologists and gynecologic oncologists in identifying which women who have endometrial cancer may have Lynch syndrome could have a significant clinical impact. The revised Bethesda criteria focus primarily on individuals who have colon cancer [4]; a more multidisciplinary set of guidelines that would provide all clinicians with criteria for screening would be useful for the early identification of women who have Lynch syndrome.

A study by Berends and colleagues [34] examined a cohort of women under age 50 years who had endometrial cancer and determined the prevalence of germline mutations in *hMLH1*, *hMSH2*, or *hMSH6*. Among 63 women tested, they identified 5 individuals who had germline mutations (8%). In

those women who had endometrial cancer, who were less than 50 years of age, and who had a first-degree relative with a Lynch syndrome–associated cancer, the prevalence of a mismatch-repair gene mutation was 23%. The authors recommend that women who have endometrial cancer, who are under age 50 years, and who have a first-degree relative who has colon or other Lynch syndrome–associated cancer should be considered for genetic testing. The authors recently completed a study examining 100 women under age 50 years who had endometrial cancer and found that 9 of 100 (9%) had germline mutations in *hMLH1*, *hMSH2*, or *hMSH6* [24]. They also found that women under age 50 years who had endometrial cancer and who had a first-degree relative who had colon or other Lynch syndrome–associated cancer had a high likelihood of having a germline mutation. Therefore, gynecologic oncologists and gynecologists caring for young women who have endometrial cancer can identify Lynch syndrome in their patients by asking a directed question about cancer in a first-degree relative.

Individuals who have synchronous or metachronous colon and endometrial tumors are likely to have Lynch syndrome. In a study by Millar and colleagues [25], 7 of 40 women who had synchronous or metachronous colon and endometrial cancers (18%) had a germline *hMLH1* or *hMSH2* mutation. Individuals who have synchronous endometrial and ovarian cancers have been identified in Lynch syndrome families. Synchronous endometrial and ovarian cancers occur in about 10% of all ovarian cancers and in 5% of all endometrial cancers, however, and are not likely to be an accurate indicator of Lynch syndrome [26]. Soliman and colleagues [27] found that only 7% of women who had synchronous endometrial and ovarian cancers had molecular findings consistent with a DNA mismatch-repair defect. Each of these patients had either a personal history of a Lynch syndrome–associated cancer or a first-degree relative who had a Lynch syndrome–associated cancer. Clearly, further guidelines need to be developed to assist the gynecologist or gynecologic oncologist in identifying individuals who have Lynch syndrome.

Endometrial cancer phenotype in Lynch syndrome

As discussed previously, sporadic MSI-H endometrial cancer caused by *hMLH1* methylation (acquired or nonhereditary cancer) is associated almost exclusively with endometrioid tumors, higher International Federation of Gynecology and Obstetrics grade, and advanced stage [23,28–31]. MSI-H tumors caused by inherited DNA mismatch-repair defects have been shown to include a broader spectrum of tumor histologies, including endometrioid adenocarcinoma, papillary serous carcinoma, clear cell carcinoma, and malignant mixed Müllerian tumor [32]. In fact, the spectrum of endometrial tumors associated with Lynch syndrome more closely mirrored that of the general population than those associated with *hMLH1* methylation. In a series on Lynch syndrome–associated endometrial cancer described by

Broaddus and colleagues [32], 78% were stage I, 10% were stage II, and 12% were stage III or IV. Deep myometrial invasion (> 50% of the uterine wall) occurred in 26% of cases. Only 44% of the endometrioid tumors were grade 1, with the majority being grade 2 or 3. In all, nearly 25% of all cancers had pathologic features (deep myometrial invasion; cervix involvement; lymph node or adnexal metastasis) that would necessitate adjuvant therapy following surgical staging.

A few other studies have described the clinicopathologic features of Lynch syndrome–associated endometrial cancer. Vasen and colleagues [28] identified 125 women who had endometrial cancer from families fulfilling Amsterdam criteria from seven countries. At the time of the study, genetic testing was not available. The median age of diagnosis was 48 years (range, 27–72 years). Information on presenting symptoms, histology, and grade of tumor was not reported. Sixty-one percent of the 125 patients had a second primary cancer, most commonly colon cancer, either before or after the diagnosis of endometrial cancer. The investigators reported excellent survival, with only 12% dying of endometrial cancer.

A study by Boks and colleagues [33] also examined survival of patients who had endometrial cancer and Lynch syndrome. They compared 50 patients who had endometrial cancer and Lynch syndrome (based either on germline test results or revised Amsterdam criteria) with 100 age- and stage-matched women who had sporadic endometrial cancer. The overall 5-year cumulative survival rates were similar: 88% for women who had Lynch syndrome and 82% for women who had sporadic endometrial cancer. In the cohort of women who had Lynch syndrome, the majority (78%) had early-stage disease, and 92% had endometrioid histology. Among the 22% of women who had Lynch syndrome and advanced-stage disease, it was unclear whether prognosis was better than in a population that had advanced-stage sporadic disease. Additional studies will be needed to determine if endometrial cancer associated with Lynch syndrome has a more favorable survival than sporadic endometrial cancer. Comparing outcomes in advanced-stage patients may be important, because the prognosis for early-stage endometrial cancer is very favorable overall.

Typical endometrioid endometrial cancer develops by a stepwise pathway from normal endometrium to complex hyperplasia with atypia to carcinoma. It is unclear whether Lynch syndrome–associated endometrial cancer follows this pattern. In one study, two patients who had known mutations had endometrial hyperplasia without concurrent endometrial cancer, and three patients had endometrial hyperplasia with concurrent endometrial cancer. The authors demonstrated loss of the appropriate mismatch-repair protein by immunohistochemistry in both the hyperplasias and the cancers, suggesting that the mismatch-repair defect may occur early in endometrial carcinogenesis [34]. Zhou and colleagues [35] examined *PTEN* mutations, an early and frequent event in sporadic endometrial cancer, in Lynch syndrome–associated tumors. They examined 41 endometrial cancers from

mutation-positive Lynch families and found that 68% demonstrated weak or absent staining for *PTEN* by immunohistochemistry. Eighteen of 20 cases had somatic *PTEN* mutations involving the 6(A) tracts in exon 7 or 8. The authors conclude that *PTEN* mutations are critical in the pathogenesis of both sporadic and Lynch syndrome–associated endometrial cancers. Additional studies of the histologic and molecular phenotype of endometrial cancer in women who have Lynch syndrome are necessary to define more clearly the differences between sporadic endometrial cancer and endometrial cancer associated with Lynch syndrome.

Clinical management

Screening and prevention

Currently, there have been limited studies evaluating screening for endometrial cancer in women who have Lynch syndrome. Nevertheless, clinical guidelines have been established that recommend screening for endometrial cancer beginning at age 25 to 35 years [36]. Modalities for endometrial cancer screening include transvaginal ultrasound and in-office endometrial sampling.

Studies have shown that transvaginal ultrasound to evaluate the thickness of the endometrial stripe does not have clear benefit. In premenopausal women, the thickness of the endometrial stripe changes with the menstrual cycle and is unlikely to be a sensitive or specific test for endometrial cancer. Two studies have reported experience with ultrasound as a screening modality for endometrial cancer. Dove-Edwin and colleagues [37] examined the outcome of endometrial cancer surveillance by ultrasound in 269 women who had Lynch syndrome. Women who were screened included those who were mutation positive, who had Lynch syndrome based on Amsterdam criteria, or who did not fulfill Amsterdam criteria but had a family history suggestive of Lynch syndrome. No cancers were detected in 522 ultrasounds. Two interval cases of endometrial cancer occurred, however. One patient had a normal surveillance ultrasound 2 years before developing postmenopausal bleeding. The second patient had a normal surveillance ultrasound 6 months before diagnosis of stage I endometrial cancer. The authors conclude that an ultrasound may not be an effective method to detect early endometrial cancer. In a study by Rijcken [38], 41 women who had Lynch syndrome were enrolled in a screening program. Overall, 179 transvaginal ultrasounds were performed, and 17 were defined as abnormal based on the thickness or irregularity of the lining. Fourteen of these patients had a follow-up endometrial biopsy that was within normal limits. In one patient who had a thickened endometrium on ultrasound (27 mm), biopsy revealed complex atypical hyperplasia. Two additional patients had ultrasounds that described an irregular endometrium; both had focal complex atypical hyperplasia on biopsy. Only one patient in this cohort developed endometrial

cancer, and she had a normal screening transvaginal ultrasound but developed vaginal bleeding 8 months later. At the time of diagnosis, she had stage IB, grade 2, endometrioid adenocarcinoma.

An endometrial biopsy is an office procedure that provides adequate tissue for pathologic diagnosis and is a reasonable screening modality. Studies performed in women presenting with abnormal vaginal bleeding have shown that the sensitivity of an office endometrial biopsy is equivalent to a dilatation and curettage performed in the operating room [39]. The authors' current recommendations for patients who are known mutation carriers include an annual office endometrial biopsy. An annual transvaginal ultrasound to evaluate the ovaries also is recommended. Annual CA-125 testing may be included, but false positives are common in the premenopausal age range.

The oral contraceptive pill has been shown to decrease risk of endometrial cancer by 50% in women at general-population risk [40]. In addition, the oral contraceptive pill also has been shown to decrease substantially the risk of ovarian cancer. A chemoprevention study in women who have Lynch syndrome and are using the oral contraceptive pill or medroxyprogesterone acetate is currently being conducted at the M.D. Anderson Cancer Center. Although the end point for this study will not be reduction in incidence of disease, the authors will examine the effect of these agents on surrogate molecular biomarkers in the endometrium.

Prophylactic surgery

A recently published article in the *New England Journal of Medicine* from the authors' consortium examined the efficacy of prophylactic hysterectomy in a large number of women who had documented germline mutations associated with the Lynch syndrome [41]. Sixty-one women who underwent prophylactic hysterectomy were matched with 210 women who did not undergo hysterectomy. None of the women who underwent prophylactic hysterectomy developed endometrial cancer, whereas 69 women in the control group (33%) developed endometrial cancer. No studies of prophylactic gynecologic surgery had been published previously for women who had Lynch syndrome. These findings helped provide substantive data supporting this prevention strategy for women who have Lynch syndrome and, importantly, provided a basis for consensus groups to make clinical recommendations. Based on this study, the Cancer Genetics Consortium's most recent published guidelines state, ''Evidence supports the efficacy of prophylactic hysterectomy and oophorectomy'' [42]. Women who have Lynch syndrome should be counseled that prophylactic hysterectomy and bilateral salpingo-oophorectomy is a reasonable management option to consider. When childbearing is complete, a hysterectomy and bilateral salpingo-oophorectomy can be performed either through laparoscopy or a traditional laparotomy. For women who have Lynch syndrome and are undergoing colon surgery,

concurrent prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy can be considered also.

For gynecologists or gynecologic oncologists performing prophylactic hysterectomy and bilateral salpingo-oophorectomy in women who are known mutation carriers, the possibility of finding an occult endometrial or ovarian cancer should be considered. An incidental cancer has been reported in an asymptomatic 48-year-old woman who had a known *hMSH2* mutation undergoing prophylactic vaginal hysterectomy and bilateral salpingo-oophorectomy. On the final pathologic review, she was found to have a grade 2 endometrial cancer with 5 out of 12 mm of invasion of the uterine wall and involvement of the endocervical glands. Because the endometrial cancer was not identified at the time of surgery, surgical staging was not performed. The patient therefore underwent a second restaging operation performed by laparotomy [31]. Currently the authors recommend that a preoperative endometrial biopsy be performed in women who are known mutation carriers and who are undergoing prophylactic hysterectomy. In addition, the authors recommend that the uterus be examined intraoperatively by a pathologist for occult disease so that the appropriate surgical staging can be performed as necessary.

Summary

Defective DNA mismatch repair occurs in approximately 20% to 25% of all cases of endometrial cancer. A majority of these cases are noninherited or acquired and result from methylation or silencing of the *hMLH1* promoter. For women who have inherited defects in DNA mismatch repair, or Lynch syndrome, the onset of endometrial cancer usually occurs at a younger age, and the risk of developing a second cancer is high. Gynecologic oncologists and gynecologists play a key role in identifying these individuals. In addition to asking about family history of endometrial and colon cancer, tumor studies including MSI testing and immunohistochemical analysis for the mismatch-repair proteins can assist in differentiating acquired from inherited defective DNA mismatch repair. Although studies have shown a preponderance of endometrioid cancers associated with acquired MSI, inherited MSI endometrial cancers have a broader spectrum of disease.

References

[1] Black D, Soslow RA, Levine DA, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. J Clin Oncol 2006;24(11):1745–53.

[2] Vasen HF, Mecklin JP, Khan PM, et al. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34(5): 424–5.

[3] Gurin CC, Federici MG, Kang L, et al. Causes and consequences of microsatellite instability in endometrial carcinoma. Cancer Res 1999;59(2):462–6.

[4] Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96(4):261–8.
[5] Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342(2):69–77.
[6] Caduff RF, Johnston CM, Svoboda-Newman SM, et al. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 1996; 148(5):1671–8.
[7] Fiumicino S, Ercoli A, Ferrandina G, et al. Microsatellite instability is an independent indicator of recurrence in sporadic stage I-II endometrial adenocarcinoma. J Clin Oncol 2001; 19(4):1008–14.
[8] Maxwell GL, Risinger JI, Alvarez AA, et al. Favorable survival associated with microsatellite instability in endometrioid endometrial cancers. Obstet Gynecol 2001;97(3):417–22.
[9] Wong YF, Ip TY, Chung TK, et al. Clinical and pathologic significance of microsatellite instability in endometrial cancer. Int J Gynecol Cancer 1999;9(5):406–10.
[10] Tibiletti MG, Furlan D, Taborelli M, et al. Microsatellite instability in endometrial cancer: relation to histological subtypes. Gynecol Oncol 1999;73(2):247–52.
[11] MacDonald ND, Salvesen HB, Ryan A, et al. Frequency and prognostic impact of microsatellite instability in a large population-based study of endometrial carcinomas. Cancer Res 2000;60(6):1750–2.
[12] Baldinu P, Cossu A, Manca A, et al. Microsatellite instability and mutation analysis of candidate genes in unselected Sardinian patients with endometrial carcinoma. Cancer 2002; 94(12):3157–68.
[13] Basil JB, Goodfellow PJ, Rader JS, et al. Clinical significance of microsatellite instability in endometrial carcinoma. Cancer 2000;89(8):1758–64.
[14] Catasus L, Machin P, Matias-Guiu X, et al. Microsatellite instability in endometrial carcinomas: clinicopathologic correlations in a series of 42 cases. Hum Pathol 1998;29(10): 1160–4.
[15] Zighelboim I, Goodfellow PJ, Gao F, et al. Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. J Clin Oncol 2007;25(15):2042–8.
[16] Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214–8.
[17] Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997;6(1):105–10.
[18] Vasen HF, Stormorken A, Menko FH, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001;19(20):4074–80.
[19] Green J, O'Driscoll M, Barnes A, et al. Impact of gender and parent of origin on the phenotypic expression of hereditary nonpolyposis colorectal cancer in a large Newfoundland kindred with a common MSH2 mutation. Dis Colon Rectum 2002;45(9):1223–32.
[20] Watson P, Vasen HF, Mecklin JP, et al. The risk of endometrial cancer in hereditary nonpolyposis colorectal cancer. Am J Med 1994;96(6):516–20.
[21] Wijnen J, de Lecuw W, Vasen H, et al. Familial endometrial cancer in female carriers of MSH6 germline mutations. Nat Genet 1999;23(2):142–4.
[22] Hendriks YM, Wagner A, Morreau H, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004;127(1):17–25.
[23] Lu K, Dinh M, Kohlmann W, et al. Gynecological malignancy as a "sentinel cancer" for women with HNPCC. Gynecol Oncol 2004;92:421.
[24] Lu KH, Schorge JO, Rodabaugh KJ, et al. Prospective determination of prevalence of Lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007;25(33).

[25] Millar AL, Pal T, Madlensky L, et al. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum Mol Genet 1999; 8(5):823–9.
[26] Soliman PT, Slomovitz BM, Broaddus RR, et al. Synchronous primary cancers of the endometrium and ovary: a single institution review of 84 cases. Gynecol Oncol 2004;94(2):456–62.
[27] Soliman PT, Broaddus RR, Schmeler KM, et al. Women with synchronous primary cancers of the endometrium and ovary: do they have Lynch syndrome? J Clin Oncol 2005;23(36): 9344–50.
[28] Vasen HF, Watson P, Mecklin JP, et al. The epidemiology of endometrial cancer in hereditary nonpolyposis colorectal cancer. Anticancer Res 1994;14(4B):1675–8.
[29] Smith RA, Cokkinides V, Eyre HJ. American Cancer Society guidelines for the early detection of cancer, 2006. CA Cancer J Clin 2006;56(1):11–25, quiz 49–50.
[30] National Comprehensive Cancer Network. Hereditary predisposition: HNPCC follow-up (surveillance). 2006.
[31] Chung L, Broaddus R, Crozier M, et al. Unexpected endometrial cancer at prophylactic hysterectomy in a woman with hereditary nonpolyposis colon cancer. Obstet Gynecol 2003; 102(5 Pt 2):1152–5.
[32] Broaddus RR, Lynch HT, Chen LM, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. Cancer 2006; 106(1):87–94.
[33] Boks DE, Trujillo AP, Voogd AC, et al. Survival analysis of endometrial carcinoma associated with hereditary nonpolyposis colorectal cancer. Int J Cancer 2002;102(2):198–200.
[34] Berends MJ, Hollema H, Wu Y, et al. MLH1 and MSH2 protein expression as a pre-screening marker in hereditary and non-hereditary endometrial hyperplasia and cancer. Int J Cancer 2001;92(3):398–403.
[35] Zhou XP, Kuismanen S, Nystrom-Lahti M, et al. Distinct PTEN mutational spectra in hereditary non-polyposis colon cancer syndrome-related endometrial carcinomas compared to sporadic microsatellite unstable tumors. Hum Mol Genet 2002;11(4):445–50.
[36] Burke W, Petersen G, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA 1997;277(11):915–9.
[37] Dove-Edwin I, Boks D, Goff S, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. Cancer 2002;94(6):1708–12.
[38] Rijcken FE, Mourits MJ, Kleibeuker JH, et al. Gynecologic screening in hereditary nonpolyposis colorectal cancer. Gynecol Oncol 2003;91(1):74–80.
[39] Dijkhuizen FP, Mol BW, Brolmann HA, et al. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 2000;89(8):1765–72.
[40] Combination oral contraceptive use and the risk of endometrial cancer. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. JAMA 1987;257(6):796–800.
[41] Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354(3):261–9.
[42] Chen S, Wang W, Lee S, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA 2006;296(12):1479–87.

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Management of Abnormal Uterine Bleeding and the Pathology of Endometrial Hyperplasia

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Endometrial carcinoma is the most common malignancy of the female genital tract, and effective measures for cancer prevention would impact a large number of patients. Efficient algorithms for the prevention of endometrial cancer ideally require availability of proper risk identification, effective screening tests, and a clear definition of precancerous lesions. Early diagnosis of invasive carcinoma is also desirable, which allows for appropriate treatment while surgical cure is still possible. Unfortunately, screening tests for endometrial carcinoma are not available, because the endometrium is not as accessible as the cervix, which is successfully screened by the Pap test. There is a common lay misconception that the Pap test screens for all gynecologic cancers, including endometrial and ovarian carcinoma. Most endometrial carcinomas are discovered on endometrial biopsy (EMB) during the work-up for abnormal uterine bleeding (AUB). AUB is a common clinical symptom, however, and most patients presenting with AUB do not have an underlying cancer. Consideration of cancer risk and appropriate work-up of patients with AUB is paramount in the prevention and early diagnosis of endometrial carcinoma or its precursor lesions. Once an endometrial abnormality is diagnosed, treatment options vary, and the efficacy of certain therapies is still unclear. This article discusses risk factors

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for endometrial cancer, the diagnostic approach, treatment options, and the histopathology of endometrial hyperplasia including recent molecular advances in the knowledge of endometrial hyperplasia and carcinoma.

The clinical problem, risk factors, and diagnostic approach

Endometrial adenocarcinoma is the most common gynecologic malignancy in the United States, with approximately 36,000 new cases [1] and 7350 deaths per year. Endometrial cancer is the eighth leading cause of cancer deaths in United States women, and is most common in the sixth to seventh decade of life, with an average age of 60 years [2]. The overall lifetime risk for women to develop endometrial cancer is 2% to 3%. Younger women are at a much lower risk for endometrial cancer, with only 8% of cases occurring under the age of 45 years, and 25% occurring in premenopausal women [3,4]. These latter cases, however, are much more difficult to diagnose because of a plethora of nonmalignant gynecologic pathology in the premenopausal patient.

Disorders of the menstrual cycle account for up to 30% of outpatient visits to gynecologists [5]. Menstrual abnormalities, generally known as "abnormal uterine bleeding," can be divided into absence of menstrual flow, abnormal amount of flow, and irregularity of flow. The differential diagnosis of AUB includes hormonal abnormalities, medications and other iatrogenic causes, systemic diseases, benign or malignant tumors, pregnancy and related conditions, trauma, and dysfunctional uterine bleeding as a diagnosis of exclusion. In most patients the cause of AUB is benign, but a malignancy must always be considered in the differential diagnosis.

When defining AUB, patients are usually classified into reproductive and postmenopausal age groups. In the childbearing age group, AUB can be defined as any change in menstrual periods, such as frequency, duration, amount of flow, or bleeding between cycles. In postmenopausal women, AUB is defined as vaginal bleeding at least 12 months after the cessation of regular menses, or as unpredictable bleeding after use of hormone replacement therapy (HRT) for 12 months or more [6]. A more general definition of AUB includes prepubertal bleeding, menorrhagia, metrorrhagia, postcoital, and postmenopausal bleeding. Key terms in the description of AUB include amenorrhea (no menses for >90 days); menorrhagia (excessive or prolonged bleeding at regular intervals); polymenorrhea (bleeding intervals <21 days); oligomenorrhea (bleeding intervals >35 days); metrorrhagia (irregular bleeding intervals); menometorrhagia (prolonged and excessive bleeding at irregular intervals); intermenstrual bleeding; and dysfunctional uterine bleeding (excessive bleeding unrelated to anatomic or systemic disease) as a diagnosis of exclusion [7].

Anovulatory cycles are common in premenopausal and perimenopausal women. Under normal conditions, pituitary follicle-stimulating hormone stimulates ovarian estrogen secretion, and the endometrial lining builds

up in anticipation of ovulation. After ovulation has occurred, progesterone from the resulting corpus luteum induces secretory changes in the endometrium, before normal menstrual shedding ends the cycle. In anovulatory cycles no corpus luteum is formed, no progesterone is produced to induce the secretory phase, and normal menses does not occur. The continued unopposed estrogen production leads to further buildup of proliferative or disordered proliferative endometrium, which ultimately breaks down in erratic shedding. Anovulation can also occur in patients with polycystic ovarian syndrome ([PCOS] Stein-Leventhal syndrome), or with systemic illnesses or stress, termed "hypothalamic dysfunction" [5–7]. Continuous prolonged exposure to estrogen during anovulatory cycles can lead to endometrial hyperplasia and endometrial carcinoma. Other benign causes of AUB in premenopausal women include leiomyomas, adenomyosis, and endometriosis. Light irregular bleeding can be caused by cervicitis, endocervical polyps, and cervical cancer, or by vaginitis and vulvitis [5].

AUB in the postmenopausal patient is always concerning and should be considered of malignant origin until proved otherwise; however, endometrial cancer accounts for AUB symptoms in only about 10% of postmenopausal women. The most common causes of postmenopausal vaginal bleeding are endometrial atrophy (60%–80%); estrogen replacement therapy (15%–25%); endometrial polyps (2%–12%); and endometrial hyperplasia (5%–10%) [2].

Most risk factors for endometrial carcinoma relate to long-term exposure to unopposed estrogen, such as obesity, PCOS, and exogenous iatrogenic administration [1,2]. In obese patients, androstenedione derived from the adrenal glands undergoes aromatization in the peripheral adipose tissues. This peripheral conversion to estrone leads to chronically elevated levels of this weak circulating estrogen. Epidemiologic studies have shown that women who are 21 to 50 pounds overweight are three times more likely to develop endometrial cancer, and those overweight by greater than 50 pounds have a 10 times increased risk [2]. Among women who use HRT, patients taking unopposed estrogen have a four to eight times greater likelihood of developing endometrial cancer than patients on combination HRT [2]. Patients with PCOS are exposed to increased estrogen because of anovulatory cycles [8].

Tamoxifen is an effective treatment for hormone-responsive breast cancer, but is associated with a twofold to sevenfold elevated risk for endometrial carcinoma. Approximately 40% to 50% of patients on tamoxifen therapy develop a proliferative endometrial abnormality, such as proliferative polyposis or hyperplasia. Long-term tamoxifen therapy is also associated with the uterine cancer malignant mixed müllerian tumor, increasing the risk by 8- to 15-fold. A comparison of tamoxifen- and non–tamoxifen-associated endometrial carcinomas in breast cancer patients found similar genetic alterations in both, such as PTEN (phosphatase and tensin homolog deleted from chromosome 10), p53, and K-*ras* mutations [9].

Women with PCOS present with menstrual irregularities, hirsutism, and obesity. Long-term sequelae of this disorder include anovulatory infertility, and an increased risk for endometrial carcinoma and for cardiovascular diseases secondary to diabetes mellitus, dyslipidemia, and systolic hypertension. According to the 2003 Rotterdam consensus criteria, at least two of the following criteria should be present for a diagnosis of PCOS: oligo-ovulation or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries. Other endocrine diseases, such as congenital adrenal hyperplasia, androgen-secreting tumors, or Cushing's syndrome, must be excluded [8]. There are currently no studies that demonstrate PCOS as a clear cause for endometrial carcinoma, but many case reports indicate an association between the two diseases. Patients with AUB who meet the criteria for PCOS should have an EMB. A few studies have assessed the risk for endometrial cancer in PCOS patients [10], but a meta-analysis to calculate a relative cancer risk in patients with chronic anovulation or PCOS is difficult to perform, because of the differing diagnostic criteria and lack of adequate controls in existing studies [10].

Several hereditary syndromes carry an increased risk of endometrial cancer. Hereditary nonpolyposis colorectal cancer is caused by mutations in DNA mismatch repair genes including hMLH-1, hMSH-2, hMSH-6, and PMS-2 [11,12]. Women with hereditary nonpolyposis colorectal cancer have a 40% to 50% lifetime risk for endometrial cancer, compared with 3% in the general population. Cowden disease is caused by mutations of the PTEN gene on chromosome 10q. The syndrome is also called multiple hamartoma syndrome, because patients present with hamartomatous neoplasms of the skin, mucosal surfaces, gastrointestinal tract, bones, central nervous system, eyes, and genitourinary tract. Approximately 20% to 36% of patients with Cowden disease develop breast carcinoma, and some also develop endometrial cancer. Bannayan-Zonana syndrome is also caused by a PTEN mutation, but these patients tend to have far fewer neoplasms [13].

No established screening test is available for endometrial cancer at this time. Ninety percent of women diagnosed with endometrial cancer have AUB or vaginal discharge as the presenting symptom [2]. Timely clinical evaluation of AUB remains the key in the prevention of uterine malignancies and in the practice of gynecologic medicine. Guidelines from the American College of Obstetrics and Gynecology recommend that an EMB be performed in women with AUB who are 35 years of age or older, especially when irregular bleeding was present for more than 6 months and associated with menorrhagia. Endometrial surveillance is merited with EMB and possibly imaging studies, such as transvaginal ultrasound. Patients with irregular bleeding of less than 3 months duration and without menorrhagia may be followed clinically [5].

Historically, dilation and curettage has been considered the gold standard for the diagnosis and often the treatment of endometrial disease, but

requires general anesthesia [2]. An EMB is typically performed as an office-based procedure, but obtains less tissue for diagnosis. Initial concerns arose that focal hyperplasias or carcinomas could be missed by the EMB procedure. But studies have shown that the diagnostic accuracy of the EMB ranges from 90% to 98% when compared with dilation and curettage. In perimenopausal women the EMB has a slightly less diagnostic accuracy when compared with postmenopausal women, with detection rates of 91% versus 99%, respectively [5]. EMB has also been reported to miss up to 18% of focal endometrial lesions, and the sensitivity for detecting atypical endometrial hyperplasia has been as low as 81% [6]. The Pipelle procedure uses a suctioning device to collect endometrial tissue that has been separated from the endometrial lining, and was paramount in detection rates of cancer in both premenopausal and postmenopausal women (99.6% and 91%, respectively), according to a meta-analysis [14]. When various endometrial sampling techniques were compared, hysteroscopy-directed biopsies provided the most accurate evaluation with the highest specificity and sensitivity [15].

Hysteroscopy, sonohysteroscopy, and transvaginal ultrasound are all valuable modalities to evaluate the uterus. Hysteroscopy visualizes the uterine cavity and can identify endometrial polyps, submucosal leiomyoma, and other lesions [6,16]. Biopsies can be taken under direct visual control, and the sensitivity (96%) and specificity (100%) for endometrial cancer are higher than for the blind Pipelle biopsy [17]. Hysteroscopy, however, is more invasive and requires anesthesia, special equipment, and is more time-consuming. For saline-infusion sonography and sonohysteroscopy, sterile saline is instilled into the uterine cavity and allows imaging of the endometrium [18]. Saline-infusion sonography has been combined with directed EMB, resulting in a sensitivity of 95% to 97% and specificity of 70% to 98% [6]. Transvaginal ultrasound can also be used to evaluate the endometrial layer and measure the thickness of the endometrial stripe. A thinned layer related to postmenopausal atrophy can be distinguished from thickened endometrium or polyps [2]. An endometrial stripe thicker than 4 to 6 mm may be used as a cutoff for further investigation, such as EMB. The sensitivity of transvaginal ultrasound with endometrial stripe evaluation for detecting endometrial cancer has been reported at 91% to 96%, but with a specificity as low as 58% [6,17]. Transvaginal ultrasound has also been evaluated as a screening tool for endometrial cancer in women without gynecologic symptoms. One study evaluated transvaginal ultrasound in postmenopausal women who were treated with idoxifene for hormone-responsive breast cancer [19]. Using a 6-mm cutoff for endometrial stripe thickness, transvaginal ultrasound had a high negative (99%) but a low positive predictive value (2% but low sampling rate of 45%) for endometrial carcinoma. The authors concluded that transvaginal ultrasound is not an adequate screening test in asymptomatic women. The diagnostic usefulness of three-dimensional ultrasound with Doppler blood flow

studies to detect abnormalities in the uterine cavity is currently being evaluated.

Clinical management of abnormal uterine bleeding

First-line treatment for AUB usually consists of nonsteroidal anti-inflammatory drugs, such as mefenamic acid and the antifibrinolytic agent tranexamic acid. Tranexamic acid reduces menstrual loss by about 50% and mefenamic acid by one third. Both drugs are taken during menstruation and also help relieve menstrual cramps [20]. Commonly used hormonal therapies consist of progestins, which may stop endometrial growth, allow organized endometrial sloughing to decrease bleeding, and are protective for the development of hyperplasia and cancer. Progestins increase the prostaglandin $F_{2\alpha}$/prostaglandin E ratio by stimulating arachidonic acid formation in the endometrium, which may also contribute to decreasing AUB. Progestins are administered orally or locally through an intrauterine device (IUD), depending on whether the source of bleeding is thought to be ovulatory or anovulatory [21]. There is no consensus on the best dose and regimen of progestins, but typically oral progestins are used for 7 to 14 days. Combination oral contraceptive pills are also commonly used to regulate menstrual bleeding [2,21].

Progesterone delivered by an IUD has been shown to reduce menstrual loss by 97% [21]. It has been suggested that the IUD is superior to cyclic progestins and combination oral contraceptive pills [2], and may be the most effective and underused method for controlling AUB [21]. Two review articles address the effectiveness of the IUD for AUB. One systematic review of studies evaluating surgery versus medical therapy for heavy menstrual bleeding indicated an equal quality of life improvement with the IUD and with hysterectomy [18]. The other review evaluated studies comparing oral progesterone with progesterone-releasing IUD for heavy menstrual bleeding. The authors concluded that the IUD is more effective than cyclic norethisterone (for 21 days) as treatment for heavy bleeding [22]. Other hormonal methods of controlling AUB include danazol and gonadotropin-releasing hormone agonists.

Endometrial ablation has been gaining support as a more effective treatment for AUB, compared with conventional hormone therapy. It is useful for patients who failed hormonal therapy and avoids hysterectomy. Indications for endometrial ablation include disabling uterine bleeding, failed medical therapies, contraindications to medical treatment, unexplained bleeding on HRT, or poor surgical risk for hysterectomy [23]. Endometrial hyperplasia is a relative contraindication and any genital tract malignancy is an absolute contraindication to endometrial ablation. It is paramount to exclude hyperplasia or cancer before ablating the endometrium. First-generation techniques for endometrial ablation included laser ablation

and rollerball electrocoagulation, required a high level of technical skill, and were associated with higher complication rates [23]. Second-generation devices, such as the thermal balloon ablation system and impedance-controlled electrocoagulation, are considered global ablation technologies, require less technical skill, and have fewer complications. One review reported the overall effectiveness of endometrial ablation in the therapy of AUB at 80% to 85% [23].

The management of endometrial hyperplasia is overall less clear. Although atypical endometrial hyperplasia is managed with hysterectomy, there is no consensus for management of nonatypical hyperplasia. Hyperplasia without atypia may be treated with high-dose progestins, combination oral contraceptive pills, IUD, or observation [18]. These modalities have limited effectiveness for complex hyperplasia, however, and often the hyperplasia is resistant to therapy or recurs on cessation of therapy [24]. High-dose progesterone may be used for 21 days for simple hyperplasia. A small study of 34 patients with nonatypical endometrial hyperplasia compared thermal balloon ablation with traditional progestin therapy. Six or 12 months after treatment, normal endometrium was found on biopsy in 76% of patients treated with thermal balloon ablation, and in 65% of those treated with progestin. These preliminary results indicated at least equivalent results for ablation versus progestin therapy, considering the number of patients who required subsequent hysterectomy [24]. Endometrial ablation may play an important role in the treatment of nonatypical hyperplasia. Hysterectomy remains the final option when the previously mentioned therapies have failed, and AUB with failed medical therapy is reported as the indication for 20% of hysterectomies performed in the United States. It is of paramount importance to exclude a uterine malignancy before using any of these treatment options.

Patients with endometrial carcinoma require total abdominal hysterectomy and bilateral salpingo-oophorectomy with surgical staging, including abdominal exploration, collection of peritoneal cytology, and possible selective pelvic and para-aortic lymphadenectomy [2]. The management of atypical endometrial hyperplasia is less apparent. Most women with atypical endometrial hyperplasia undergo hysterectomy [18]. But endometrial carcinoma has been reported to co-exist with atypical complex hyperplasia, with invasive cancers found in up to 42.6% of hysterectomies performed for atypical complex hyperplasia [1]. It is advisable that the surgeon request intraoperative pathologic evaluation by frozen section to exclude an invasive tumor in the hysterectomy specimen [25].

Because approximately 8% of endometrial carcinomas occur in patients younger than 45 years who may wish to preserve fertility, alternatives to hysterectomy have been studied. High-dose daily progesterones (40–400 mg megestrol, 200–800 mg medroxyprogesterone) have been used in patients desiring future fertility along with repeat endometrial sampling every 3 months. This resulted in a resolution rate of 50% to 80% but

a recurrence rate of 30% to 40% [3]. Progestin therapy may be used for younger patients with well-differentiated endometrial adenocarcinoma who have no evidence of myometrial or cervical invasion by MRI [3]. Small studies have also used IUD delivery of progestins and gonadotropin-releasing hormone agonists to treat early stage endometrial cancer in younger patients [26]. These treatments are all designed to delay definitive surgery with staging, but by no means can replace surgery after a pregnancy is completed [4].

Pathologic evaluation of endometrial biopsies and the pathology of endometrial hyperplasia

Adequate pathologic interpretation of the EMB or curettage specimen is essential for the management of patients with AUB, especially for the detection of precancerous or malignant lesions. Most endometrial specimens from patients with AUB are either normal (proliferative or secretory phase at reproductive age, inactive or atrophic in menopause) or abnormal but without representing an increased risk for cancer. Common abnormal histologic patterns not associated with an increased cancer risk are caused by the hormonal changes of anovulatory cycles, hormonal changes of perimenopause, or HRT. Prolonged exposure to unopposed estrogen caused by anovulatory cycles or exogenous estrogen typically results in disordered proliferative endometrium. Delayed ovulation can superimpose progestin-related changes on a previously estrogen-exposed endometrium and result in histologic patterns with mixed proliferative and secretory changes. Progestin therapy can lead to a pattern with small inactive glands and stroma with changes resembling decidua (ie, pseudodecidualization). Prolonged or declining estrogen exposure commonly leads to breakdown of endometrial glands and stroma with subsequent shedding. Anatomic abnormalities that cause AUB include benign endometrial or endocervical polyps and submucosal leiomyoma. When endometrial hyperplasia enters the differential diagnosis, the cancer risk depends on the type of hyperplasia and the presence or absence of cytologic atypia. Complex atypical hyperplasia is associated with a subsequent cancer risk of up to 42.6%, compared with around 1% for simple nonatypical hyperplasia (Table 1) [1,27,28].

The 1994 World Health Organization (WHO) classification of endometrial hyperplasia is the most commonly used, and comprises four categories: (1) simple hyperplasia without atypia, (2) complex hyperplasia without atypia, (3) simple atypical hyperplasia, and (4) complex atypical hyperplasia. Fig. 1 shows photomicrograph images of normal proliferative endometrium; simple hyperplasia without atypia; complex atypical hyperplasia; endometrial intraepithelial neoplasia ([EIN] discussed later); and a low-grade endometrioid carcinoma. Pathologists assign cases into one category based on architectural features and the degree of cytologic atypia [29]. Simple hyperplasias have endometrial glands with predominately simple (tubular or

Table 1
Number of patients diagnosed with endometrial hyperplasia that progressed to endometrial carcinoma (ie, cancer risk)

Diagnosis	Kurman et al 1985 [27]	Baak et al 2001 [36]	Baak et al 2005 [28]	Trimble et al 2006 [1]
Simple hyperplasia	1 (1%) of 93	1 (1.5%) of 65	2 (0.7%) of 289	
Complex hyperplasia	1 (3%) of 29	0 (0%) of 6	6 (9%) of 65	
Simple atypical hyperplasia	1 (8%) of 13	3 (8%) of 38	5 (7.5%) of 67	
Complex atypical hyperplasia	10 (29%) of 35	7 (30%) of 23	11 (20%) of 56	
Total	13 (8%) of 170	11 (8%) of 132	24 (5%) of 477	
Morphometric score according to Baak et al [28,36]				
D score >1 ("favorable")		0 (0%) of 86		
D score 0–1 ("uncertain")		1 (5%) of 20		
D score ≤0.0 ("unfavorable" or EIN)		10 (38%) of 26		
Total		11 (8%) of 132		
Non-EIN			2 (0.6%) of 359	
EIN			22 (19%) of 118	
Total			24 (5%) of 477	
Atypical endometrial hyperplasia				123 (42.6%) of 289 had concurrent carcinoma

Abbreviation: EIN, endometrial intraepithelial neoplasia.

cystic) shapes, lack gland crowding, and have a low gland-to-stroma ratio. Complex hyperplasias show gland crowding with an increased ratio of glands relative to the stroma. Complex glands have irregular shapes with branching and infoldings. Cytologic atypia is present when epithelial cells or nuclei lose their normally polarized columnar shape (ie, loss of polarity), and when nuclear enlargement or variation in size and shape are present. An abnormal nuclear staining quality with chromatin clumping or clearing can also signify cytologic atypia [29]. Some weaknesses of the 1994 WHO hyperplasia classification have emerged. Studies have revealed disappointing levels of interobserver reproducibility for the four hyperplasia categories, even among experienced gynecologic pathologists [30–32]. This is because the histologic appearance of any hyperplasia category can vary substantially from patient to patient, and even in the same endometrial specimen. Because the endometrium constantly responds to the endocrine environment, each hyperplasia case can have a different degree and combination of

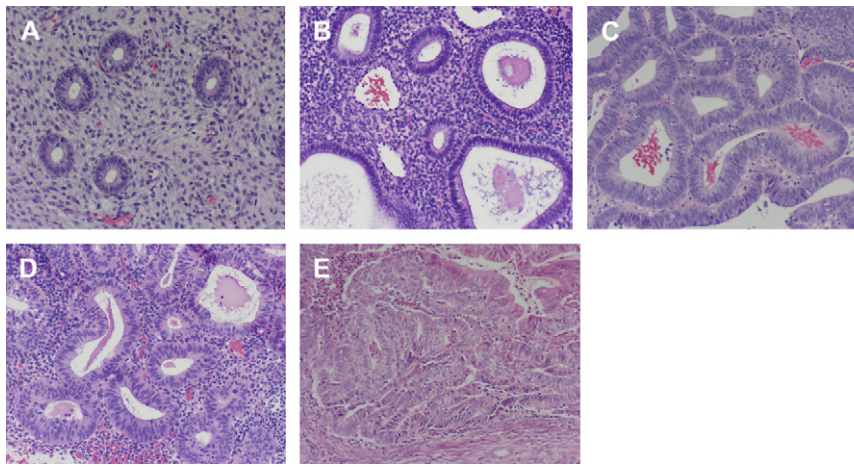

Fig. 1. Photomicrographs of endometrial biopsies with normal proliferative endometrium (*A*), simple hyperplasia without atypia (*B*), complex atypical hyperplasia (*C*), endometrial intraepithelial neoplasia (*D*), and a low-grade endometrioid carcinoma (*E*) (hematoxylin-eosin stains, original magnification ×200).

morphologic abnormalities. Furthermore, cytologic atypia can be found in nonhyperplastic lesions, such as benign reparative or metaplastic changes, which can be misinterpreted as hyperplastic atypia. This diagnostic dilemma translates into a clinical problem for patient management, because the different hyperplasia types are associated with different cancer risks. Complex atypical hyperplasia is now widely accepted as a cancer precursor. Although simple nonatypical hyperplasia has a cancer risk of approximately 1%, this risk increases to up to 42.6% with complex atypical hyperplasia (see Table 1) [1,27,28]. A recent study by the Gynecologic Oncology Group found invasive endometrioid adenocarcinoma in hysterectomy specimens of 42.6% of patients in whom the preoperative diagnosis was atypical endometrial hyperplasia [1]. Because of the clinical implication of assessing cancer risk for individual patients, two research tools were used to address this problem, morphometry and molecular studies, which were then elegantly combined into the concept of EIN [33]. Fig. 1 shows examples of endometrial biopsies with normal proliferative endometrium, simple hyperplasia without atypia, complex atypical hyperplasia, EIN, and a well-differentiated endometrioid carcinoma for comparison.

In the late 1980s researchers analyzed histologic sections of atypical endometrial hyperplasia using computerized image analysis (ie, morphometry) [34]. They measured architectural and cytologic features by overlaying a grid onto a microscopic field of hyperplastic endometrial tissue. Through subsequent statistical analysis, one architectural and one cytologic parameter emerged as the best prognosticators to predict progression of atypical hyperplasia to carcinoma. An increase in the number of endometrial glands

relative to the stroma (volume percentage stroma) emerged as the single best prognosticator for subsequent cancer development. On the cytologic level, the standard deviation of the shortest nuclear axis was the best morphometric prognosticator. A parameter called ''outer surface density of the glands'' also added to the discriminating power [34]. The investigators condensed these three morphometric parameters into a formula to calculate a D score, with values ranging from −4 to +4. Lesions with a D score of less than or equal to 0.0 were associated with high cancer risk (see Table 1), and are today termed ''endometrial intraepithelial neoplasia.'' Patients with a D score greater than 1 had a low cancer risk, and values between 0 and less than or equal to 1 were considered uncertain (see Table 1) [35,36].

A prospective multicenter study published in 2001 evaluated the D score for prediction of outcome of endometrial hyperplasia [36]. Endometrial curettings from 132 patients diagnosed as hyperplasia were analyzed by morphometry, assigned a D score, and patients were followed for up to 10 years. Overall, 11 (8%) of the 132 patients were subsequently diagnosed with endometrial carcinoma. Only 1 (1%) of 71 patients with nonatypical hyperplasia developed cancer, whereas 10 (16%) of 61 with atypical hyperplasia did develop cancer (see Table 1). Twenty-six patients had a D score of less than or equal to 0.0 (classified as ''unfavorable'' or EIN), of which 10 (38%) developed cancer. None of the 86 cases with D score greater than 1 (''favorable'') developed cancer, and only 1 (5%) of 20 cases with D score between 0 and 1 (classified as ''uncertain'') developed cancer (see Table 1). The D score had a sensitivity of 100% and a specificity of 82%, higher values than the WHO classification with a sensitivity of 91% and a specificity of 58%. Positive and negative predictive values of the D score were 38% and 100%, respectively, compared with 15% and 99% of the WHO classification. The authors concluded that the D score is a better marker for cancer prediction than the WHO classification, and that it is highly reproducible and cost-effective [36].

Another series of morphometric studies analyzed the nuclear chromatin pattern in normal endometrium, hyperplasias, and carcinomas [37]. The researchers recorded digital images from histologic sections; measured over 90 morphometric nuclear features (eg, nuclear area and optical density); and then created nuclear signatures for each diagnostic category. Although the nuclei from endometrial lesions comprised a highly heterogeneous population, statistical analysis revealed significant differences in nuclear subsets from nonatypical hyperplasias, atypical hyperplasias, and endometrial adenocarcinomas. The nuclear changes also showed a trend of progression from normal endometrium toward adenocarcinoma [37]. A later study compared nuclei from atypical hyperplasias with and without co-occurring adenocarcinoma, and found statistically significant morphometric (karyometric) differences among the two. The authors concluded that karyometric analysis of nuclear subpopulations can correctly classify an estimated 85% of cases [38].

Molecular pathology of endometrial hyperplasia and carcinoma

Endometrial carcinomas are classified into two major types: the endometrioid type (type I) and the serous papillary type (type II). Type I cancers typically arise in a background of atypical hyperplasia, are associated with prolonged estrogen exposure, and have a better prognosis. Type II cancers are usually associated with endometrial atrophy, occur in older patients, and have a worse prognosis. Both types are not only separated by clinical and prognostic features, but also have different molecular alterations and evolve through a dualistic pathway of molecular pathogenesis [12]. Type I carcinomas arise through a sequence of genetic damages that occur in the evolution from normal benign endometrium to atypical complex hyperplasia to invasive carcinoma, and a comprehensive model of this pathogenesis has been formulated [12,39]. The most commonly described molecular alterations in this sequence are listed in Table 2, but the understanding of the pathogenesis of endometrial hyperplasia and endometrioid carcinoma is still incomplete.

The most common molecular alterations of endometrial hyperplasia and endometrioid carcinoma are mutations in the PTEN and K-*ras* genes and microsatellite instability (MIS). PTEN mutations are the earliest known alteration to occur, and were found in 16.7% to 36% of atypical hyperplasias [40–44]. PTEN mutations are now regarded as an early and potentially initiating event in endometrial carcinogenesis. More recent studies found mutated PTEN at rates increasing from nonatypical to atypical hyperplasia to invasive carcinoma [43,44]. Abnormal PTEN expression by immunohistochemistry was described even in rare histologically normal proliferative endometrium [45]. PTEN is a tumor suppressor gene. It is one of the most commonly mutated genes in malignancies from many organ systems, and plays a role in regulating the cell cycle, apoptosis, and cell migration and differentiation [46]. The PTEN gene is located on chromosome 10q23, and its protein contains a tyrosine phosphatase domain with the second messenger phosphatidylinositol-(3,4,5)-triphosphate as its main substrate [46].

The K-*ras* proto-oncogene encodes a protein that is located on the inner plasma membrane and is involved in signal transduction pathways through its GTPase activity. Ras mutations are common in many human malignancies, including 90% of pancreatic and 50% of colorectal carcinomas. The K-*ras* gene mutation occurs in up to one third of endometrioid-type endometrial carcinomas and about half as many hyperplasias, and is thought to be an early event (see Table 2) [47–53].

MIS is caused by loss of function of mismatch repair genes. Microsatellites are short repetitive sequences that are distributed throughout the genome. When DNA replication errors occur, they are normally repaired by nuclear enzymes of the DNA mismatch repair system. When one of the repair enzymes is inactivated, some replication errors are not corrected. Genetic damage can accumulate and facilitate tumor formation in this

Table 2
Molecular alterations in endometrial hyperplasia and endometrioid endometrial carcinoma

Gene or Genetic damage	Atypical hyperplasia	Endometrioid carcinoma	Comment or Conclusion	References
PTEN mutation	16.7%–36%	26%–39%	Early event in carcinogenesis, may be initiating event, may occur before or after MIS	[40–44]
		83% in Mutter et al [42]		
K-*ras* mutation	13%–22%, 0% of 5 cases [51], 11 (55%) of 20 atypical and nonatypical hyperplasias with synchronous carcinoma [50]	15%–35%		[47–53]
Microsatellite instability	4.2%–50%	38%–44%	Early event in carcinogenesis	[11,44,54,55]
	0% of 27 cases selected for age $\leq$50 [55]	28% of 18 cases selected for age $\leq$50 [55]		
Beta-catenin mutation, abnormal IHC	Abnormal in 13% (mutation) to 35% (by IHC)	Abnormal in 23% (mutation) to 80% (by IHC)		[56,57]
E-cadherin, P-cadherin, and p120^{ctn}, abnormal IHC	↘ in 40%, 9.5%, 57.1%, respectively	↘ in 50%, 27.7%, 60.3%, respectively		[57]
Clonality by X-chromosome inactivation	Two of four cases monoclonal, two cases inconclusive	Nine of 10 monoclonal, grade 1		[58]
	Seven (77.8%) of nine monoclonal	Fifteen (68.3%) of 22 monoclonal	Normal endometrium polyclonal, three of three polyps monoclonal	[59]
	Four cases monoclonal	Five cases monoclonal	Five simple hyperplasias and normal endometria polyclonal	[60]

(*continued on next page*)

Table 2 (*continued*)

Gene or Genetic damage	Atypical hyperplasia	Endometrioid carcinoma	Comment or Conclusion	References
Comparative genomic hybridization	Genetic gains and losses in 12 (66.7%) of 18 cases, most common on chromosomes 1, 4, 16, 20	NA	Genetic gains and losses more frequent in atypical complex than in nonatypical (41.4%) hyperplasias	[61]
	Genetic gains and losses most common on chromosomes 1, 8, 10	Genetic abnormalities found in atypical hyperplasias also occurred in carcinomas		[62]
	Genetic gains and losses most common on chromosomes 1, 4, 20	Genetic gains and losses most common on chromosomes 1, 3, 8	Different patterns of chromosomal alterations in hyperplasias and carcinomas, except for chromosome 1	[63]
FISH	Chromosome 1 or 17 alterations in all five (100%) cases	Chromosome 1 or 17 alterations in 26 (90%) of 29 cancers	FISH assays performed on ThinPrep cytology preparations	[64]

Percentages of cases positive for a genetic alteration or abnormal IHC staining are given. Because this article is focused on endometrial hyperplasia and precancer, only references that include hyperplasia are listed.

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MIS, microsatellite instability; NA, not analyzed.

process called MIS. Approximately 30% to 40% of sporadic type I endometrial carcinomas have MIS, most commonly because promoter hypermethylation inactivates the repair enzyme hMLH-1. Because MIS also occurs in a subset of endometrial hyperplasias (see Table 2), it is thought to be an early event in endometrial carcinogenesis [11,44,54,55].

Abnormal expression of molecules involved in the regulation of cell adhesion was also found in hyperplasias and carcinomas by immunohistochemistry and mutational analysis. Beta-catenin is normally expressed in the cell membrane, and in some malignancies this staining distribution translocates to the nucleus. Beta-catenin showed a stepwise decrease in cell membrane staining from normal endometrium through atypical hyperplasias to low- and high-grade carcinomas. Nuclear accumulation of beta-catenin staining was more pronounced in atypical hyperplasias and

carcinomas, compared with nonatypical hyperplasia [56]. Mutations in exon 3 of the beta-catenin gene were found in a subset of atypical hyperplasias and a higher number of carcinomas (see Table 2) [56]. Abnormal expression of beta-catenin, E- and P-cadherin, and $p120^{ctn}$ was also more frequent in carcinomas compared with atypical hyperplasias (see Table 2) [57]. Malignant tumors and most precancers are clonal populations of neoplastic cells. When atypical hyperplasias were analyzed for clonality, various numbers of cases were found to be clonal proliferations (see Table 2) [58–60].

The damage that occurs to individual chromosomes in the evolution of a neoplastic process was investigated by several authors using comparative genomic hybridization. Gains and losses of genetic material were more frequent in atypical compared with nonatypical hyperplasias [61]. One study found that the gains and losses of atypical hyperplasias were also present in carcinomas [62], but another reported that the gains and losses in hyperplasias were different from those in carcinomas (see Table 2) [63]. Baloglu and colleagues [62] concluded that atypical endometrial hyperplasia is closely related to endometrioid carcinoma and should be considered a precancer, whereas simple hyperplasia is a benign disorder. Because abnormalities on chromosomes 1 and 17 were known to occur in endometrial cancers, one study used fluorescence in situ hybridization with probes for chromosomes 1 and 17 to test endometrial specimens for abnormalities on these chromosomes [64]. Cytology specimens were collected by transvaginal sampling using a brush and prepared with a liquid-based method. Alterations of chromosomes 1 and 17 were detected in all of five atypical hyperplasias and in 26 (90%) of 29 endometrioid carcinomas (see Table 2). This study is one of the first that analyzed specimens collected preoperatively, and was able to identify not only carcinomas but atypical hyperplasias with the fluorescence in situ hybridization technique [64]. Gene expression microarray analysis found a plethora of genes that were differentially expressed between normal endometrium and carcinomas [65]. Another gene chip study identified genes that are differentially expressed between the different histologic types of endometrial carcinomas, including endometrioid, serous papillary, and clear cell carcinomas [66]. There is much hope that these promising studies will lead to the discovery of molecular markers for endometrial cancer and precancer to allow early detection of these lesions, or triage of women with concomitant endometrial cancer who should have appropriate referral to a gynecologic oncologist.

The concept of endometrial intraepithelial neoplasia

EIN has emerged as a novel diagnostic entity over the past decade, and fulfills all the criteria required for a precancer as outlined by the Precancer Consensus Conference in 2006 [67]. EIN is defined by its histopathologic features, its cancer risk as substantiated by clinical outcome data, and by specific molecular genetic alterations [33,68]. In 41% of patients diagnosed

with EIN, an endometrial carcinoma is found within 1 year, thought to represent a synchronous tumor present at the time of the diagnostic procedure. If no cancer is found within a year, patients have a 45-fold increased risk of developing a carcinoma [33].

The histopathologic criteria required for a diagnosis of EIN are a combination of architectural and cytologic changes. Architecturally, EIN lesions are composed of crowded glands with a gland-to-stoma ratio of greater than 1, and have to be more than 1 mm in greatest diameter [33]. EIN lesions are most commonly focal, but in approximately 20% of cases the entire endometrium is involved. Cytologically, nuclear or cytoplasmic features must differ between lesional cells and the normal background, and must include differences in nuclear polarity, nuclear pleomorphism, or altered cytoplasmic differentiation. When considering a diagnosis of EIN, pathologists have to compare the lesional cells with the surrounding normal glands. If no normal glands are present, nuclei must be "highly abnormal" to establish a diagnosis of EIN. Finally, it is crucial to exclude a benign lesion that can mimic EIN. Disordered proliferative or secretory endometrium, normal basalis, or benign polyps can have areas of gland crowding. Repair changes can have cytologic atypia with enlarged nuclei or altered cytoplasm that may stand out from the surrounding tissue. Finally, an invasive carcinoma has to be excluded before a diagnosis of EIN is made [33].

The value of EIN as a clinicopathologic entity has been established by follow-up studies that predict the associated cancer risk (see Table 1) [28,34,35]. Initially, EIN lesions were defined by morphometric studies that assigned a D score to each case (as described previously), but the diagnosis was highly reproducible by pathologists even without morphometry. Finally, molecular alterations define EIN as a clonal lesion that shares molecular abnormalities with endometrioid carcinoma. These include mutations of the PTEN and K-*ras* genes, and MIS. In contrast to the 1994 WHO classification, the EIN concept clearly separates the neoplastic clonal proliferations of EIN lesions as precancers from the benign architectural changes of unopposed estrogens called "benign endometrial hyperplasia" [69]. The histologic changes produced by unopposed estrogens (nonatypical hyperplasias) are quite unlike localized EIN lesions. Finally, morphometric analysis defined the specific pathologic changes of EIN that were associated with increased cancer risk [36]. Because EIN and complex atypical hyperplasia are defined by different diagnostic criteria, only 79% of atypical endometrial hyperplasias translate to EIN, and approximately one third of all EIN diagnoses are garnered from nontypical hyperplasia categories [70].

Type II endometrial carcinoma and endometrial intraepithelial carcinoma

Type II endometrial cancers include serous papillary carcinoma and clear cell carcinoma. These are biologically different from endometrioid (type I) carcinomas in that they occur in older patients, are not related to unopposed

estrogen exposure, and are typically associated with endometrial atrophy rather than hyperplasia. Approximately 10% of all endometrial cancers show serous papillary and 4% clear cell histology. These tumors are high grade, more often high stage, and have a worse prognosis than type I tumors. The most frequent mutations in type II carcinomas are in the p53 gene, followed by inactivation of the tumor suppressor genes p16 and beta-catenin. Her2/neu gene amplification can sometimes be found. The two histologic patterns (type I versus type II) of endometrial malignancies correspond to a dualistic model of molecular carcinogenesis [12,71,72]. Because type II carcinomas are so aggressive and usually fatal, it would be extremely useful if a precancerous lesion could be identified that is detectable in endometrial biopsies before invasive cancer develops.

A putative precursor for serous carcinoma called "endometrial intraepithelial carcinoma" has been described [73–80]. In most studies, however, endometrial intraepithelial carcinoma was found in endometria that also harbored invasive serous papillary carcinoma, and other researchers do not accept endometrial intraepithelial carcinoma as a true precancer. Between 78% and 100% of endometrial intraepithelial carcinoma lesions are p53 positive by immunohistochemistry [74,76,79,80], and p53 mutations were detected in 78% of endometrial intraepithelial carcinoma lesions after microdissection [78]. The glucose transporter GLUT1 and p63 were expressed in 83% and 92% of endometrial intraepithelial carcinoma lesions, and were also detected in the associated serous carcinomas [80]. A putative precursor for clear cell carcinoma was recently reported that stained for p53 with a staining score slightly lower than for clear cell carcinoma [81].

Future developments

Knowledge in the molecular pathogenesis of atypical complex hyperplasia, EIN, and endometrial carcinoma has evolved considerably since the first genetic alterations were discovered. It is hoped that the ongoing accelerated acquisition of novel data will soon lead to the development of screening tests and of molecular markers that can be tested on biopsy tissue or blood. Ideally, clinicians will be able to identify patients at risk before clinical symptoms, such as AUB, develop. Precancerous endometrial lesions could potentially be treated with chemoprevention, as already occurs with breast cancer, and invasive carcinomas could be treated curatively when discovered at the earliest possible stage. Some promising molecular markers for endometrial precancers already exist, but this knowledge has to be translated into clinical practice. In addition, new technologies, such as genomics and proteomics, will likely lead the way toward discovery of more diagnostic markers for early diagnosis and prevention of endometrial neoplasms.

References

[1] Trimble CL, Kauderer J, Zaino R, et al. Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia: a gynecologic oncology group study. Cancer 2006;106(4):812–9.

[2] Hillard PJ. Benign diseases of the female reproductive tract. In: Berek JS, editor. Berek & Novak's gynecology. 14th edition. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 431–504.

[3] Seli E, Tangir J. Fertility preservation options for female patients with malignancies. Curr Opin Obstet Gynecol 2005;17(3):299–308.

[4] Rackow BW, Arici A. Endometrial cancer and fertility. Curr Opin Obstet Gynecol 2006; 18(3):245–52.

[5] Fazio SB, Ship AN. Abnormal uterine bleeding. South Med J 2007;100(4):376–82 [quiz 383, 402].

[6] Albers JR, Hull SK, Wesley RM. Abnormal uterine bleeding. Am Fam Physician 2004;69(8): 1915–26.

[7] Mishell DR Jr. Abnormal uterine bleeding. In: Stenchever MA, Droegemueller W, Herbst AL, et al, editors. Comprehensive gynecology. 4th edition. St. Louis: Mosby; 2001. p. 1079–97.

[8] Lane DE. Polycystic ovary syndrome and its differential diagnosis. Obstet Gynecol Surv 2006;61(2):125–35.

[9] Prasad M, Wang H, Douglas W, et al. Molecular genetic characterization of tamoxifen-associated endometrial cancer. Gynecol Oncol 2005;96(1):25–31.

[10] Gadducci A, Gargini A, Palla E, et al. Polycystic ovary syndrome and gynecological cancers: is there a link? Gynecol Endocrinol 2005;20(4):200–8.

[11] Esteller M, Catasus L, Matias-Guiu X, et al. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. Am J Pathol 1999;155(5):1767–72.

[12] Matias-Guiu X, Catasus L, Bussaglia E, et al. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol 2001;32(6):569–77.

[13] Miller C. Cowden disease (multiple hamartoma syndrome). eMedicine from WebMD. Available at: http://www.emedicine.com/derm/topic86.htm. Accessed October 15, 2007.

[14] Dijkhuizen FP, Mol BW, Brolmann HA, et al. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 2000;89(8):1765–72.

[15] Cooper JM, Erickson ML. Endometrial sampling techniques in the diagnosis of abnormal uterine bleeding. Obstet Gynecol Clin North Am 2000;27(2):235–44.

[16] de Kroon CD, Jansen FW. Saline infusion sonography in women with abnormal uterine bleeding: an update of recent findings. Curr Opin Obstet Gynecol 2006;18(6):653–7.

[17] Mahoney SF, Armstrong A. Accurate diagnosis of postmenopausal bleeding. Nurse Pract 2005;30(8):61–3.

[18] Clark TJ. Outpatient hysteroscopy and ultrasonography in the management of endometrial disease. Curr Opin Obstet Gynecol 2004;16(4):305–11.

[19] Fleischer AC, Wheeler JE, Lindsay I, et al. An assessment of the value of ultrasonographic screening for endometrial disease in postmenopausal women without symptoms. Am J Obstet Gynecol 2001;184(2):70–5.

[20] Banu NS, Manyonda IT. Alternative medical and surgical options to hysterectomy. Best Pract Res Clin Obstet Gynaecol 2005;19(3):431–49.

[21] Singh RH, Blumenthal P. Hormonal management of abnormal uterine bleeding. Clin Obstet Gynecol 2005;48(2):337–52.

[22] Lethaby AE, Cooke I, Rees M. Progesterone or progestogen-releasing intrauterine systems for heavy menstrual bleeding. Cochrane Database of Systematic Reviews 2005;4:CD002126.

[23] Vilos GA. Hysteroscopic and nonhysteroscopic endometrial ablation. Obstet Gynecol Clin North Am 2004;31(3):687–704, xi.

[24] Järvelä IY, Santala M. Treatment of non-atypic endometrial hyperplasia using thermal balloon endometrial ablation therapy. Gynecol Obstet Invest 2005;59(4):202–6.
[25] Karamursel BS, Guven S, Tulunay G, et al. Which surgical procedure for patients with atypical endometrial hyperplasia? Int J Gynecol Cancer 2005;15(1):127–31.
[26] Jadoul P, Donnez J. Conservative treatment may be beneficial for young women with atypical endometrial hyperplasia or endometrial adenocarcinoma. Fertil Steril 2003;80(6):1315–24.
[27] Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia: a long-term study of untreated hyperplasia in 170 patients. Cancer 1985;56(2):403–12.
[28] Baak JP, Mutter GL, Robboy S, et al. The molecular genetics and morphometry-based endometrial intraepithelial neoplasia classification system predicts disease progression in endometrial hyperplasia more accurately than the 1994 World Health Organization classification system. Cancer 2005;103(11):2304–12.
[29] Scully RE, Bonfiglio TA, Kurman RJ, et al. Uterine corpus. World Health Organization: histological typing of female genital tract tumors. New York: Springer-Verlag; 1994. p. 13–31.
[30] Bergeron C, Nogales FF, Masseroli M, et al. A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. Am J Surg Pathol 1999; 23(9):1102–8.
[31] Kendall BS, Ronnett BM, Isacson C, et al. Reproducibility of the diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated carcinoma. Am J Surg Pathol 1998; 22(8):1012–9.
[32] Zaino RJ, Kauderer J, Trimble CL, et al. Reproducibility of the diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. Cancer 2006;106(4):804–11.
[33] Mutter GL, Zaino RJ, Baak JP, et al. Benign endometrial hyperplasia sequence and endometrial intraepithelial neoplasia. Int J Gynecol Pathol 2007;26(2):103–14.
[34] Baak JP, Nauta JJ, Wisse-Brekelmans EC, et al. Architectural and nuclear morphometrical features together are more important prognosticators in endometrial hyperplasias than nuclear morphometrical features alone. J Pathol 1988;154(4):335–41.
[35] Ørbo A, Baak JP, Kleivan I, et al. Computerised morphometrical analysis in endometrial hyperplasia for the prediction of cancer development: a long-term retrospective study from northern Norway. J Clin Pathol 2000;53(9):697–703.
[36] Baak JP, Ørbo A, van Diest PJ, et al. Prospective multicenter evaluation of the morphometric D-score for prediction of the outcome of endometrial hyperplasias. Am J Surg Pathol 2001;25(7):930–5.
[37] Garcia FA, Davis JR, Alberts DS, et al. Nuclear chromatin patterns in normal, hyperplastic and atypical endometrium. Anal Quant Cytol Histol 2001;23(2):144–50.
[38] Garcia FA, Davis JR, Alberts DS, et al. A karyometric approach to the characterization of atypical endometrial hyperplasia with and without co-occurring adenocarcinoma. Anal Quant Cytol Histol 2003;25(6):339–46.
[39] Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol 2006;24(29):4783–91.
[40] Levine RL, Cargile CB, Blazes MS, et al. PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. Cancer Res 1998;58(15):3254–8.
[41] Maxwell GL, Risinger JI, Gumbs C, et al. Mutation of the PTEN tumor suppressor gene in endometrial hyperplasias. Cancer Res 1998;58(12):2500–3.
[42] Mutter GL, Lin MC, Fitzgerald JT, et al. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000;92(11):924–30.
[43] Sun H, Enomoto T, Fujita M, et al. Mutational analysis of the PTEN gene in endometrial carcinoma and hyperplasia. Am J Clin Pathol 2001;115(1):32–8.
[44] Kanaya T, Kyo S, Sakaguchi J, et al. Association of mismatch repair deficiency with PTEN frameshift mutations in endometrial cancers and the precursors in a Japanese population. Am J Clin Pathol 2005;124(1):89–96.

[45] Mutter GL, Ince TA, Baak JP, et al. Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 2001;61(11):4311–4.
[46] Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000; 100(4):387–90.
[47] Enomoto T, Inoue M, Perantoni AO, et al. K-*ras* activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res 1991;51(19):5308–14.
[48] Enomoto T, Fujita M, Inoue M, et al. Alterations of the p53 tumor suppressor gene and its association with activation of the c-K-*ras*-2 protooncogene in premalignant and malignant lesions of the human uterine endometrium. Cancer Res 1993;53(8):1883–8.
[49] Sasaki H, Nishii H, Takahashi H, et al. Mutation of the Ki-*ras* protooncogene in human endometrial hyperplasia and carcinoma. Cancer Res 1993;53(8):1906–10.
[50] Tsuda H, Jiko K, Yajima M, et al. Frequent occurrence of c-Ki-*ras* gene mutations in well differentiated endometrial adenocarcinoma showing infiltrative local growth with fibrosing stromal response. Int J Gynecol Pathol 1995;14(3):255–9.
[51] Esteller M, Garcia A, Martinez-Palones JM, et al. The clinicopathological significance of K-*ras* point mutation and gene amplification in endometrial cancer. Eur J Cancer 1997;33(10):1572–7.
[52] Mutter GL, Wada H, Faquin WC, et al. K-*ras* mutations appear in the premalignant phase of both microsatellite stable and unstable endometrial carcinogenesis. Mol Pathol 1999; 52(5):257–62.
[53] Lagarda H, Catasus L, Arguelles R, et al. K-*ras* mutations in endometrial carcinomas with microsatellite instability. J Pathol 2001;193(2):193–9.
[54] Hardisson D, Moreno-Bueno G, Sanchez L, et al. Tissue microarray immunohistochemical expression analysis of mismatch repair (hMLH1 and hMSH2 genes) in endometrial carcinoma and atypical endometrial hyperplasia: relationship with microsatellite instability. Mod Pathol 2003;16(11):1148–58.
[55] Sutter C, Dallenbach-Hellweg G, Schmidt D, et al. Molecular analysis of endometrial hyperplasia in HNPCC-suspicious patients may predict progression to endometrial carcinoma. Int J Gynecol Pathol 2004;23(1):18–25.
[56] Saegusa M, Hashimura M, Yoshida T, et al. beta-Catenin mutations and aberrant nuclear expression during endometrial tumorigenesis. Br J Cancer 2001;84(2):209–17.
[57] Moreno-Bueno G, Hardisson D, Sarrio D, et al. Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. J Pathol 2003;199(4):471–8.
[58] Mutter GL, Chaponot ML, Fletcher JA. A polymerase chain reaction assay for non-random X chromosome inactivation identifies monoclonal endometrial cancers and precancers. Am J Pathol 1995;146(2):501–8.
[59] Jovanovic AS, Boynton KA, Mutter GL. Uteri of women with endometrial carcinoma contain a histopathological spectrum of monoclonal putative precancers, some with microsatellite instability. Cancer Res 1996;56(8):1917–21.
[60] Esteller M, Garcia A, Martinez-Palones JM, et al. Detection of clonality and genetic alterations in endometrial pipelle biopsy and its surgical specimen counterpart. Lab Invest 1997;76(1):109–16.
[61] Kiechle M, Hinrichs M, Jacobsen A, et al. Genetic imbalances in precursor lesions of endometrial cancer detected by comparative genomic hybridization. Am J Pathol 2000;156(6):1827–33.
[62] Baloglu H, Cannizzaro LA, Jones J, et al. Atypical endometrial hyperplasia shares genomic abnormalities with endometrioid carcinoma by comparative genomic hybridization. Hum Pathol 2001;32(6):615–22.
[63] Muslumanoglu HM, Oner U, Ozalp S, et al. Genetic imbalances in endometrial hyperplasia and endometrioid carcinoma detected by comparative genomic hybridization. Eur J Obstet Gynecol Reprod Biol 2005;120(1):107–14.
[64] Susumu N, Aoki D, Noda T, et al. Diagnostic clinical application of two-color fluorescence in situ hybridization that detects chromosome 1 and 17 alterations to direct touch smear and

liquid-based thin-layer cytologic preparations of endometrial cancers. Int J Gynecol Cancer 2005;15(1):70–80.
[65] Mutter GL, Baak JP, Fitzgerald JT, et al. Global expression changes of constitutive and hormonally regulated genes during endometrial neoplastic transformation. Gynecol Oncol 2001;83(2):177–85.
[66] Risinger JI, Maxwell GL, Chandramouli GV, et al. Microarray analysis reveals distinct gene expression profiles among different histologic types of endometrial cancer. Cancer Res 2003; 63(1):6–11.
[67] Berman JJ, Albores-Saavedra J, Bostwick D, et al. Precancer: a conceptual working definition—results of a consensus conference. Cancer Detect Prev 2006;30(5):387–94.
[68] Mutter GL. Available at: www.endometrium.org. 2003. Accessed October 10, 2007.
[69] Mutter GL, Baak JP, Crum CP, et al. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. J Pathol 2000;190(4):462–9.
[70] Silverberg SG, Kurman RJ, Nogales F, et al. Epithelial tumors and related lesions. In: Tavassoli FA, Devilee P, editors. World Health Organization classification of tumors. Pathology and genetics of tumors of the breast and female genital organs. Lyon, France: International Agency for Research on Cancer (IARC) Press; 2003. p. 221–32.
[71] Lax SF, Kendall B, Tashiro H, et al. The frequency of p53, K-*ras* mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer 2000;88(4):814–24.
[72] Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch 2004;444(3):213–23.
[73] Ambros RA, Sherman ME, Zahn CM, et al. Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation. Hum Pathol 1995;26(11):1260–7.
[74] Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorigenesis. Hum Pathol 1995;26(11):1268–74.
[75] Spiegel GW. Endometrial carcinoma in situ in postmenopausal women. Am J Surg Pathol 1995;19(4):417–32.
[76] Zheng W, Khurana R, Farahmand S, et al. p53 immunostaining as a significant adjunct diagnostic method for uterine surface carcinoma: precursor of uterine papillary serous carcinoma. Am J Surg Pathol 1998;22(12):1463–73.
[77] Wheeler DT, Bell KA, Kurman RJ, et al. Minimal uterine serous carcinoma: diagnosis and clinicopathologic correlation. Am J Surg Pathol 2000;24(6):797–806.
[78] Baergen RN, Warren CD, Isacson C, et al. Early uterine serous carcinoma: clonal origin of extrauterine disease. Int J Gynecol Pathol 2001;20(3):214–9.
[79] Soslow RA, Pirog E, Isacson C. Endometrial intraepithelial carcinoma with associated peritoneal carcinomatosis. Am J Surg Pathol 2000;24(5):726–32.
[80] Idrees MT, Schlosshauer P, Li G, et al. GLUT1 and p63 expression in endometrial intraepithelial and uterine serous papillary carcinoma. Histopathology 2006;49(1):75–81.
[81] Fadare O, Liang SX, Ulukus EC, et al. Precursors of endometrial clear cell carcinoma. Am J Surg Pathol 2006;30(12):1519–30.

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Cervical Cancer Prevention—Cervical Screening: Science in Evolution

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Cervical cancer burden

Over the past several decades, our understanding of cervical carcinogenesis has increased greatly. Cervical cancer is the most well-understood of human cancers and potentially one of the most preventable. Cervical cancer screening began in the 1950's with the introduction of the Papanicolaou smear, which was considered a milestone in cancer prevention efforts. During the 1960's and 1970's, various sexually transmitted organisms (eg, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *herpes simplex virus type 2*) were implicated as the etiologic agent in cervical carcinogenesis. In the late 1970's and early 1980's, Zur Hausen identified human papillomavirus (HPV) in cases of cervical cancer. By the late 1990's, HPV was convincingly established as a common sexually transmitted infection and that approximately 15 "carcinogenic" HPV genotypes cause virtually all cervical cancer worldwide. Cancer develops rarely (on a per event basis) from an almost universal exposure to HPV, resulting in approximately 500,000 cases and 275,000 related deaths annually (2002 estimates) worldwide [1].

Initially, cervical abnormalities less than cancer were considered to progress in a biological lock-step evolution from mild to moderate to severe dysplasia to carcinoma in situ—or in the cervical intraepithelial neoplasia (CIN) terminology—from grades 1 through 3. Viewed by light microscopy,

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cell and tissue abnormalities appear to form a continuum of severity. However, it is understood now that the majority of low-grade morphological changes are associated with transient HPV infections, which will clear spontaneously, and that high-grade changes can occur de novo from persistent HPV infection without necessarily progressing through lower-grade appearing abnormalities. CIN 2 is no longer considered a separate biological stage in neoplastic development, but rather, it is viewed as a mixture of lesions, some of which are destined to regress and some represent precancer. Histologic CIN 3 is accepted as the most robust surrogate for precancer, although as an added measure for patient safety, CIN 2 is the threshold for treatment in the United States.

Figure 1 presents the current view of cervical carcinogenesis, which unites pathological/morphological changes with HPV virologic states [2]. In this unified view, cervical carcinogenesis can be divided into 1) infection with one or more of the 15 or so carcinogenic types of HPV, which may or may not be associated with low grade cellular changes, 2) viral persistence (failure to clear viral infection), 3) proliferation/progression of a clone of persistently infected cells to a pathologic precancer, CIN 3, often accompanied by early molecular alternations of the virus and host cells, and 4) accumulation of genetic mutations resulting in the capacity for invasion.

Molecular HPV testing has been incorporated into clinical management [3]. Most cytologic findings of atypical squamous cells of undermined significance (ASCUS) are now clarified by triaging with HPV testing. Only HPV-positive ASCUS, with the equivalent risk of low-grade squamous intraepithelial lesion cytology, is referred for colposcopic follow-up, while HPV-negative ASCUS should return to routine screening. For women 30 and older, dual primary screening with HPV and cytology is an option. If both tests are negative, the screening interval is extended to 3 years based on the very low risk for precancer. Studies comparing HPV testing versus cytology in the screening setting demonstrate significantly greater sensitivity, but slightly decreased specificity for HPV testing alone compared to

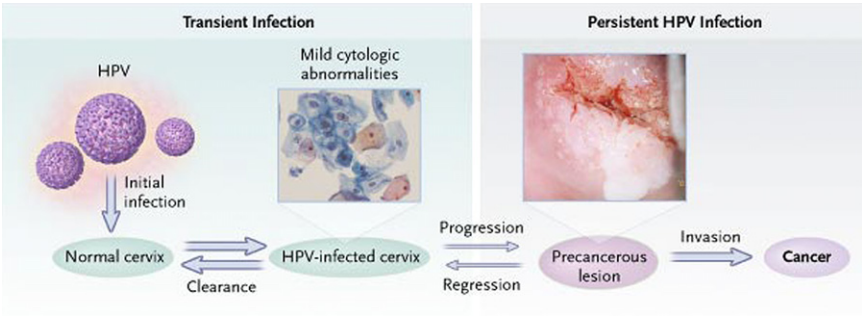

Fig. 1. Steps in cervical carcinogenesis. (*From* Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 2003;348:490; with permission. Copyright © 2003, Massachusetts Medical Society.)

cytology alone [4–6]. There is a wealth of evidence supporting the use of HPV testing in primary screening in women age 30 years and older.

Finally, the development of prophylactic HPV vaccines holds great promise for the primary prevention of cervical cancer. The introduction of vaccines will not eliminate the need for screening (at least for now), but one will need to consider rational strategies to integrate vaccination and screening to ensure women's safety and avoid costly duplication of prevention efforts in the future.

Cervical cancer burden

Worldwide, approximately 500,000 new cases of cervical cancer (approximately one case per minute) are diagnosed and 275,000 related-deaths occur annually, making it the second or third most common female cancer and cancer-related cause of mortality, and it is the cause of about one tenth of all female cancer deaths [1]. Cervical cancer has an especially profound societal impact, because a substantial fraction of cervical cancer occurs in women who are in their 30's and 40's, while they are still raising or supporting families. Cervical cancer is a particularly vexing problem in developing countries and regions where more than 80% of all cervical cancer occurs, and it is often the most common cancer in women. Cervical cancer accounts for 7% of all female malignancies in developed countries, which is in sharp contrast to 24% in developing countries [1,7]. This disparity is attributed primarily to the differences in screening and treatment of precancerous lesions [1].

The incidence and mortality of cervical cancer in the United States has declined significantly since the 1950s by more than 70% [8–10]. This decline is attributed mainly to the introduction of the Papanicolaou (Pap) test in the 1940s. Cervical cancer, which in this country was the number one killer of women, is now ranked twelfth in cancer deaths for women in the United States [11,12]. The American Cancer Society estimates that, in 2007, approximately 11,000 women will be diagnosed with cervical cancer, and approximately 3,600 women will die of it [13].

The anatomic accessibility of the cervix to direct examination and the long preclinical stage during which approximately 95% of precursor lesions can be treated conservatively and successfully [14], make cervical precancer an ideal target for secondary prevention efforts such as screening and treatment. The Pap test probably is the most widely used cancer-screening test, even though it never has been evaluated in a randomized, controlled trial and will not be, because it has been accepted as an effective screening tool, and it would be unethical to not screen women. Additionally numerous convincing epidemiologic data show that, since the introduction of Pap in countries with well-organized screening programs with wide population coverage, both the incidence of and mortality from cervical cancer have decreased significantly. The best data to support these observations come

from Nordic countries, where organized cytology-based screening programs have been launched, and the results have been tracked through cancer registries. The incidence of cervical cancer has fallen by more than 50% in Finland, Sweden, Denmark, and Iceland, where organized cervical screening programs were established in the 1960s [15]. Despite the availability of a Pap screening program in England, the incidence of cervical cancer remained relatively constant until the introduction of an organized screening program in 1988, which led to a dramatic reduction in subsequent years [15].

In the United States, the decline in incidence and mortality in cervical cancer is not experienced uniformly in all segments of the population. In the 1990s, the incidence of cervical cancer was at least 33% higher, and the mortality from cervical cancer was 71% higher in high poverty counties than in low-poverty counties in the United States [16]. The age-adjusted incidence rates between 2000 and 2004 were 8.5 per 100,000 among white women, 11.4 per 100,000 among African American women, and 13.8 per 100,000 among Hispanic women [12]. The incidence of and mortality from cervical cancer are also higher among other minority ethnic groups in the United States, including some Asian populations, than in the general population [17].

The Healthy People 2010 objective of 90% Pap screening for women aged 18 years or older has not yet been achieved by any state or territory [18]. Unscreened populations of women in the United States historically include older women, uninsured and impoverished women, migrant and minority women, and those residing in rural areas [19]; in particular, women from the Mississippi Delta and the Black Belt region, United States–Mexico Border populations, and Appalachia have some of the highest rates of cervical cancer in the United States. Data from the 1994 and 2003 US National Health Interview Survey showed that screening trends have remained unchanged from the 1994 survey to the 2003 survey: 77% and 76% of women reported having had a Pap test in the past 3 years, respectively [20,21]. Additionally, they show that age is still a risk factor for inadequate screening; in the 1994 survey screening was higher among 18- to 44-year-old women (82%) than among women 65 years or older (57%). This trend remained virtually unchanged in the 2003 survey: 81% of the 18- to 44-year-old women and 57% of women 65 years old or older women had had a Pap test in the previous 3 years. Race is also another risk factor for inadequate screening. The 2003 survey showed that although screening rates among African Americans and whites were similar (76% and 80%, respectively), screening rates were much lower in other race groups (69%).

Internationally, organizing screening programs in developing countries, where the burden of cervical cancer is the greatest, has remained a challenge. There are many obstacles to screening, generally attributed to a lack of infrastructure and resources—technical, medical, and financial—and a lack of awareness and education about cervical cancer among women and health care providers. Moreover, in Africa and South America, which bear the

biggest cervical cancer burden, there are competing health care needs such as HIV/AIDS, infectious diseases such as malaria, tuberculosis, and high infant and maternal mortality rates. In addition there is a lack of trained clinicians, adequate laboratory supplies, personnel, and treatment facilities. Finally, there are considerable cultural barriers; women may be reticent to seek routine pelvic screening, especially in the absence of any symptoms [22–24], which underscores the profound need for an acceptable and reliable screening method that focuses on timely detection of early lesions and treatment of the lesions to reduce cervical cancer burden. Interested readers are referred to an excellent review specific to this topic [25].

An important aspect of the success of cytology screening in developed countries is attributed to repeated screening of women during the long natural history of cervical cancer development. The repeated nature of cytology screening makes it cost prohibitive for resource-poor countries. Moreover, if an abnormality is detected, the need for multiple visits (a first visit to perform the screening test, a second visit for tissue confirmation, and possibly a third visit for treatment) can lead to loss of follow-up of women who may be at greatest risk of cervical cancer, further compounding a complex issue. People who run screening programs in resource-poor settings must consider these limitations and develop approaches sustainable in, and suitable for, those settings (eg, screening methods that target the etiologic agent human papillomavirus [HPV], the appropriate age to initiate screening, the screening interval, and a protocol that allows screening and treatment at one visit).

Cervical transformation zone

Two embryologically distinct cell types make up the cervical epithelium. The ectocervix, the part of the cervix that extends into the vagina, is made of nonkeratinized, stratified, squamous epithelium, similar to the lining of the vagina. The endocervix, the part of the cervix that leads to the uterus, is lined by mucus-secreting, columnar epithelium [26]. The junction of the columnar and the stratified squamous epithelial cells, ''the squamocolumnar junction'', recedes toward the endocervix with age, as columnar cells are replaced with stratified squamous epithelium in a process known as ''squamous metaplasia'', which leads to the formation of the transformation zone. In general, because of the rapid turnover of cells, transformation zones throughout the body are susceptible to carcinogens and carcinogenesis, but the molecular underpinnings of this vulnerability have not been described. The cervix is uniquely susceptible to HPV-induced cervical carcinogenesis when contrasted with the vagina. Although the vagina has a much greater surface area than the cervix, vaginal cancer is one of the rarest of all malignancies. Approximately 75% to 80% of all cervical cancers are squamous cell carcinoma [27]. Adenocarcinomas account for most of the remaining 25%.

Screening test characteristics

A simple 2 × 2 screening table (Table 1) illustrates key concepts to understanding clinical diagnostics and the performance of screening tests when identifying individuals who have and do not have disease.

Although the validity of a screening test as measured by sensitivity and specificity is important from a public health view in clinical settings, a different set of questions is of importance to the clinician. First, if the test results are positive in a patient, what is the probability or risk of disease for that patient? That is, what is the positive predictive value of the test? Similarly, if a patient's test results are negative, what is the probability that the patient does not have the disease? This is the negative predictive value of a test and provides reassurance against disease among test-negative women.

The performance of a screening test will also depend on which disease end point is considered. Cervical cancer prevention studies use surrogate end points to evaluate risk because no one would willingly permit a woman to develop cervical cancer under observation. Histologically confirmed CIN 3 is the best surrogate for cervical cancer, but the risk of CIN 2 and CIN 3 is studied also, because that is the threshold for treatment.

Table 1 shows results of a dichotomous screening test (in this example cytology, Pap-positive versus Pap-negative) compared with the true disease state of the population screened (CIN 3 or cancer). Four outcomes are possible.

A. True positives (test positive results in individuals who have CIN 3/cancer)
B. False positives (test positive results in individuals who do not have CIN 3/cancer)
C. False negatives (test negative results in individuals who have CIN 3/cancer)
D. True negatives (test negative results in individuals who do not have CIN 3/cancer)

Sensitivity is the ability of the test to correctly identify those who have the disease (A/[A + C]). Specificity is the ability of the test to correctly identify those who do not have the disease (D/[B + D]). The positive predictive value (A/[A + B]) of any abnormal result is the risk of disease among women who have that test result (absolute risk). The negative predictive value (D/[C + D]) is the reassurance that a woman who has a normal test result is not at risk of disease until the next expected visit.

Table 1
The 2 × 2 table

Test result	Cancer risk	No cancer
Positive screening test (eg, positive Pap)	A: true positives	B: false positives
Negative screening test (eg, negative Pap)	C: false negatives	D: true negatives

The sensitivity of the Pap test for detecting CIN 2 or worse lesions (based on studies in countries with cytology-based screening) was estimated to be 53% (48.6%–57.4%), and specificity was estimated to be 96.3% (96.1%–96.5%) [28]. Test sensitivity must be distinguished from program sensitivity. The former is a measure of the sensitivity of a single test at one point in time to detect the endpoint. The latter is the combined or overall sensitivity of a series of tests at intervals determined by the screening program to detect the endpoint. Repeat screening at regular intervals can compensate somewhat for the limitations in the sensitivity of a technique.[1] In the context of cervical screening, two main types of errors contribute to lower sensitivity. Sampling error occurs when a cervical lesion is present, but cells representative of the abnormality are not present on the glass slide specimen. Sampling error may occur if the lesion is not sampled or if abnormal cells collected on the sampling implement are not transferred to the slide. Factors that contribute to sampling error include small size of the lesion, inaccessible location of the lesion (eg, high in the endocervical canal), or inappropriate sampling technique. Laboratory error occurs when cells indicative of an intraepithelial lesion or carcinoma are present in the specimen but are not identified as abnormal when the result is reported. Factors that may contribute to laboratory error include the presence of only a few abnormal cells, the small size of the abnormal cells, the presence of inflammation or blood obscuring cells, or diagnostic misinterpretation of the significance of identified cell abnormalities. Even under optimal screening conditions, sampling and laboratory errors cannot be eliminated entirely. Therefore, it is an unrealistic expectation that any test or program can achieve perfect sensitivity, and as a consequence, there can be no absolute (100%) assurance of safety with a negative test.

Screening based on cytology

Conventional Papanicolaou test and liquid-based cytology

Cytologic evaluation of cervical cells was introduced in the 1940s by George Papanicolaou, after whom the Pap test is named. It probably is the most widely used cancer-screening technique in the United States and in other developed countries. The test involves gently scraping cells from the surface of the cervix and microscopic examination of the fixed and stained cells for abnormal morphologic cell changes.

Specimen collection

Cervical sample collection for cytology involves the clinician visualizing the cervix and sampling the squamocolumnar junction, because this is the

[1] This assumes that one false negative test is not highly correlated with the next false negative test. If true, the programmatic performance is the product of $[1\text{-(false negative)}]^n$; where n = number of screens.

region where majority of cervical lesions occur. It has been demonstrated that the use of either the combination of a spatula and cervical brush or a broom-shaped device that samples both the ectocervix and endocervix simultaneously increases the detection of abnormalities [29].

Conventional Papanicolaou test

In the conventional Pap test, the cellular sample collected with either a spatula or brush is spread quickly and evenly over the surface of a glass slide to thin out the large clumps while avoiding excessive manipulation that can damage the cells. Studies have shown that, after smear preparation, more than half of the material remains on the collection device, which is discarded, and thus is lost for microscopic analysis [30]. To preserve morphologic details, slides are fixed by immersion in alcohol or sprayed with fixative. Air-drying of the sample may limit the interpretability of the specimens.

Specimen adequacy depends on a number of parameters including number and types of epithelial cells present and morphologic preservation. Additionally, the smear must not be obscured by factors such as blood, neutrophils, inflammation, or air-drying that may limit microscopic visualization of the cells [31]. The adequacy of the smear depends on sampling of the transformation zone, with an ''adequate'' specimen consisting of well-preserved, evenly distributed squamous and glandular cells. The presence of both epithelial cell types provides indirect evidence that the squamocolumnar junction has been sampled.

The diagnostic evaluation of a Pap test is labor intensive and subjective. The slide may consist of more than 100,000 cells, of which only a small number may be abnormal. Microscopic screening is performed by trained cytotechnologists who must be able to detect the rare abnormal cells among thousands of cytologically normal cells. Any identified abnormal or questionable cytologic changes are referred to a pathologist for interpretation.

Liquid-based cytology

As mentioned earlier, with conventional smear techniques only a fraction of the cellular material collected from the cervix is transferred to the glass slide. With LBC, instead of spreading the cervical cells on the glass slide, the sampling device is vigorously rinsed or stirred in a vial of preservative/fixative, producing a suspension of cells. In principle these modifications in sampling have several advantages over a conventional Pap test. More of the cellular sample is eluted (and therefore retained in the suspension) and a random sampling of cells is transferred to the slide in an even (monolayer) preparation [30,32]. These processing techniques also allow removal of extraneous material such as blood, providing better visualization of the cells.

In the United States and many other countries, LBC has largely replaced the conventional Pap smear. Although several studies have shown increased

sensitivity of LBC compared to conventional Pap smears, a recent review did not find evidence that LBC reduced the proportion of unsatisfactory slides or that it detected more HSIL. Moreover, it highlighted that study design could affect findings [33]. Results of a recently published, randomized clinical trial conducted in Italy also failed to demonstrate that LBC was more sensitive than Pap for detection of CIN 2 or worse lesions, but LBC resulted in more positive tests, which led to a lower positive predictive value. However, the investigators found fewer unsatisfactory results with LBC than with conventional Pap tests [34].

A significant added benefit of LBC is that the residual specimens are available for additional testing, such as "reflex" HPV testing in cases of equivocal ASCUS cytology results [35,36]. This ability to test for HPV from the same specimen eliminates the need for an additional patient visit to collect a separate sample.

Computerized screening technologies

To reduce the false-negative results associated with diagnostic evaluation of Pap slides (ie, to improve on the human visual system used to identify abnormal cells), a number of new approaches have been developed using computer image analysis technology for automated slide analysis to help cytotechnologists focus on abnormal areas of the slide.

Several computer imaging devices and programs have been developed to improve the sensitivity of cytology. One such device, which can be used on conventionally prepared Pap slides and LBC slides, uses a high-speed video microscope imaging interpretation software [37]. This device was approved by the FDA in 1998 for initial (or primary) screening of conventional Pap slides and again in 2001 for initial screening of LBC slides. It also has received FDA approval for secondary screening of previously evaluated negative specimens by routine manual screening.

As a primary screener, the computer identifies approximately 25% of samples—those with the lowest rank score—as least likely to contain an abnormality; these slides are not reviewed by a cytotechnologist. The remaining 75% of specimens undergo manual microscopic screening. In addition, of the cases reviewed as negative by the cytotechnologist, the subset with the highest rank score is determined by the computer then is subjected to a second round of manual screening.

In secondary screening, the device identifies approximately 20% of previously diagnosed "negative" cases as most likely to contain an abnormality; these slides then undergo repeat manual screening by the cytotechnologists. However, when used for secondary screening, these technologies are cost-effective only if incorporated into a less-frequent screening strategy [38]. Another automated imaging device is intended to be used with a proprietary LBC system. The imager rapidly scans every cell and cell cluster and identifies 22 areas of interest or "fields of view" for every slide. The cytotechnologist can focus on and review the 22 fields of view that the imager

has selected thus reducing the amount of time needed to screen each slide. A recent report from Australia compared this imager with manually read conventional cytology and showed that the imager detected 1.29 more cases of histologic high-grade squamous disease per 1000 women screened than did manually read slides, and more of the imager-read slides were satisfactory for examination and contained more low-grade cytologic abnormalities [39].

Evaluation of cytology results

Pap test results may be reported using a variety of terminology systems. A translation table (Table 2) is helpful to convert from one nomenclature to another. When cytology emerged as a diagnostic discipline in the 1940s and 1950s, Papanicolaou devised a numeric classification (I–V) to communicate the degree of confidence that cancer cells were present in a specimen. As used initially by Papanicolaou, the numeric designations were:

Class I: benign
Class II: minor cellular abnormalities considered benign
Class III: cells suspicious for but not diagnostic of cancer
Class IV: cells fairly conclusive for malignancy
Class V: cells diagnostic of cancer

As the field of cytology expanded, this numeric code gave way to terminology systems that designated the degree of abnormality identified with categories for four grades of dysplasia: mild, moderate, severe, and carcinoma-in situ [CIS]. Richart [40] introduced the term ''cervical intraepithelial neoplasia'' (CIN), grades 1, 2, and 3, to promote the concept of a disease continuum of precursors to invasive cancer. The CIN system is based on morphologic criteria and tissue architecture: the proportional thickness of the epithelium involved by disorderly growth and cytologic atypia. Mild and moderate dysplasia correspond roughly to CIN 1 and CIN 2, respectively. CIN 3, however, encompasses severe dysplasia and CIS, thus eliminating a difficult and sometimes arbitrary diagnostic distinction between almost versus complete full-thickness abnormality.

Table 2
Cervical diagnostic terminology

Dysplasia	CIN	Bethesda
Atypia	Atypia	ASCUS
HPV	HPV	LSIL
Mild dysplasia	CIN 1	HSIL
Moderate dysplasia	CIN 2	
Severe dysplasia	CIN 3	
CIS		

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papilloma virus; LSIL, low-grade squamous intraepithelial lesion.

Koilocytosis, a diagnostic term indicating cellular changes of perinuclear cytoplasmic cavitation, was recognized by Meisels [41] to be a manifestation of genital HPV infection. Initially, HPV cellular changes were considered distinct from "true" dysplasia or CIN and were not considered part of the precursor pathway to cervical cancer. As techniques for identifying HPV became more sensitive, however, HPV DNA was found in more than 99% of cervical neoplasia studied [42,43] . The pathogenesis of cervical neoplasia and cervical cancer now is known to be caused by HPV, based on epidemiologic, virologic, and experimental evidence. Therefore, isolation of koilocytotic atypia or HPV effect as a distinct entity from dysplasia/CIN is no longer biologically valid.

The Bethesda system, developed at a National Cancer Institute workshop in 1988 [44] and refined in 1991 and 2001 [45], collapses the cytologic diagnostic subcategories of intraepithelial lesions into low- and high-grade squamous intraepithelial lesions, abbreviated as "LSIL" and "HSIL," respectively. This division is based on the concept of HPV-induced cellular changes as discrete processes. LSIL is the result of an acute infection with any HPV type resulting in mild, usually transient cytologic effects. HSIL is the result of persistent infection with predominantly carcinogenic HPV types and the interplay of a variety of factors, including host immune response, which poses greater risk of invasion [46]. Although the CIN classification remains widely used in cervical histopathology, the Bethesda system is used more commonly to report Pap test results.

The Bethesda system also introduced the term "atypical squamous cells of undetermined significance" (ASCUS) to reflect equivically abnormal changes that are quantitatively or qualitatively insufficient to establish a definitive interpretation of squamous intraepithelial lesion. ASCUS is not a single biologic entity and therefore is associated with highly variable clinical outcomes. It does represent an improvement, however, over older classifications that used "atypia" to encompass reactive changes and HPV-associated cell changes in addition to equivocal findings. In the Bethesda system, reactive changes are categorized as "benign," and HPV associated cell changes are subsumed under "squamous intraepithelial lesion."

Abnormal Pap test results are not distributed evenly among the previously described categorizations. In a well-screened population (eg, in the United States), cytologic interpretation of low-grade and equivocal lesions are common, with relatively few HSIL and cancers. In the United States, cancers represent less than 0.1% of cytologic interpretations, and high-grade lesions constitute approximately 0.6% [47]. By contrast, LSIL and ASCUS account for an estimated 5% of all Pap test results, translating to 3 million women in the United States annually.

Follow-up and management of abnormalities

Screening without treatment is unethical and a drain of resources without patient benefit. Loss to follow-up with a multi-step management strategy is

a significant problem. In two studies, 13% and 15% of cervical cancers that occurred in women who had had an abnormal Pap test were attributed to lack of patient notification or to patient noncompliance with recommended treatment [48,49]. One-stop screening, diagnosis, and treatment clinics have been established in a few high-risk areas to address this problem [50,51]. This labor-intensive approach to screening cannot feasibly be applied as yet on a large scale, however.

The Atypical Squamous Cells of Undetermined Significance–Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS) was a randomized clinical trial launched by the US National Cancer Institute to provide empiric evidence for the best clinical management of women who had minimally abnormal cervical cytology (ASCUS and LSIL). It showed that among women who had ASCUS, HPV testing for carcinogenic HPV DNA (HPV triage) was at least as sensitive as universal immediate colposcopy for identifying women who had CIN 3 or worse, and reduced by half the number of women who needed referral for colposcopy. Among women who had LSIL, HPV DNA triage was not as useful, because most LSIL lesions were HPV-positive [52]. It further showed that the 2-year risks of CIN 2 or worse were virtually identical for HPV-positive ASCUS and LSIL, hence indicating that HPV-positive ASCUS is biologically equivalent to LSIL. Further analysis of ALTS data has shown that only 1.4% of women who have HPV-negative ASCUS have CIN 3 or worse lesions detected in the subsequent 2 years. This risk is similar to that of women who have negative cytology in the absence of HPV testing. This finding suggests that women who have HPV-negative ASCUS might return to routine screening intervals, which may be longer than 1 year, depending on the patient's age and past screening history [53].

Women who have HPV-positive ASCUS and those who have LSIL or worse lesions are managed similarly by referring them to colposcopy with directed biopsy as needed per ASCCP management algorithms [3]. Women who have histologically confirmed CIN 2 or worse lesions are managed by ablation or excision of the lesion and the transformation zone.

New screening techniques for cervical cancer

Despite the success of cytology programs in developed countries in reducing the burden of cervical cancer, there is interest in development of technologies to enhance the accuracy of cervical cancer screening and thereby make it more cost effective. As described earlier, some efforts have been directed at improving the quality of cytology (eg, LBC methods), while others have focused on improving the laboratory microscopic screening process (eg, computerized imaging). Additionally, molecular assays, based on detecting HPV, the etiologic agent for cervical cancer, are being considered.

Carcinogenic human papillomavirus *testing*

In the 1990s data from multiple, international epidemiologic studies established that infection with one of a group of 15 carcinogenic HPV types is a necessary cause of cervical cancer [42,43] and its immediate precursor, CIN 3. Other HPV types are classified as low risk because they are not associated with cervical cancer, although HPV 6 and HPV 11 cause 90% of genital warts.

HPV is the most common acute sexually transmitted viral infection in the United States [54] and internationally [55], although different cultural, social, and sexual norms can result in significant regional variation. Most infections, even by carcinogenic HPV types, are benign and clear within 1 to 2 years. Women who do not clear the carcinogenic HPV infection (ie, who have HPV persistence) are at elevated risk of developing precancer and, if not detected and treated, cancer [56].

Testing based on detection of HPV DNA in cervical specimens has been introduced in the United States and in some European countries to improve the efficiency and to maximize the sensitivity of cervical cancer screening. As mentioned earlier, the results of the ALTS trial and other studies showed that testing for carcinogenic HPV is cost effective and more sensitive than repeat conventional cytology for detection of precancer in women who have ASCUS [57–62]. Carcinogenic HPV testing is approved as an adjunctive test with cytology for primary cervical cancer screening for women 30 years and older and is more sensitive, with very high negative predictive value (hence providing reassurance that test-negative women are at low risk for developing cervical precancer and cancer). It is less specific than cytology, however, because HPV infections are very common [28,57]. HPV testing also is added to follow-up of women after colposcopy when cancer is not detected [63], and it is used as a follow-up strategy among women who have undergone treatment of a lesion [64–66].

One FDA-approved DNA-based molecular assay is available commercially. It collectively targets detection of 13 carcinogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). HPV DNA testing can be performed directly from residual LBC specimens or from a separately collected sample. This signal-amplification assay uses a technique combining antibody capture of HPV DNA and RNA probe hybrids and chemiluminescent signal for detection. Several companies currently are conducting clinical trials to evaluate new tests for carcinogenic HPV, and results should be forthcoming shortly.

Because the incidence and prevalence of HPV is age-associated, age plays a crucial role in determining the target population for HPV DNA screening. The high prevalence of HPV infection among young sexually active women often is not associated with precancer, rather HPV infection and HPV-associated mild lesions always almost clear spontaneously. This precludes the use of HPV testing as a primary screening strategy among

young women close to the onset of sexual activity. As HPV DNA prevalence declines sharply with age, viral persistence increases, while the sensitivity of HPV DNA for cervical neoplasia remains high. Furthermore, the incidence of cervical lesions starts to increase in the late twenties to early thirties or 10 to 15 years after onset of sexual activity, and the incidence of cervical cancer begins to increase in the late thirties. Hence the positive predictive value of a HPV DNA test for cervical precancer and cancer improves with age. Moreover, the accuracy of cytology declines with age because of poor sampling of the squamocolumnar junction, which migrates into the endocervical canal and becomes more difficult to sample, but the detection of morphologic look-alikes that are unrelated to cervical carcinogenesis increases. Taken together, these factors suggest that HPV testing at younger ages is inefficient; but it is a cost-effective primary screening strategy in older women provided that the screening interval is lengthened among HPV-negative women.

New biomarkers

There is a demand for new screening tests that may be more specific and have better predictive values for cervical cancer to compensate for false-negative rates associated with cytology and the high false-positive rates associated with HPV DNA tests. Some of the new tests are modifications of the existing technologies (and not a new biomarker), and some are based on new biomarkers of disease (that measure host-viral interaction). These new biomarkers may be used eventually as stand-alone tests in combination with cytology, or they may be used as a triage test for carcinogenic HPV-positive women. Although assays are being developed continually, only completely standardized assays should be used in clinical practice, because lack of rigorous research and standardization can influence analytic performance of the tests [67].

Type-specific human papillomavirus testing

An important goal of new applications of HPV testing is to improve specificity while maintaining clinical sensitivity. Although testing for carcinogenic HPV is highly sensitive, there is significant variability in the risks associated with each HPV type. In particular, it is now known that HPV 16 is the most important HPV type worldwide. It is present in about 50% of cervical cancers and is the most prevalent HPV type in invasive cervical cancers. HPV 16 and HPV 18 are represented in approximately two thirds of cervical cancers [7,68]. HPV 16 also is the most common genotype in the general population, accounting for approximately 20% of the infections among cytologically normal women, 20% of infections among women who have equivocal lesions, and approximately 25% of infections in women who have mild abnormalities [56]. Hence, if HPV 16 could be identified

reliably and its cytopathologic manifestations treated, 50% of cervical cancers, in theory, could be prevented. Longitudinal studies have shown that type-specific detection of HPV 16 and 18 identifies women at the greatest risk of cervical precancer and cancer [69–71]. These type-specific HPV detection assays are based on polymerase chain reaction amplification of the viral DNA from the tissue or exfoliated cells collected from the site of infection and on the detection of HPV types in the amplified products. There are several primer sets designed to allow amplification of many HPV types, but the detection part of the assays can identify between 27 and 41 different types. At least four primers (MY09/MY11, PGMY09/PGMY11, GP5+/6+, and SPF10) give roughly similar results. Although at this writing there are no commercially available HPV genotyping assays, several companies are evaluating HPV genotype–specific assays in clinical trials.

Cellular markers

The new generation of screening tests targets biomarkers that can help discriminate between the rare infection that has the potential for progression to precancer and cancer and the majority of the infections, which will regress. Two novel biomarkers—mRNA expression of E6/E7 transcripts and $p16^{INK4a}$ (referred to as "*p16*" henceforth)—are markers of disease progression and show promising results in initial studies, but large-scale evaluations comparing them to other markers such as HPV typing are lacking [72]. Both E6/E7 mRNA expression and *p16* rely on the basic molecular events involved in cervical carcinogenesis. E6 and E7 are two main HPV oncoproteins, and their persistent over-expression is a necessary step in HPV-induced carcinogenesis [72,73]. They are expressed at low levels early in the viral lifecycle, are important in inducing cellular transformation, and target many cellular functions, most importantly degradation of the human tumor suppressor gene *p53* by E6 and inactivation of retinoblastoma (*pRB*) by E7 dysregulation. Therefore the detection of E6/E7 mRNA of high-risk HPV types may indicate a further step in the progression to cancer [72].

The biomarker *p16*, a cyclin-dependent kinase inhibitor, is expressed at very low levels in normal cells, but it is overexpressed in precancer and cancer, indicating progressive steps from a productive HPV infection toward a transforming infection. Its overexpression can be detected by immunoassays designed to detect the protein on cytology slides [74] or in cellular lysates using an ELISA format [74].

Although different groups have evaluated these two biomarkers, neither has been evaluated in large, formal epidemiologic studies, so their utility as primary screening methods needs further study. Other candidate biomarkers are being developed as screening tools, but data thus far are based on a few pilot projects. Interested readers are referred to two excellent, comprehensive recent review articles by Cuzick [72] and Wentzensen [74].

Cervical cancer screening in resource-poor settings

Self-collected samples for detection of human papillomavirus

Women are able to self-collect samples by inserting a swab in the vagina up to the vault and rotating it in the vaginal vault. The swab then is placed in transport media and collected for further testing for HPV DNA. Several studies have evaluated the diagnostic accuracy of self-collection using swabs, tampons, or brushes. A meta-analysis of studies comparing usefulness of self-collected samples with clinician-collected samples (mostly performed in less-developed countries) showed that self-collection had an overall sensitivity of 74% and specificity of 84%, compared with clinician samples [72,75]. Although not as good as clinician-collected samples, this sensitivity is comparable to cytology for CIN 2 or worse lesions, for which the sensitivity of cytology is less than 70% [72]. This lower sensitivity of self-sampling may be acceptable if women who otherwise would not be screened are encouraged to participate [76].

Visual techniques

Visual inspection with acetic acid (VIA), also known as ''direct visual inspection'' or ''acetic acid test,'' is a very low-cost approach to screening that may be an option for areas that do not have access to any cervical screening [77]. VIA, at its most basic, consists of unmagnified evaluation of the cervix (transformation zone) after the application of 3% to 5% dilute acetic acid for visual signs of a high-grade lesion or cancer. The test is considered positive if clear and well-defined acetowhite areas are detected near the squamocolumnar junction (transformation zone). A similar technique, visual inspection with magnification, employs a low-power magnification device to inspect the cervix after treating it with acetic acid. An advantage of VIA is that it gives immediate results, making it possible to treat abnormal lesions at the same visit. Several cross-sectional studies, mostly performed in less-developed countries, have evaluated VIA with mixed results. The sensitivity of VIA in detecting high-grade lesions and cervical cancer has ranged from 49% to 96%, and specificity has ranged from 49% to 98% [25,78]. There were limitations to the studies, however. Most suffered from verification bias because the true disease status for a large majority of the individuals in the studies was not known, and second, because most used colposcopy, which also has a low sensitivity, as the reference standard for disease verification [79]. Because VIA and colposcopy are based on the same visual technique, precancerous lesions missed by VIA are likely to be missed by colposcopy.

See-and-treat options

The current cervical cancer screening programs practiced in high-resource settings include at least a three-visit intervention: screening and triage

of equivocal results; colposcopy with directed biopsy for diagnostic purposes; treatment; and post-treatment follow-up. To overcome the obstacles to establishing the infrastructure for cytology-based screenings in resource-poor settings, many studies have investigated combining screening and treatment in a single visit or providing treatment a short time after screening. A randomized trial was performed in South Africa of 6555 nonpregnant women between age 35 and 65 years to determine the safety and efficacy of two screen-and-treat options. All women were screened with both HPV and VIA. Women who were HPV positive or who had a positive VIA test were assigned randomly to cryotherapy or to delayed treatment. At 6 and 12 months after randomization the prevalence of histologically defined high-grade CIN was significantly lower in both screen-and-treat arms than in the delayed-evaluation group [80]; moreover, prevalence was much lower in the HPV DNA arm than in the VIA arm.

Summary

No screening test is 100% effective in detecting all cervical cancer cases. Secondary prevention of cervical cancer, as practiced in high-resource regions, includes screening, triage of equivocal lesions, colposcopically guided biopsy of abnormal results, treatment, follow-up after treatment, and return to routine screening. The screening program in the United States is estimated to cost $6 billion annually [81]. Tests with better performance characteristics, fewer visits per screening cycle, and fewer screening cycles per lifetime are needed. New technologies are in development and, if used wisely, can improve the efficiency of cervical cancer prevention and reduce overtreatment. If used poorly, they can drive up cost of corresponding benefits [82].

Whichever validated screening method is chosen, the key to success of cervical cancer screening programs (ie, reducing cervical cancer incidence) is to ensure broad coverage of services and follow-up of abnormalities. Different countries and settings may vary the details of the screening program (eg, the age to initiate screening, the screening interval, and age to stop screening) based on considerations of cost effectiveness and societal priorities for cancer prevention.

Many new technologies are well beyond the financial capabilities of developing countries that are seeking to establish or improve existing screening programs. Cost-effectiveness analyses, however, will assist in developing a more rationally based screening program that may improve sensitivity at no or little extra cost. Using a new technology or a combination of technologies will increase the cost of a screening event, but modelling studies show that the gain in sensitivity may allow less frequent screening that theoretically could result in cost-neutral implementation [38].

Equally important is considering screening practices, given the development of effective prophylactic vaccines against HPV types 16 and 18 and against the two low-risk types 6 and 11 (discussed in more detail elsewhere

in this issue). In theory, preventing HPV 16 and 18 infections can cut cervical cancer rates by 70% worldwide. At present, however, these vaccines are expensive and require three doses, which may make the programs very difficult to implement in resource-poor settings. In developed countries the impact of vaccination may be to reduce the number of screen-detected abnormalities. Because HPV 16 and 18 cause the most obvious morphologic abnormalities, the positive predictive value of an abnormal cytology for CIN 3 will likely decrease with widespread vaccination. Current bivalent and quadravalent vaccines will not eliminate the need for screening for several reasons: 1) many women are beyond the age cohort for vaccination; 2) women infected prior to vaccination may not derive protection; 3) the vaccines do not protect against all carcinogenic HPV types, therefore women are still at risk of precancer and cancer caused by other types of HPV. However, we will need to consider rational strategies to integrate vaccination and screening to ensure women's safety and avoid costly duplication of prevention efforts in the future [81].

References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55:74–108.
[2] Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 2003;348:489–90.
[3] Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 Consensus Guidelines for the Management of Women With Abnormal Cervical Screening Tests. J Low Genit Tract Dis 2007;11:201–22.
[4] Naucler P, Ryd W, Tornberg S, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007;357:1589–97.
[5] Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 2007;357:1579–88.
[6] Bulkmans N, Berkhof J, Rozendaal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007, Oct 3; [Epub ahead of print].
[7] Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine 2006; 24(Suppl 3):S11–25.
[8] Saraiya M, Ahmed F, Krishnan S, Richards TB, Unger ER, Lawson HW. Cervical cancer incidence in a prevaccine era in the United States, 1998–2002. Obstet Gynecol 2007;109: 360–70.
[9] Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. CA Cancer J Clin 1999; 49:8–31, 1.
[10] Wingo PA, Cardinez CJ, Landis SH, et al. Long-term trends in cancer mortality in the United States, 1930–1998. Cancer 2003;97:3133–275.
[11] Solomon D, Schiffman M. Cervical cancer screening. In: Golman MB, Hatch MC, editors. Women and Health. San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press; 2000. p. 942–8.
[12] Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Mortality - All COD, Public-Use With State, Total U.S. (1969–2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics

Branch, released April 2006. Underlying mortality data provided by NCHS (www.cdc.gov/nchs). www.seer.cancer.gov. 2006.
[13] American Cancer Society. Cancer Facts and Figures 2007. Atlanta: American Cancer Society; 2007.
[14] Dobbs SP, Asmussen T, Nunns D, Hollingworth J, Brown LJ, Ireland D. Does histological incomplete excision of cervical intraepithelial neoplasia following large loop excision of transformation zone increase recurrence rates? A six year cytological follow up. BJOG 2000;107:1298–301.
[15] Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine 2006;24(Suppl 3):S63–70.
[16] Singh GK, Miller BA, Hankey BF, Edwards BK. Persistent area socioeconomic disparities in U.S. incidence of cervical cancer, mortality, stage, and survival, 1975–2000. Cancer 2004; 101:1051–7.
[17] Saslow D, Castle PE, Cox JT, et al. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin 2007;57:7–28.
[18] Hughes E, McCracken M, Roberts H, et al. Surveillance for certain health behaviors among states and selected local areas–behavioral risk factor surveillance system, United States, 2004. MMWR Surveill Summ 2006;55:1–124.
[19] Brown CL. Screening patterns for cervical cancer: how best to reach the unscreened population. J Natl Cancer Inst Monogr 1996;7–11.
[20] 1994 National Health Interview Survey public use data-file. Morbidity and Mortality Weekly Report. 1996.
[21] National Center for Health Statistics. 2003 NATIONAL HEALTH INTERVIEW SURVEY (NHIS). 2004.
[22] Sankaranarayanan R, Black RJ, Swaminathan R, Parkin DM. An overview of cancer survival in developing countries. IARC Sci Publ 1998;135–73.
[23] Hernandez-Avila M, Lazcano-Ponce EC, de Ruiz PA, Romieu I. Evaluation of the cervical cancer screening programme in Mexico: a population-based case-control study. Int J Epidemiol 1998;27:370–6.
[24] Safaeian M, Kiddugavu M, Gravitt PE, et al. Comparability of self-collected vaginal swabs and physician-collected cervical swabs for detection of human papillomavirus infections in Rakai, Uganda. Sex Transm Dis 2007;34:429–36.
[25] Denny L, Quinn M, Sankaranarayanan R. Chapter 8: Screening for cervical cancer in developing countries. Vaccine 2006;24(Suppl 3):S71–7.
[26] Schiffman MH, Brinton LA, Devesa SS, Fraumeni JF. Cervical Cancer. In: Schottenfeld D, Fraumeni JF, editors. Cancer Epidemiology and Prevention. Second edition. New York, Oxford: Oxford University Press; 1996. p. 1090–116.
[27] Sheets E, Goodman H. The Cervix. In: Berkowitz R, Barbieri R, editors. Kistner's Gynecology Principles and Practice. 6th Edition. St. Louis: Mosby; 1995. p. 103–36.
[28] Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006;119: 1095–101.
[29] Boon ME, de Graaff Guilloud JC, Rietveld WJ. Analysis of five sampling methods for the preparation of cervical smears. Acta Cytol 1989;33:843–8.
[30] Hutchinson ML, Isenstein LM, Goodman A, et al. Homogeneous sampling accounts for the increased diagnostic accuracy using the ThinPrep Processor. Am J Clin Pathol 1994;101: 215–9.
[31] Solomon D, Henry M. Specimen adequacy. Compendium on Quality Assurance, Proficiency Testing and Workload Limitations in Clinical Cytology. Chicago: Tutorials of Cytology; 1995. p. 90–4.
[32] Watts G. Commentary: Liquid automation refreshes Dr Papanicolaou. BMJ 2007;335: 35–6.

[33] Davey E, Barratt A, Irwig L, et al. Effect of study design and quality on unsatisfactory rates, cytology classifications, and accuracy in liquid-based versus conventional cervical cytology: a systematic review. Lancet 2006;367:122–32.
[34] Ronco G, Cuzick J, Pierotti P, et al. Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial. BMJ 2007;335:28.
[35] Ferenczy A, Franco E, Arseneau J, Wright TC, Richart RM. Diagnostic performance of Hybrid Capture human papillomavirus deoxyribonucleic acid assay combined with liquid-based cytologic study. Am J Obstet Gynecol 1996;175:651–6.
[36] Sherman ME, Schiffman MH, Lorincz AT, et al. Cervical specimens collected in liquid buffer are suitable for both cytologic screening and ancillary human papillomavirus testing. Cancer 1997;81:89–97.
[37] Tripath Inc. FocalPoint (TM) slide profiler - System Product Insert. 2007.
[38] Brown AD, Garber AM. Cost-effectiveness of 3 methods to enhance the sensitivity of Papanicolaou testing. JAMA 1999;281:347–53.
[39] Davey E, d'Assuncao J, Irwig L, et al. Accuracy of reading liquid based cytology slides using the ThinPrep Imager compared with conventional cytology: prospective study. BMJ 2007; 335:31.
[40] Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol 1969;105:386–93.
[41] Meisels A, Fortin A. Condylomatous lesions of the cervix and vagina. I. Cytologic patterns. Acta Cytol 1976;20:505–9.
[42] Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796–802.
[43] Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.
[44] Solomon D. The 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses. JAMA 1989;262:931–4.
[45] Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2001;287(16):2114–9.
[46] Schiffman MH, Liaw KL, Herrero R, Sherman M, Hildesheim A, Solomon D. Epidemiologic support for a simplified view of cervical carcinogenesis. Eur Bull 1998;1: 2–6.
[47] Liaw KL, Glass AG, Manos MM, et al. Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. J Natl Cancer Inst 1999;91:954–60.
[48] Janerich DT, Hadjimichael O, Schwartz PE, et al. The screening histories of women with invasive cervical cancer, Connecticut. Am J Public Health 1995;85:791–4.
[49] Carmichael JA, Jeffrey JF, Steele HD, Ohlke ID. The cytologic history of 245 patients developing invasive cervical carcinoma. Am J Obstet Gynecol 1984;148:685–90.
[50] Burger RA, Monk BJ, Van Nostrand KM, Greep N, nton-Culver H, Manetta A. Single-visit program for cervical cancer prevention in a high-risk population. Obstet Gynecol 1995;86: 491–8.
[51] Megevand E, Van WW, Knight B, Bloch B. Can cervical cancer be prevented by a see, screen, and treat program? A pilot study. Am J Obstet Gynecol 1996;174:923–8.
[52] Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). Arch Pathol Lab Med 2003;127:946–9.
[53] Safaeian M, Solomon D, Wacholder S, Schiffman M, Castle P. Risk of precancer and follow-up management strategies for women with human papillomavirus-negative atypical squamous cells of undetermined significance. Obstet Gynecol 2007;109:1325–31.
[54] Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813–9.

[55] Clifford GM, Gallus S, Herrero R, et al. 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;366:991–8.
[56] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890–907.
[57] Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: A summary of meta-analyses. Vaccine 2006;24(Suppl 3): S78–89.
[58] Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001;93:293–9.
[59] Arbyn M, Buntinx F, Van RM, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. J Natl Cancer Inst 2004;96:280–93.
[60] Manos MM, Kinney WK, Hurley LB, et al. Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. JAMA 1999;281: 1605–10.
[61] Kulasingam SL, Kim JJ, Lawrence WF, et al. Cost-effectiveness analysis based on the atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion Triage Study (ALTS). J Natl Cancer Inst 2006;98:92–100.
[62] Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of human papillomavirus DNA testing in the United Kingdom, The Netherlands, France, and Italy. J Natl Cancer Inst 2005;97: 888–95.
[63] Walker JL, Wang SS, Schiffman M, Solomon D. Predicting absolute risk of CIN3 during post-colposcopic follow-up: results from the ASCUS-LSIL Triage Study (ALTS). Am J Obstet Gynecol 2006;195:341–8.
[64] Kreimer AR, Guido RS, Solomon D, et al. Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease. Cancer Epidemiol Biomarkers Prev 2006;15: 908–14.
[65] Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. Gynecol Oncol 2005;99:S7–11.
[66] Gok M, Coupe VM, Berkhof J, et al. HPV16 and increased risk of recurrence after treatment for CIN. Gynecol Oncol 2007;104:273–5.
[67] Dunn ST, Allen RA, Wang S, Walker J, Schiffman M. DNA extraction: an understudied and important aspect of HPV genotyping using PCR-based methods. J Virol Methods 2007;143: 45–54.
[68] Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121: 621–32.
[69] Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072–9.
[70] Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 2005;97:1066–71.
[71] Bulk S, Berkhof J, Bulkmans NW, et al. Preferential risk of HPV16 for squamous cell carcinoma and of HPV18 for adenocarcinoma of the cervix compared to women with normal cytology in The Netherlands. Br J Cancer 2006;94:171–5.
[72] Cuzick J, Mayrand MH, Ronco G, Snijders P, Wardle J. Chapter 10: New dimensions in cervical cancer screening. Vaccine 2006;24(Suppl 3):S90–7.
[73] zur HH, de Villiers EM. Human papillomaviruses. Annu Rev Microbiol 1994;48:427–47.

[74] Wentzensen N, von Knebel DM. Biomarkers in cervical cancer screening. Dis Markers 2007; 23:315–30.
[75] Ogilvie GS, Patrick DM, Schulzer M, et al. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. Sex Transm Infect 2005;81:207–12.
[76] Bais AG, van Kemenade FJ, Berkhof J, et al. Human papillomavirus testing on self-sampled cervicovaginal brushes: an effective alternative to protect nonresponders in cervical screening programs. Int J Cancer 2007;120:1505–10.
[77] Singh V, Sehgal A, Luthra UK. Screening for cervical cancer by direct inspection. BMJ 1992; 304:534–5.
[78] Sankaranarayanan R, Gaffikin L, Jacob M, Sellors J, Robles S. A critical assessment of screening methods for cervical neoplasia. Int J Gynaecol Obstet 2005;89(Suppl 2):S4–12.
[79] Jeronimo J, Schiffman M. Colposcopy at a crossroads. Am J Obstet Gynecol 2006;195: 349–53.
[80] Denny L, Kuhn L, De SM, Pollack AE, Dupree W, Wright TC Jr. Screen-and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. JAMA 2005;294:2173–81.
[81] Schiffman M. Integration of human papillomavirus vaccination, cytology, and human papillomavirus testing. Cancer 2007;111:145–53.
[82] Moriarty AT. A rock and a hard place: HPV testing and financial gain. Diagn Cytopathol 2007;35:463–4.

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Prophylactic Human Papillomavirus Vaccination: A Breakthrough in Primary Cervical Cancer Prevention

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The identification of the obligate viral etiology of most cervical cancers, and our improved understanding of the human papillomavirus (HPV) viral life cycle and related host response has lead to the development of a prophylactic vaccine for the primary prevention of cervical cancer. This approach holds tremendous promise for the long-term prevention of cervical cancer, and it is not dependent on modifying human sexual behavior to ameliorate behavioral risk factors associated with this disease. The implementation of prophylactic-vaccine-based strategies for cervical cancer prevention, however, will present important clinical and public policy challenges, some of which will be discussed in this article.

The burden of human papillomavirus–related disease

HPV is the most common sexually transmitted viral infection. In the United States each year more than 6 million people are infected with genital HPV [1]. It is estimated that at any one time about 15% of the population, or 20 million individuals, in this country are currently infected, as evidenced

This article is based in part on the American Cancer Society Guideline for human papillomavirus vaccine use to prevent cervical cancer and its precursors [124].

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by the recovery of HPV DNA in genital sampling [2,3]. Nearly half of these infections occur among individuals between 15 and 25 years of age; in this cohort the point prevalence can be as high 27% to 46% [4–7]. Conservative estimates suggest that at least half of all sexually active men and women are exposed to HPV at some point in their lifetime and that approximately 80% of sexually active women will be infected by 50 years of age [8]. HPV is causally associated with nearly all cases of cervical cancers [9], and HPV type 16 or 18 is implicated in more than 70% of cases [10,11]. Additionally HPV 16 and 18 are responsible for more than half of the premalignant (cervical intraepithelial neoplasia [CIN] grade 2 and 3) lesions diagnosed annually in this country [12].

Among HPV-related malignancies, cervical cancer is the second most common cause of cancer death in women worldwide. More than half a million new cases are diagnosed each year worldwide, and 80% of incident cases occur in developing countries, where it is the most common cancer in women [13]. Worldwide there were 280,000 cervical cancer deaths last year [14]. By contrast, 9710 cases of invasive cervical cancer were diagnosed in the United States in 2006, and in that same year approximately 3700 women died from this disease [15].

Other HPV-related cancers contribute to the significant morbidity and mortality associated with this virus. Anal cancer is diagnosed in about 4000 people annually and kills another 620 women and men in this country. Most of these tumors (80%–90%) are related to HPV 16 or 18 [16,17]. Likewise, about 3870 new cases of vulvar cancer and 870 vulvar cancer deaths occur each year, and more than 40% are HPV related [18,19]. Other rare malignancies, including penile [20], vaginal [21], urethral [22], and head and neck cancers [23–25], have been found to contain carcinogenic HPV types.

Benign manifestations of this infection also are associated with significant morbidity. As many as 1.4 million individuals in the United States have genital warts, and more than 0.5 million new cases are diagnosed annually [26]. The overwhelming majority of cases (90%) are related to HPV infection with types 6 or 11 [27]. Approximately 10% of men and women develop genital warts at some point in their lives [28]. Condyloma or genital warts are benign growths that often recur within the first 6 months of initial diagnosis, requiring multiple treatment sessions [29], and in rare instances, can become locally invasive and require extensive surgical resection [30].

Juvenile laryngeal papillomatosis occurs in about 1 in 200,000 children under age 18 years, most often before the age of 4 years, and is characterized by recurrent benign tumors that may lead to respiratory obstruction. Because of the high recurrence rate, surgical removal often must be repeated multiple times [31]. In rare circumstances papillomas may transform to carcinoma; this transformation has been reported to occur in the larynx, esophagus, and bronchi [32,33]. HPV types 6 and 11 are most frequently demonstrated in respiratory papillomas. Some investigators have found that HPV 11 is most often associated with progression to cancer [34].

Current paradigm for prevention of cervical cancer

Until very recently, population-based cervical cancer prevention has relied almost exclusively on secondary prevention, the detection of early pre-neoplastic changes by exfoliative cervical cytology (Box 1), and has been implemented through a variety of opportunistic (in the United States) and organized (in Western Europe) screening programs [35]. The introduction of cytologic screening programs in unscreened populations has been shown to reduce cervical cancer rates by 60% to 90% within 3 years of implementation [36]. In this country, the incidence of cervical cancer has decreased by 75% and mortality has decreased by 74% in the 50 years following the introduction of the Papanicolaou (Pap) smear [37,38].

Despite these successes, cervical cytology itself has important and well-characterized limitations. Most important is the limited single-test sensitivity,

Box 1. Framework for cervical cancer prevention

Primary prevention: HPV information and prevention
Behavioral modification
- Sexual precautions

Prophylactic vaccine

Secondary prevention: CIN detection/treatment
Behavioral modification
- Prevention of sexually transmitted infections
- Tobacco cessation

Screening programs
- Pap smear and HPV

Medical therapeutics
- Excisional therapy
- Therapeutic vaccines
- Chemo-preventives
- Retinoids
- Indole carbinol
- Immune response modulators

Tertiary prevention: cervical cancer therapy and control
Behavioral modification
- Tobacco cessation

Medical therapies
- Radical surgery
- Radiation therapy
- Chemotherapy
- Therapeutic vaccines

Surveillance

which can range from 49% to 67% (with a specificity estimated to range between 62% and 97%) for dry-slide conventional cytology [39]. These test performance characteristics are marginally improved with the incorporation of liquid-based thin-layer cytology. The imperfect reproducibility of cytologic diagnoses between and within observers has been a recurrent finding associated with the subjective nature of cytologic interpretation [40]. Such test-specific shortcomings are compounded by provider and patient follow-up errors, which also contribute significantly to the morbidity and mortality associated with this disease [41]. The relatively recent addition of adjunctive oncogenic HPV testing in screening for women older than 30 years represents a significant improvement in the existing cytology-based screening paradigm by exploiting our improved understanding of the epidemiology of this disease [35,42].

The failure of some women at risk to receive regular screening tests also contributes to the burden of cervical cancer. Half of all women who develop cervical cancer in this country have never been screened, and an additional 10% will have not been screened in 5 years before their diagnosis [41,43,44]. Failure to participate in screening is a complex and multi-factorial phenomenon, which may be related to a variety of factors including personal preferences, cultural factors, and systemic health care access issues.

Important significant racial and ethnic disparities exist between non-Hispanic white women and other racial/ethnic groups in this country in the incidence of cervical cancer and in the mortality, and survival associated with the diagnosis of cervical cancer [45–47]. The incidence of cervical cancer remains about 60% higher among black women (10.5/100,000) than among white women (6.6/100,000), and cervical cancer mortality among black women is the highest (4.7/100,000) of any racial or ethnic group [38]. Rates are particularly high among African American women living in the rural South and also in some urban areas [48]. Other racial/ethnic/geographic groups that experience cervical cancer incidence and mortality higher than the general population include (1) Hispanics living in the United States–Mexico border areas [49]; (2) white (non-Hispanic) women living in Appalachia and northeastern rural communities [50]; (3) native American women living in the Northern Plains and Alaskan Natives [51]; and (4) Vietnamese-Americans [47]. For all groups, disparities in incidence and mortality tend to increase with age [49]. Cervical cancer remains a major health issue for these communities, largely because of poverty and poor access to health care, which is exacerbated by socio-cultural barriers [52]. It is very remarkable that more than half of cervical cancer deaths in the United States occurs in foreign-born women [53].

The gains achieved through the existing screening programs is caused by a combination of factors including (1) the slow progression from precancerous lesions to invasive disease providing opportunity for early detection; (2) the identification of cytologic abnormalities before invasion; (3) frequent repetitive screening; and (4) effective minimally morbid therapy for

premalignant disease [54]. Prevention of invasive cervical cancer requires formal and informal programs with the infrastructure to collect and process specimens and systems to provide results, follow-up, and appropriate therapy in the event of abnormal screening results.

Human papillomavirus awareness and vaccine acceptability

A variety of studies have assessed the degree of awareness and knowledge regarding HPV among adolescents [55–57], university students [58–62], and young adults [57,63], including women with prior abnormal screening [64–67]. In general these studies find a relatively limited understanding of this disease entity. In one study of more than 1000 women attending a well-woman clinic, only 30% had heard of HPV [63]. Another survey of more than 500 inner-city high school students reported that 87% of students had not heard of HPV [56]. Studies that assessed knowledge of other common sexually transmitted infections found that knowledge of HPV was the lowest or one of the lowest areas assessed [55–57].

The most frequently asked questions at the American Social Health Association National HPV and Cervical Cancer Prevention Resource Center include questions regarding HPV transmission, pregnancy effects, infection source, prevention/treatment options, and infection duration [68]. Focus groups with low-income women led to similar conclusions. Investigators recommended that effective education about HPV must include (1) information about transmission, prevention, treatment, and cancer risk; (2) messages tailored to different age and risk groups; (3) clarification of carcinogenic and noncarcinogenic HPV types and their consequences; and (4) reassurance of low overall individual cancer risk [69].

Several studies among young women [70–72], parents of adolescents [73–76], and providers [77,78] have suggested an overall high acceptability for a prophylactic HPV vaccine. The most influential factors include perception of efficacy, safety, severity of infection, perceived risk, physician recommendation, and, for providers, guidelines and professional society recommendations. Acceptability among parents and providers seems to be higher for older adolescents [76]. Some parents expressed concern that a vaccine would increase unsafe sexual behavior [73,75], whereas another study reported that sexual transmission did not affect parental attitudes [76].

Most parents, young women, and adolescents have minimal knowledge of HPV and its association with cervical cancer [56,63]. Several studies indicate that vaccine acceptance is improved with increased knowledge [71,73,79,80]. In one study of 575 parents of 10- to 15-year-old children, brief education significantly increased acceptance of an HPV vaccine, particularly for parents who initially were undecided [73]. Results from a randomized intervention study designed to assess the impact of a brief HPV informational brochure (such as provided in doctors' offices) on parental acceptance of HPV vaccines for their 8- to 12-year-old children, however,

showed that the observed increase in knowledge related to receipt of the brochure did not increase acceptance of the vaccine. Attitudes and life experiences seemed to be more important factors [81]. Acceptance also may be influenced by whether the vaccine is perceived as a vaccine to reduce the risk of cervical cancer or as a vaccine to prevent a sexually transmitted infection. Findings from these acceptability studies are limited by their small sample size and narrow population-based sampling, but experts conclude that education of parents and providers should emphasize the risk of HPV infection in adolescents and the importance of vaccinating children before the onset of sexual activity.

Prophylactic human papillomavirus vaccination

Recent scientific discoveries of the viral etiology of cervical and other related types of cancer, and the development of prophylactic vaccines present the first realistic opportunity for primary prevention. Two prophylactic HPV vaccines have been developed based on the recombinant expression of the L1 major capsid protein and subsequent self-assembly into viruslike particles (VLPs) that resemble the outer shell of the virus. VLPs contain no DNA and are not live/attenuated viruses. Injection of the HPV VLPs elicits a strong and sustained type-specific response [82,83]. The quadrivalent prophylactic HPV vaccine (Gardasil, Merck & Co., Inc., Whitehouse Station, NJ) protects against HPV types 6, 11, 16, and 18. The bivalent HPV vaccine (Cervarix, GlaxoSmithKline, Middlesex, UK) protects against types 16 and 18. The goal of prophylactic vaccination is to reduce the incidence of HPV-related cervical, vulvar, vaginal, and anal premalignant and invasive disease, and the diagnostic and therapeutic interventions associated with these disease entities. The quadrivalent product also is expected to protect against genital warts, which potentially should be associated with a reduction in vertical transmission associated with laryngeal papillomatosis.

Efficacy

The prophylactic vaccines for HPV types 16 [82,84–86], 16 and 18 [87,88], and 6, 11, 16, and 18 [89] have been demonstrated to prevent persistent HPV 16 and 18 infections and HPV 16– and 18–related CIN2/3 [85,86,89]. The enrollment criteria for these trials restricted the participants' age, lifetime number of sex partners, past histories of cervical abnormality, and prevalent HPV 16 or 18 infections. In the study population, however, and with follow-up data for more than 5 years, these studies consistently demonstrate nearly 100% efficacy in the prevention of persistent type-specific HPV infections and CIN 2/3 among subjects adherent to the study protocol (per protocol analysis group), and who did not have evidence of the specific viral type found in the vaccine formulation prior to prophylactic vaccination. The quadrivalent product also protected against HPV 6–, 11–, 16–, and

18–related external genital lesions including genital warts, VIN, and VaIN. For women who had normal cytology at baseline and no carcinogenic HPV types within 90 days of study enrollment, the bivalent vaccine reduced the rate of HPV 16/18–associated abnormal cytologic results by 93%. Although the ultimate goal of these products is to prevent malignancy, persistent HPV infection and infection-related CIN2/3 were used as valid and appropriate intermediate clinical end points because of ethical and practical considerations that preclude the use of invasive disease as an end point.

Quadrivalent vaccine FUTURE II

The FUTURE II [90] trial focused solely on high grade cervical disease (ie, CIN 2, 3, AIS, cancer) endpoints, and after 3 years of follow-up, among 12,167 women aged 15 to 26 years who completed the vaccination regimen per protocol and were negative for the respective HPV vaccine type at entry through 1 month following the third vaccine dose (vaccine arm = 5305 participants; placebo arm = 5260 participants), vaccine efficacy was 98% (95% confidence interval [CI], 86%–100%) for preventing HPV 16– or HPV 18–related CIN2/3 and adenocarcinoma in situ (AIS) [91]. There was only one case of CIN3 in the vaccine group, compared with 41 cases of CIN plus an additional AIS case in the control group.

Quadrivalent vaccine FUTURE I

By contrast, FUTURE I [91] considered cervical and vulvovaginal disease endpoints. In 3 years of follow-up, for 5455 subjects aged 16 to 23 years who completed the quadrivalent vaccine regimen, did not violate the protocol, and had no virologic evidence of infection with the specific HPV vaccine type at study entry through 1 month following the third vaccine dose; the vaccine prevented 100% (95% CI=94–100) of HPV 6/11/16/18-related cervical lesions of any grade. Vaccine efficacy was 100% (95% CI, 94%–100%) for preventing HPV 6/11/16/18–related external genital warts or vulvar/vaginal intraepithelial neoplasia (VIN/VaIN) of any grade.

Intent-to-treat analyses were conducted to evaluate the impact of the quadrivalent vaccine with respect to HPV 6–, 11–, 16–, and 18–related cervical and other genital disease in all women who were randomized into both trials and received at least one dose of vaccine. No other restrictions were applied. Thus the overall impact of the vaccine on prevalent disease was estimated among women regardless of baseline HPV 6, 11, 16, or 18 polymerase chain reaction (PCR) status (ie, prevalent infection at study entry) and previous infection. The endpoint analyses included events arising from HPV infections and disease related to the vaccine specific HPV present at the time of vaccination as well as those arising from infections that were acquired after vaccination. Impact was measured starting 1 month after dose one, and the follow-up was for 3 years.

The majority of CIN and lower genital disease (ie, warts, VIN, and VaIN) detected in the group that received quadrivalent vaccine occurred

as a consequence of HPV infection that was present at enrollment. In FUTURE II [102], efficacy for HPV 16/18–related CIN2/3 or AIS was estimated at 44% (95% CI, 26%–58%), with 83 cases of high-grade disease in the vaccine arm compared with 148 among the placebo arm. For FUTURE I [103], the efficacy for HPV 6/11/16/18–related CIN or AIS was 55% (95% CI, 40%–66%), with 71 and 155 events in the vaccine control arms, respectively. There was also a 73% (95% CI=58%–83%) efficacy for HPV 6/11/16/18-related anogenital and vaginal lesions, with 28 and 102 cases identified among vaccine and placebo groups, respectively. An interim analysis of combined phase II and III quadrivalent vaccine studies (median follow-up, 1.9 years) demonstrated a 12.2% (95% CI=3.2%–25.3%) reduction in CIN2/3 compared with placebo regardless of HPV type [92]. This likely would represent the efficacy among the general population, however the greatest prophylactic benefit likely will apply to young women (median age, 16 years) reporting on average two and no more than four lifetime sexual partners at the time of vaccination. It is very important to note that when subjects entered these studies with evidence of current or past HPV infection (by PCR- or serology-positive for HPV related vaccine types), there was no significant protection from subsequent disease demonstrated by administration of the prophylactic quadrivalent vaccine [98].

When subjects entered these studies with evidence of current or past HPV infection (ie, were PCR- or serology-positive for HPV vaccine types), the administration of the quadrivalent prophylactic HPV vaccine demonstrated no clear evidence of protection from subsequent disease [92]. Additionally, although safety data is abundant and compelling, at this time efficacy is unknown for younger girls and results are not yet available for males.

Bivalent vaccine

The phase II bivalent vaccine trial provides information regarding vaccine efficacy and durability of the immune response. Approximately 776 women aged 15 to 25 years who completed the three-dose vaccination regimen were followed for 25 to 53 months (mean follow-up was 48 months). Vaccine efficacy was 100% (95% CI, 42.4%–100%) for preventing HPV 16– or HPV 18–related CIN 2 or 3; this included no cases in the vaccine group and five cases in the placebo group. Additionally there was a case of persistent HPV 16 or 18 among vaccinated women, compared to 23 cases among controls receiving placebo (96% efficacy) [88].

The most recent interim analysis of the placebo controlled trial of the bivalent prophylactic vaccine involving 18,644 young women was published recently. Among women (aged 15–24) who completed the 3-dose vaccination regimen (per protocol group) and participated in an extended follow-up study, bivalent vaccine efficacy 93% and 83% for preventing HPV16- or HPV18-related CIN2 or greater, with a total of two cases in the vaccine group and 21 in the placebo group. The results failed to reach significance

for HPV 18 because of the low number of events. The efficacy for preventing persistent (12 months) HPV 16- or 18-related (the obligate precursor of high grade cervical disease and cancer) was estimated to be 80% for HPV 16 and there was a trend toward significance for HPV 18 and the related viral types 33, 45, and 52.

Duration of protection

There are limited data on the duration of HPV vaccine-induced immunity, and there are no available immune correlates of vaccine or naturally induced immunity. In naturally occurring HPV infections, many women do not develop detectable HPV antibodies. In the case of HPV 16, the available serologic assays detect type-specific antibodies in only 54% to 60% of infected women [93–95]. Longitudinal follow-up of phase III and postlicensure studies will require more than serologic measurement of HPV vaccine-induced antibody titers. Long-term surveillance, beyond the available 5-year data, will require assessment of type-specific infections in vaccine recipients to measure duration of vaccine efficacy against HPV vaccine types adequately. Such post licensure follow-up will be critical to identify waning immunity and the need for booster immunizations.

Safety and reproductive toxicity

Remarkably few substantive safety issues have emerged for either vaccine product during the course of clinical trials. Injection-site reactions were the most common event reported by 83% of the recipients of the quadrivalent prophylactic HPV vaccine and 73% of the placebo recipients in the phase IIb randomized, controlled trial [89]. The most common injection-site experiences were erythema, pain, and swelling, with severe intensity being reported more often in the vaccine recipients. The most common systemic adverse experiences, which were reported by a similar proportion of vaccine and placebo recipients (69%), were fever, headache, and nausea. Temperature elevation ($\geq 37.8^{\circ}$C) was reported by 11.4% and 9.6% of vaccine and placebo recipients, respectively. There were no agent-related deaths during the trial. Five vaccine and two placebo recipients had serious vaccine-related experiences. These included one case each of bronchospasm, gastroenteritis (possibly related to a study procedure), headache with hypertension (definitely related), injection-site pain and movement impairment (probably related), and vaginal hemorrhage (probably related) in the vaccine group. Placebo-related serious adverse experiences included a case of hypersensitivity and one case of chills with headache and fever. The discontinuation rate was very low (0.2%) for both the vaccine and placebo groups [89].

A number of women became pregnant in the period shortly after vaccination. Pregnancy occurred in 10.7% of quadrivalent HPV vaccine and 12.6% of placebo recipients. Pregnancy outcomes were evaluated with respect to time from the injection to the onset of pregnancy. Sixty-two percent

of the vaccine recipients and 60% of the placebo recipients who became pregnant had a live birth. Fifty-six women receiving the quadrivalent HPV vaccine and 58 women receiving placebo became pregnant within 30 days of the injection, and 512 recipients of the quadrivalent HPV vaccine and 509 placebo recipients became pregnant more than 30 days after the injection. Spontaneous pregnancy loss occurred in 26.1% of pregnant women in both groups. Among women becoming pregnant within 30 days of vaccination, five delivered infants with congenital anomalies, in contrast to none of the women receiving the placebo. The five apparently unrelated anomalies (pyloric stenosis with ankyloglossia, congenital megacolon, hydronephrosis, hip dysplasia, and club foot) were judged to be unrelated to vaccination. For women becoming pregnant more than 30 days after vaccination, 10 quadrivalent HPV vaccine recipients and 16 placebo recipients had pregnancies with congenital anomalies [96]. A postmarketing pregnancy registry will be crucial to evaluate further reproductive toxicities and pregnancy outcomes among the increasing population of women who will be exposed to the vaccine.

During the phase IIb randomized, controlled trial, safety data for the bivalent vaccine were collected by daily diary for 7 days and by interview 30 days after each injection. Serious adverse events and pregnancy outcomes were collected throughout the duration of the trial. The vaccine seemed to be generally safe and well tolerated. As with the quadrivalent prophylactic HPV product, injection-site adverse events including pain, redness, or swelling were reported more often among vaccine recipients than among placebo recipients (94% versus 88%). Systemic adverse events including headaches, fatigue, and gastrointestinal systems were reported by a similar proportion of vaccine and placebo recipients (86%). Most adverse events were recorded as mild or moderate in intensity. Overall, 16.6% of the vaccine recipients and 13.6% of the placebo recipients had a temperature elevation ($\geq 37.5^{\circ}C$). Only one vaccine recipient and no placebo recipients discontinued treatment because of a serious adverse experience. There were no deaths in the trial considered to be secondary to vaccine or procedure. Pregnancy and congenital anomaly data for this vaccine have not been published at this time [87,88].

Age to vaccinate

Ideally, vaccination should occur before the onset of sexual intercourse in order to maximize the benefit of this intervention. Implementation of this approach is impractical, however, in real-world settings and instead public health decisions are based on age thresholds at which exposure is likely to occur. For the prophylactic vaccine products, the lower age limit is bound by the age of study participants (9 years of age), and data in this pediatric population are available only for safety and immunogenicity and not for disease end points. The lower age limits for vaccine efficacy studies of the quadrivalent vaccine and the bivalent vaccine are 16 and 15 years,

respectively. Because of the prophylactic nature of this vaccine, it is important to take into account the risk of prior infection, which is best estimated by a history of sexual activity. According to national survey data, 24% of girls report being sexually active by age 15 years, 40% by age 16 years, and 70% by age 18 years [97]. Seven percent of high school students (male and female) reported having initiated intercourse before age 13 years, and 10% of sexually active ninth graders reported having had four or more lifetime sex partners [95]. HPV acquisition often occurs soon after onset of insertional sexual activity. Among adolescents and young women aged 13 to 21 years, 70% had evidence of HPV infection within 5 to 7 years of onset of sexual intercourse [98]. In another study, 39% of college-aged women acquired HPV within 24 months of onset of sexual activity [99]. These epidemiologic studies probably underestimate the true exposure to HPV infections because transient infections are likely to be undetected, and test or sampling errors can depress the recovery of cervico-vaginal HPV [100]. Vaccination before sexual intercourse is critical to achieve optimal effectiveness.

Unlike vaccine performance in young populations, the efficacy and potential benefit of HPV vaccines for women older than 19 years is less clear compelling. Women older than 19 years who have not begun to have sexual activity will almost certainly derive the full benefit from HPV vaccination. For many 19- to 26-year-olds who have not been exposed to all four vaccine HPV types, there will likely be some protective benefit specifically against viral types not previously exposed to. Many currently and/or previously sexually active women in this age group will have been exposed to HPV 16 and/or 18 and will have less benefit from prophylactic vaccination. Brown and colleagues [101] tested sexually active adolescent girls (median number of sex partners of two) every 2 months and found the cumulative prevalence of HPV 16 and 18 is 31% and 20%, respectively, at 2.2 years. The risk of exposure to carcinogenic and noncarcinogenic HPV types increases with the number of lifetime sex partners [5,94,99,102]. Current population-based national survey data confirm that 50% of females over the age of 19 years have had four or more sexual partners [103], with a median number of four [104]. Such considerations are important when considering the generalizability of vaccine trials and their implications for public health policy.

Based on these data, the American Cancer Society has concluded that currently evidence is insufficient to recommend for or against vaccination of women aged 19 through 26 years, and there is no evidence for women over age 26 years. In the quadrivalent vaccine clinical trials, there was no clear evidence of protection from disease caused by HPV types for which study participants were PCR positive and/or seropositive at the time they entered the trial [92]. Given that about half of all women aged 19 to 26 years report four or more lifetime partners, the likelihood of prior HPV exposure is significant, and the likely benefit of prophylactic HPV vaccination will more likely approximate vaccine efficacy levels suggested by the intent to treat analyses.

Although vaccine trial data have not demonstrated equivalent efficacy for already exposed women, equivalent safety has been demonstrated. It should be noted however, that there is no role for pre-vaccination HPV testing, and there is currently an absence of an FDA approved type specific HPV test.

Impact on cancer screening

Widespread prophylactic vaccination is likely to affect the performance characteristics of existing screening tests. Lowering the total prevalence of CIN2/3+ will likely decrease the positive predictive value of screening cytology while increasing the negative predictive value. Similar changes also are likely for high-risk HPV testing because the risk of high-grade disease and cancer over 10 years among carcinogenic HPV-positive women who test negative for HPV 16 and 18 is very low [105]. Therefore a smaller percentage of women who have an abnormal screening will have CIN2/3+ detected during a colposcopically directed biopsy procedure (fasl positive screen), and a smaller percentage of women who have a negative screening test result will have a missed CIN2/3+ lesion. Monitoring changes in the performance of screening tests will be crucial, since this will impact future screening practices.

The development of type-specific HPV testing may find clinical use as an adjunct to screening in a postvaccine environment [105,106].

Currently, however, there are no data to suggest alterations in cervical cancer screening for vaccinated women, and practitioners and patients are encouraged to comply with existing clinical screening guidelines [107]. Moreover, the potential public health benefit from vaccination will be compromised if patients and practitioners develop a false sense of security that translates into decreased compliance with recommended cervical cancer screening.

Cost-effectiveness analyses

Several analyses that address the potential impact of HPV vaccines are available to inform health policy. These analyses differ in objectives and choice of model structure; they were intended to provide insight into vaccine impact. The economic analyses have assumed conservatively that the cost of the vaccine and administration would be $300. The models were based on direct medical costs associated with cervical cancer and importantly did not include genital warts, other HPV-related cancers or diseases, or non–health care costs. None of the published studies modeled a quadrivalent vaccine or catch-up vaccination, and each model assumed vaccination of preadolescent girls at age 12 years.

Although a range of cost effectiveness was found across different models, the insights provided were consistent and complementary. Several variables were found to have a disproportionate impact on cost and benefits associated with vaccination. These variables included later onset and less frequent

screening, age of vaccination, duration of efficacy, and cost of vaccine. Each of these models identified vaccination strategies costing less than $50,000 per quality-adjusted life year saved [108–110]. The cost effectiveness resulting from the prevention of HPV 6/11/16/18–associated diseases is highly dependent on the price of the vaccine, including administration and visit costs. When genital wart prevention is taken into account, cost-effectiveness of the vaccine becomes more favorable, although the magnitude of this has not been estimated adequately.

Published models suggest that type-specific HPV vaccination will reduce significantly but not eliminate the risk of cervical cancer. In the context of the existing screening, a type-specific vaccine may decrease HPV 16/18–associated CIN3 and cervical cancer, although the size of the incremental clinical benefit compared with screening alone would depend on screening program effectiveness. The cost effectiveness of vaccination is likely to rely on individual and practitioner delay in the onset of screening and to do so less frequently, while adopting a conservative approach to the follow-up of women who have minimally abnormal screening results. Additionally, vaccine benefit decreases as age of vaccination increases beyond the onset of sexual activity.

Modeling data significantly also suggests that if vaccine coverage is high, vaccination of males in addition to females will offer little additive benefit in preventing HPV-related cervical disease and may not be cost effective for the prevention of cervical cancer in women [110,111]. High vaccine coverage with a female-only vaccination program is likely to protect heterosexual males against HPV 6/11/16/18 through herd immunity. In low resource developing world settings with low vaccine coverage, vaccination of both males and females may be more effective in preventing HPV-related cervical disease [112].

Guidelines for prophylactic vaccination

In 2005 the American Cancer Society (ACS) convened an expert panel to issue recommendations for the use of the quadrivalent prophylactic vaccine product that was under review by the Food and Drug Administration. The group was charged with reviewing the data regarding the vaccine based on a comprehensive literature review (PubMed), a review of bibliographies, and selected unpublished data. The work of the committee consisted of a series of telephone conferences, culminating in a 1-day working meeting. The committee worked in parallel to but independent from the Centers for Disease Control Advisory Committee on Immunization Practice, which issued its own recommendations [113]. Consensus was reached on key issues and recommendations based on a review of the available data. When data were incomplete or insufficient, gaps were acknowledged, and expert opinion was incorporated into the recommendations. The final recommendations were approved by the ACS Gynecologic Cancer Advisory Group

and the ACS Board of Directors [107]. The ACS prophylactic vaccination recommendations are summarized in Box 2.

Policy considerations

Vaccines are among the most important public health achievements; however, newer and more expensive vaccines have raised concerns about the ability of public and private programs to sustain the financing and delivery of vaccines at optimal levels. In the developed and the developing

Box 2. American Cancer Society recommendations for HPV vaccine use to prevent cervical cancer and its precursors

- Routine HPV vaccination is recommended for females aged 11 to 12 years.
- Females as young as 9 years may receive HPV vaccination.
- HPV vaccination is also recommended for females 13 through 18 years of age to catch up missed vaccine or complete the vaccination series.
- There currently are insufficient data[a] to recommend for or against universal vaccination of females aged 19 to 26 years in the general population. A decision about whether a woman aged 19 to 26 years should receive the vaccine should be based on an informed discussion between the woman and her health care provider regarding her risk of previous HPV exposure and potential benefit from vaccination. Ideally the vaccine should be administered before potential exposure to genital HPV through sexual intercourse, because the potential benefit is likely to diminish with increasing number of lifetime sexual partners.
- HPV vaccination is not currently recommended for women over age 26 years or for males.
- Screening for CIN and cancer should continue in both vaccinated and unvaccinated women according to current ACS early-detection guidelines.

[a] Insufficient evidence of benefit in 19- to 26-year-old women refers to (1) clinical trial data in women with an average of two, and not more than four, lifetime sexual partners indicating a limited reduction in the overall incidence of CIN2/3, (2) the absence of efficacy data for the prevention of HPV 16/18–related CIN2/3 in women who have had more than four lifetime sexual partners, and (3) the lack of cost-effectiveness analyses for vaccination in this age group.

Data from Saslow D, Castle PE, Cox JT, et al. American Cancer Society guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin 2007;57(1):9; with permission.

world policy and implementation issues will impact the potential impact of HPV vaccination. While in the United States, many public (federal and state) and private (insurance and industry sponsored) programs are available to decrease cost related barriers to vaccination, the cost of the HPV vaccine may add substantially to the burden of vaccine financing and delivery.

Experience with the rotavirus vaccine suggests that private sector providers may be unlikely to purchase sufficient supplies of HPV vaccines. Given that even among insured patients reimbursement may not fully cover the costs of drugs, administration, and overhead, private sector providers face the choice of not offering vaccination or referral to public sector settings [114]. Additional statutory restrictions on public sector entities may limit the ability of these entities to deliver this vaccine to vulnerable populations at disproportionate risk for cervical cancer (ie, immigrants).

Another important challenge is the need for three doses, and therefore three clinic visits, which presents barrier to widespread compliance with the full vaccine series. This may be particularly important for adolescent populations where the ability to immunize is limited by access to the individual, because this has been the challenge for hepatitis B immunization. A recent report by the National Committee on Quality Assurance has highlighted this challenge, reporting adolescent vaccination rates (for varicella and hepatitis B) ranging from 45% to 50% for managed care and Medicaid beneficiaries. This is compared to hepatitis B vaccination by age two, which ranges from 80% to 90% [115].

Summary

The theoretical impact of prophylactic vaccine–related reduction in the risk of cervical cancer will depend on a variety of factors, including the degree of vaccine coverage, the number of HPV types in the prophylactic vaccine, the durability of protection, and continued screening for cervical diseases. Even under ideal circumstances it will be at least 3 decades before the cervical cancer prevention effects of this intervention begin to be realized. Furthermore, if protection wanes with time, continued risk reduction will depend on the percent of the population having access to a booster and the efficacy of that booster. Potential vaccination-associated declines in the participation in screening programs may lead to an increase in preventable cancers [116]. Aggressive screening efforts will continue to be needed for women already exposed to HPV and will become even more important among poor communities, which already have problems with access to health care for screening and which may be the least likely to have access to vaccination.

Although vaccination will provide protection against HPV 16– and 18–associated invasive cervical cancer in the long term, the greatest potential

short-term benefit may be in reducing abnormal Pap tests and related diagnostic procedures (including colposcopy and biopsy) and genital warts, low-grade CIN, and related therapy, as well as the psychologic morbidity associated with these entities. Although not directly cancer-related, such important downstream economic benefits may be as important as the virtual elimination of cervical cancer.

References

[1] Weinstock H, Berman S, Cates W Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Reprod Health Jan-Feb 2004;36(1): 6–10.
[2] Cates W Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. Sex Transm Dis Apr 1999;26(4 Suppl):S2–7.
[3] Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med May 5 1997;102(5A):3–8.
[4] Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. Jama Jan 23-30 1991;265(4):472–7.
[5] Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med Feb 12 1998;338(7):423–8.
[6] Kulasingam SL, Hughes JP, Kiviat NB, et al. Evaluation of human papillomavirus testing in primary screening for cervical abnormalities: comparison of sensitivity, specificity, and frequency of referral. Jama Oct 9 2002;288(14):1749–57.
[7] Richardson H, Kelsall G, Tellier P, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev Jun 2003;12(6):485–90.
[8] Myers ER, McCrory DC, Nanda K, Bastian L, Matchar DB. Mathematical model for the natural history of human papillomavirus infection and cervical carcinogenesis. Am J Epidemiol Jun 15 2000;151(12):1158–71.
[9] Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol Sep 1999;189(1):12–9.
[10] Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst Jun 7 1995;87(11):796–802.
[11] Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med Feb 6 2003;348(6):518–27.
[12] Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer Jul 7 2003; 89(1):101–5.
[13] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin Mar-Apr 2005;55(2):74–108.
[14] Human Papillomavirus Infection and cervical cancer. World Health Organization. Geneva, Switzerland; 2006.
[15] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin Mar-Apr 2006; 56(2):106–30.
[16] Daling JR, Madeleine MM, Johnson LG, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer Jul 15 2004;101(2):270–80.
[17] Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003;2(31):14–9.

[18] Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine Aug 21 2006;24S3:S1–10.
[19] Trimble CL, Hildesheim A, Brinton LA, Shah KV, Kurman RJ. Heterogeneous etiology of squamous carcinoma of the vulva. Obstet Gynecol Jan 1996;87(1):59–64.
[20] Rubin MA, Kleter B, Zhou M, et al. Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol Oct 2001;159(4):1211–8.
[21] Daling JR, Madeleine MM, Schwartz SM, et al. A population-based study of squamous cell vaginal cancer: HPV and cofactors. Gynecol Oncol Feb 2002;84(2):263–70.
[22] Cupp MR, Malek RS, Goellner JR, Espy MJ, Smith TF. Detection of human papillomavirus DNA in primary squamous cell carcinoma of the male urethra. Urology Oct 1996; 48(4):551–5.
[23] Forastiere AA, Ang K, Brizel D, et al. Head and neck cancers. J Natl Compr Canc Netw May 2005;3(3):316–91.
[24] Herrero R, Castellsague X, Pawlita M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst Dec 3 2003;95(23):1772–83.
[25] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev Feb 2005;14(2):467–75.
[26] Moscicki AB. Impact of HPV infection in adolescent populations. J Adolesc Health Dec 2005;37(6 Suppl):S3–9.
[27] Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol Aug 1995;33(8):2058–63.
[28] Munk C, Svare EI, Poll P, Bock JE, Kjaer SK. History of genital warts in 10,838 women 20 to 29 years of age from the general population. Risk factors and association with Papanicolaou smear history. Sex Transm Dis Nov 1997;24(10):567–72.
[29] Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. MMWR Recomm Rep Aug 4 2006;55(RR-11):1–94.
[30] Cubilla AL, Velazques EF, Reuter VE, Oliva E, Mihm MC Jr, Young RH. Warty (condylomatous) squamous cell carcinoma of the penis: a report of 11 cases and proposed classification of 'verruciform' penile tumors. Am J Surg Pathol Apr 2000;24(4):505–12.
[31] Reeves WC, Ruparelia SS, Swanson KI, Derkay CS, Marcus A, Unger ER. National registry for juvenile-onset recurrent respiratory papillomatosis. Arch Otolaryngol Head Neck Surg Sep 2003;129(9):976–82.
[32] Go C, Schwartz MR, Donovan DT. Molecular transformation of recurrent respiratory papillomatosis: viral typing and p53 overexpression. Ann Otol Rhinol Laryngol Apr 2003;112(4):298–302.
[33] Silver RD, Rimell FL, Adams GL, Derkay CS, Hester R. Diagnosis and management of pulmonary metastasis from recurrent respiratory papillomatosis. Otolaryngol Head Neck Surg Dec 2003;129(6):622–9.
[34] Lele SM, Pou AM, Ventura K, Gatalica Z, Payne D. Molecular events in the progression of recurrent respiratory papillomatosis to carcinoma. Arch Pathol Lab Med Oct 2002;126(10): 1184–8.
[35] Saslow D, Runowicz CD, Solomon D, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin Nov-Dec 2002;52(6): 342–62.
[36] Cervix Cancer Screening, Vol. 10. Lyon: IARC Press; 2005.
[37] Devesa SS. Descriptive epidemiology of cancer of the uterine cervix. Obstet Gynecol May 1984;63(5):605–12.
[38] Ries L, Harkins D, Krapcho M, A M, Miller B, Feuer E. SEER Cancer Statistics Review, 1975–2003. National Cancer Institute Bethesda, MD; 2006.

[39] Nanda K, McCrory DC, Myers ER, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med May 16 2000;132(10):810–9.
[40] Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. Jama Mar 21 2001; 285(11):1500–5.
[41] Sawaya GF, Washington AE. Cervical cancer screening: which techniques should be used and why? Clin Obstet Gynecol Dec 1999;42(4):922–38.
[42] ACOG Practice Bulletin. Clinical Management Guidelines for Obstetrician-Gynecologists. Number 61, April 2005. Human papillomavirus. Obstet Gynecol Apr 2005;105(4):905–18.
[43] Leyden WA, Manos MM, Geiger AM, et al. Cervical cancer in women with comprehensive health care access: attributable factors in the screening process. J Natl Cancer Inst May 4 2005;97(9):675–83.
[44] Schwartz PE, Hadjimichael O, Lowell DM, Merino MJ, Janerich D. Rapidly progressive cervical cancer: the Connecticut experience. Am J Obstet Gynecol Oct 1996;175(4 Pt 2): 1105–9.
[45] Parham GP, Hicks ML. Racial disparities affecting the reproductive health of African-American women. Med Clin North Am Sep 2005;89(5):935–43, 941.
[46] Patel DA, Barnholtz-Sloan JS, Patel MK, Malone JM Jr, Chuba PJ, Schwartz K. A population-based study of racial and ethnic differences in survival among women with invasive cervical cancer: analysis of Surveillance, Epidemiology, and End Results data. Gynecol Oncol May 2005;97(2):550–8.
[47] Singh GK, Miller BA, Hankey BF, Edwards BK. Persistent area socioeconomic disparities in U.S. incidence of cervical cancer, mortality, stage, and survival, 1975–2000. Cancer Sep 1 2004;101(5):1051–7.
[48] Hall HI, Jamison PM, Coughlin SS. Breast and cervical cancer mortality in the Mississippi Delta, 1979–1998. South Med J Mar 2004;97(3):264–72.
[49] Coronado GD, Thompson B, Koepsell TD, Schwartz SM, McLerran D. Use of Pap test among Hispanics and non-Hispanic whites in a rural setting. Prev Med Jun 2004;38(6):713–22.
[50] Yabroff KR, Lawrence WF, King JC, et al. Geographic disparities in cervical cancer mortality: what are the roles of risk factor prevalence, screening, and use of recommended treatment? J Rural Health. Spring 2005;21(2):149–157.
[51] Freeman HP, Chu KC. Determinants of cancer disparities: barriers to cancer screening, diagnosis, and treatment. Surg Oncol Clin N Am Oct 2005;14(4):655–69, v.
[52] Freeman H, Wingrove B. Excess Cervical Cancer Mortality: A Marker for Low Access to Health Care in Poor Communities: National Institutes of Health; 2005.
[53] Seeff LC, McKenna MT. Cervical cancer mortality among foreign-born women living in the United States, 1985 to 1996. Cancer Detect Prev 2003;27(3):203–8.
[54] Kitchener HC, Castle PE, Cox JT. Chapter 7: Achievements and limitations of cervical cytology screening. Vaccine Aug 21 2006;24(Suppl. 3):S63–70.
[55] Clark LR, Jackson M, Allen-Taylor L. Adolescent knowledge about sexually transmitted diseases. Sex Transm Dis Aug 2002;29(8):436–43.
[56] Dell DL, Chen H, Ahmad F, Stewart DE. Knowledge about human papillomavirus among adolescents. Obstet Gynecol Nov 2000;96(5 Pt 1):653–6.
[57] Mays RM, Zimet GD, Winston Y, Kee R, Dickes J, Su L. Human papillomavirus, genital warts, Pap smears, and cervical cancer: knowledge and beliefs of adolescent and adult women. Health Care Women Int Jul-Aug 2000;21(5):361–74.
[58] Baer H, Allen S, Braun L. Knowledge of human papillomavirus infection among young adult men and women: implications for health education and research. J Community Health Feb 2000;25(1):67–78.
[59] Denny-Smith T, Bairan A, Page MC. A survey of female nursing students' knowledge, health beliefs, perceptions of risk, and risk behaviors regarding human papillomavirus and cervical cancer. J Am Acad Nurse Pract Feb 2006;18(2):62–9.

[60] Lambert EC. College students' knowledge of human papillomavirus and effectiveness of a brief educational intervention. J Am Board Fam Pract May-Jun 2001;14(3):178–83.
[61] Ramirez JE, Ramos DM, Clayton L, Kanowitz S, Moscicki AB. Genital human papillomavirus infections: knowledge, perception of risk, and actual risk in a nonclinic population of young women. J Womens Health Feb 1997;6(1):113–21.
[62] Yacobi E, Tennant C, Ferrante J, Pal N, Roetzheim R. University students' knowledge and awareness of HPV. Prev Med Jun 1999;28(6):535–41.
[63] Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Wardle J. Awareness of human papillomavirus among women attending a well woman clinic. Sex Transm Infect Aug 2003; 79(4):320–2.
[64] Gerhardt CA, Pong K, Kollar LM, Hillard PJ, Rosenthal SL. Adolescents' knowledge of human papillomavirus and cervical dysplasia. J Pediatr Adolesc Gynecol Feb 2000;13(1): 15–20.
[65] Le T, Hicks W, Menard C, et al. Human papilloma virus testing knowledge and attitudes among women attending colposcopy clinic with ASCUS/LGSIL pap smears. J Obstet Gynaecol Can Sep 2004;26(9):788–92.
[66] Pitts M, Clarke T. Human papillomavirus infections and risks of cervical cancer: what do women know? Health Educ Res Dec 2002;17(6):706–14.
[67] Waller J, McCaffery K, Nazroo J, Wardle J. Making sense of information about HPV in cervical screening: a qualitative study. Br J Cancer Jan 31 2005;92(2):265–70.
[68] Gilbert LK, Alexander L, Grosshans JF, Jolley L. Answering frequently asked questions about HPV. Sex Transm Dis Mar 2003;30(3):193–4.
[69] Anhang R, Goodman A, Goldie SJ. HPV communication: review of existing research and recommendations for patient education. CA Cancer J Clin Sep-Oct 2004;54(5):248–59.
[70] Hoover DR, Carfioli B, Moench EA. Attitudes of adolescent/young adult women toward human papillomavirus vaccination and clinical trials. Health Care Women Int Jul-Aug 2000;21(5):375–91.
[71] Kahn JA, Rosenthal SL, Hamann T, Bernstein DI. Attitudes about human papillomavirus vaccine in young women. Int J STD AIDS May 2003;14(5):300–6.
[72] Zimet GD, Mays RM, Winston Y, Kee R, Dickes J, Su L. Acceptability of human papillomavirus immunization. J Womens Health Gend Based Med Jan-Feb 2000;9(1):47–50.
[73] Davis K, Dickman ED, Ferris D, Dias JK. Human papillomavirus vaccine acceptability among parents of 10- to 15-year-old adolescents. J Low Genit Tract Dis Jul 2004;8(3): 188–94.
[74] Mays RM, Sturm LA, Zimet GD. Parental perspectives on vaccinating children against sexually transmitted infections. Soc Sci Med Apr 2004;58(7):1405–13.
[75] Olshen E, Woods ER, Austin SB, Luskin M, Bauchner H. Parental acceptance of the human papillomavirus vaccine. J Adolesc Health Sep 2005;37(3):248–51.
[76] Zimet GD, Mays RM, Sturm LA, Ravert AA, Perkins SM, Juliar BE. Parental attitudes about sexually transmitted infection vaccination for their adolescent children. Arch Pediatr Adolesc Med Feb 2005;159(2):132–7.
[77] Mays RM, Zimet GD. Recommending STI vaccination to parents of adolescents: the attitudes of nurse practitioners. Sex Transm Dis Jul 2004;31(7):428–32.
[78] Raley JC, Followwill KA, Zimet GD, Ault KA. Gynecologists' attitudes regarding human papilloma virus vaccination: a survey of Fellows of the American College of Obstetricians and Gynecologists. Infect Dis Obstet Gynecol Sep-Dec 2004;12(3-4):127–33.
[79] Gust DA, Campbell S, Kennedy A, Shui I, Barker L, Schwartz B. Parental concerns and medical-seeking behavior after immunization. Am J Prev Med Jul 2006;31(1):32–5.
[80] Kennedy AM, Brown CJ, Gust DA. Vaccine beliefs of parents who oppose compulsory vaccination. Public Health Rep May-Jun 2005;120(3):252–8.
[81] Dempsey AF, Zimet GD, Davis RL, Koutsky L. Factors that are associated with parental acceptance of human papillomavirus vaccines: a randomized intervention study of written information about HPV. Pediatrics May 2006;117(5):1486–93.

[82] Emeny RT, Wheeler CM, Jansen KU, et al. Priming of human papillomavirus type 11-specific humoral and cellular immune responses in college-aged women with a virus-like particle vaccine. J Virol Aug 2002;76(15):7832–42.
[83] Pinto LA, Edwards J, Castle PE, et al. Cellular immune responses to human papillomavirus (HPV)-16 L1 in healthy volunteers immunized with recombinant HPV-16 L1 virus-like particles. J Infect Dis Jul 15 2003;188(2):327–38.
[84] Kadish AS, Einstein MH. Vaccine strategies for human papillomavirus-associated cancers. Curr Opin Oncol Sep 2005;17(5):456–61.
[85] Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med Nov 21 2002;347(21):1645–51.
[86] Mao C, Koutsky LA, Ault KA, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol Jan 2006;107(1):18–27.
[87] Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet Nov 13-19 2004;364(9447):1757–65.
[88] Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet Apr 15 2006;367(9518):1247–55.
[89] Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol May 2005; 6(5):271–8.
[90] Future-II-Study-Group. Quadrivalent Vaccine against Human Papillomavirus to Prevent High-Grade Cervical Lesions. NEJM May 10, 2007 2007;356(19)1915–27.
[91] Future-I-Investigators. Quadrivalent Vaccine against Human Papillomavirus to Prevent Anogenital Diseases. NEJM May 10, 2007 2007;356(19)1928–43.
[92] FDA. Product Approval Information - Licensing Action (Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine). Rockville, MD; 2007.
[93] Carter JJ, Koutsky LA, Wipf GC, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J Infect Dis Nov 1996;174(5): 927–36.
[94] Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. Cancer Epidemiol Biomarkers Prev Jan 2004;13(1):110–6.
[95] Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst Apr 6 1994; 86(7):494–9.
[96] FDA. Safety and efficacy of GARDASIL® (Human Papillomavirus [Types 6,11,16,18] Recombinant Vaccine) manufactured by Merck. Vaccines and Related Biological Products Advisory Committee, May 18, 2006. Rockville, MD; 2006.
[97] Abma JC, Martinez GM, Mosher WD, Dawson BS. Teenagers in the United States: sexual activity, contraceptive use, and childbearing, 2002. Vital Health Stat 23. Dec 2004(24):1–48.
[98] Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. Jama Jun 20 2001;285(23):2995–3002.
[99] Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol Feb 1 2003;157(3):218–26.
[100] Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short-term fluctuations in the detection of cervical human papillomavirus DNA. Obstet Gynecol Aug 1996;88(2):261–8.

[101] Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis Jan 15 2005; 191(2):182–92.
[102] Peyton CL, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. J Infect Dis Jun 1 2001;183(11):1554–64.
[103] Santelli JS, Brener ND, Lowry R, Bhatt A, Zabin LS. Multiple sexual partners among U.S. adolescents and young adults. Fam Plann Perspect Nov-Dec 1998;30(6):271–5.
[104] Mosher WD, Chandra A, Jones J. Sexual behavior and selected health measures: men and women 15-44 years of age, United States, 2002. Adv Data Sep 15, 2005;2005(362):1–55.
[105] Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst Jul 20 2005;97(14):1072–9.
[106] Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst Jul 20 2005;97(14):1066–71.
[107] Saslow D, Castle PE, Cox JT, et al. American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin Jan-Feb 2007;57(1):7–28.
[108] Goldie SJ, Kohli M, Grima D, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. J Natl Cancer Inst Apr 21 2004;96(8):604–15.
[109] Kulasingam SL, Myers ER. Potential health and economic impact of adding a human papillomavirus vaccine to screening programs. Jama Aug 13 2003;290(6):781–9.
[110] Sanders GD, Taira AV. Cost-effectiveness of a potential vaccine for human papillomavirus. Emerg Infect Dis Jan 2003;9(1):37–48.
[111] Barnabas RV, Laukkanen P, Koskela P, Kontula O, Lehtinen M, Garnett GP. Epidemiology of HPV 16 and cervical cancer in Finland and the potential impact of vaccination: mathematical modelling analyses. PLoS Med May 2006;3(5):e138.
[112] Garnett GP. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. J Infect Dis Feb 1 2005;191(Suppl. 1):S97–106.
[113] Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep Mar 23 2007;56(RR-2):1–24.
[114] Davis MM, Zimmerman JL, Wheeler JR, Freed GL. Childhood vaccine purchase costs in the public sector: past trends, future expectations. Am J Public Health 2002;92(12):1982–7.
[115] Mast EE, Mahoney FJ, Alter MJ, Margolis HS. Progress toward elimination of hepatitis B virus transmission in the United States. Vaccine, elimination of hepatitis B virus transmission in the United States. Vaccine 1998;(16 Suppl):S48–51.
[116] Franco EL, Harper DM. Vaccination against human papillomavirus infection: a new paradigm in cervical cancer control. Vaccine Mar 18 2005;23(17-18):2388–94.

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Vulvo-Vaginal Cancers: Risks, Evaluation, Prevention and Early Detection

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Vulva

Vulvar cancer is a relatively rare cancer that accounts for approximately 5% of tumors related to the female reproductive tract. In 2006, there were 3740 reported cases of vulvar cancer in the United States, with 880 associated deaths [1]. The incidence is 2.2 per 100,000 and is greatest in white women. The incidence of and mortality from the disease are on the rise. Over the last three decades, the annual percentage change in vulvar cancer incidence was 0.6 and mortality was 0.3 [2]. Despite this change, the disease is curable if diagnosed early, with a survival rate of 90% if there is no nodal involvement [3]. There is a defined premalignant state similar to cervical cancer that, if identified and promptly treated, prevents the development of invasive cancer.

Vulvar cancer types

Nearly any cell on the vulva can undergo malignant degeneration, but most (90%) vulvar tumors are squamous cell carcinomas (SCCs). The remaining 10% consist of adenocarcinoma, basal cell carcinoma, melanoma, sarcoma, undifferentiated carcinoma, and metastatic cancers from various other sites [4,5]. Most adenocarcinomas arise from the Bartholin gland or skin appendages. Melanoma is the second most common malignant

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tumor of the vulva, and any changing or new pigmented lesion of the vulva should be investigated. A Bartholin mass in a woman aged 40 years or older should be considered malignant until proven otherwise.

Vulvar disease: terminology

In 1912, Bowen described a skin condition termed ''precancerous dermatosis'' [6]. Later, Knight further described the condition in lesions found adjacent to invasive vulvar cancers, suggesting an associative precancerous lesion [7]. Various terminologies have been used throughout the years to describe the condition, including lichen sclerosus et atrophicus, leukoplakia, neurodermatitis, leukeratosis, Bowen's disease, erythroplasia of Queyrat, carcinoma simplex, leukoplakic vulvitis, hyperplastic vulvitis, and kraurosis vulvae.

In 1976, the International Society for the Study of Vulvovaginal Disease recommended the replacement of these terms with vulvar dystrophies, vulvar atypia, and SCC in situ [8]. Subsequently, the International Society for the Study of Vulvovaginal Disease further revised the terminology [9]. The term ''dystrophy'' was replaced with ''nonneoplastic epithelial disorder'' (including lichen sclerosus and squamous cell hyperplasia), whereas ''atypia'' and ''carcinoma in situ'' were replaced with vulvar intraepithelial neoplasia (VIN). VIN was further subclassified as VIN I (mild dysplasia), VIN II (moderate dysplasia), and VIN III (severe dysplasia or carcinoma in situ). Because of the high interobserver variation among pathologists and understanding of human papillomavirus (HPV) infection, VIN I was subsequently removed in the 2004 International Society for the Study of Vulvovaginal Disease terminology revisions [10].

The current 2004 International Society for the Study of Vulvovaginal Disease terminology is as follows [11]:

I. VIN, usual type
   a. VIN, warty type
   b. VIN, basaloid type
   c. VIN, mixed (warty/basaloid) type
II. VIN, differentiated type
III. VIN, unclassified type

Precursors to vulvar cancer

Vulvar intraepithelial neoplasia

According to the 2004 ISVVD modified terminology, there are essentially two types of high-grade VIN. High-grade VIN usual type (''warty-basaloid'' or ''undifferentiated'' type) is associated with HPV [12]. The second type of VIN, the differentiated type, is not associated with HPV. Underlying carcinoma is associated with VIN 15% to 22% of the time [13–15]. The VIN usual type is associated with high-risk HPV subtypes, particularly type 16, and often is multifocal. Basaloid VIN generally occurs in older women

and is more likely to progress to SCC. Morphologically, these cell types have epithelial changes that resemble cervical intraepithelial neoplasms. VIN, basaloid types have cells that are uniform and fairly small with chromatin that is coarse. Nucleoli are rarely found, whereas abnormal mitoses are usually present. The keratinocytes show little to no maturation; however, keratinization or parakeratosis may be present on the surface. Conversely, VIN, warty type has larger cells with more nuclear pleomorphism. The chromatin appears coarse with clumping, whereas the nucleoli are rarely identified. Abnormal mitosis, however, is readily seen. There is a distinct granular layer and an associated dyskeratosis, parakeratosis, or hyperkeratosis [5].

VIN, differentiated type is generally not associated with HPV [16]. Aneuploidy and chromosomal changes similar to those found in invasive SCC are seen [17–20]. The most significant finding is the presence of enlarged squamous cells causing epidermal thickening. Parakeratosis is also often seen. Enlarged, hyperchromatic, irregular nuclei are present along with increased mitoses. The dermis has fibrosis and a lymphocytic infiltrate [21].

Lichen sclerosus, lichen simplex chronicus, and squamous cell hyperplasia

Lichen sclerosus is a dermatosis with unclear origin found most commonly in postmenopausal white women [5]. Friedrich and Kalra [22] reported lower levels of serum dihydrotestosterone with concomitantly elevated levels of free testosterone. A genetic predisposition also has been reported, particularly in mother/daughter pairs [23]. Although it remains debated, there may be an association between lichen sclerosus and vulvar SCC. Fifty percent of women who have vulvar SCC also have lichen sclerosus [16]. Various case reports have documented the development of carcinoma after documented biopsy-proven lichen sclerosus [24–26]. There is a progression of the disease from lichen sclerosus to lichen sclerosus with lichen simplex chronicus to lichen sclerosus with squamous cell hyperplasia to differentiated VIN and then eventually carcinoma [27].

Histologically, lichen sclerosus has a thinned epidermis, a homogenous band of altered edematous, hyalinized collagen, and a mid-dermis with cellular infiltrate [5]. Lichen sclerosus with lichen simplex chronicus also presents with acanthosis and development of epidermal thickening. This thickening is a reactive condition and responds to treatment of the lichen sclerosus [28]. Lichen sclerosus with squamous cell hyperplasia is characterized by epidermal hyperplasia without inflammation, atypia, or evidence of a specific dermatosis [9].

Although lichen sclerosus and lichen sclerosus with lichen simplex chronicus are generally not considered premalignant conditions because the rate of malignant progression is so low, the role of squamous cell hyperplasia remains controversial. Several studies have shown genetic changes in squamous cell hyperplasia adjacent to SCCs that are associated with

malignancy [17–19]. Other studies have not found the same conclusion, however, which leads us to believe that squamous cell hyperplasia is equivalent to lichen simplex chronicus [29]. Because of the possibility of cancer developing from lichen sclerosus, however, such lesions should not be ignored and warrant full assessment.

Lichen planus

Although more common in women over 40, lichen planus is a dermatosis that presents in women with a wide range of ages [30,31]. Women may have burning or pruritis or be asymptomatic. Histologic appearance of lichen planus may be variable. A predominantly lymphocytic inflammatory infiltrate is present. Colloid bodies may form from degenerated keratinocytes. Several investigators have reported an association of lichen planus with vulvar cancer [32,33]. Although the exact risk of malignancy is not known, their coexistence with lichen sclerosis suggests a similar risk.

Paget's disease

The anogenital region is the most common site of extramammary Paget's disease. This condition is of apocrine origin and commonly presents as localized pruritis and burning in white women. The lesion generally is an eczematous lesion with a patchy, velvet-like, reddish and whitish appearance [4,5]. Paget's disease of the vulva is associated with invasive vulvar cancer in upwards of 15% to 25% of cases. A common feature of the disease is that the lesions commonly extend beyond the clinically apparent margins, which must be taken into consideration when excising the disease.

Molecular biology of precursor lesions

Understanding the molecular biology of these differing preinvasive lesions may lead to better strategies for chemoprevention. The molecule p53 is a tumor suppressor gene that plays a role in arresting cell development, which results in apoptosis. It plays a key role in controlling tumor cell progression. A mutated form of this gene is commonly found in epithelial cancers. Several investigators have found various mutations or overexpression of the gene in vulvar cancer [17,34–40]. Using immunohistochemistry and polymerase chain reaction, a mutation of the gene is found in up to 78% (22%–78%), and overexpression of the gene is present in up to 69% (53%–69%) of cases of vulvar cancer. Vascular endothelial growth factor expression also has been associated with vulvar cancer [41]. It is produced by epithelial tumors to induce angiogenesis, a prerequisite for tumor growth and progression. In addition to p53 and vascular endothelial growth factor, aneuploidy of the cell line has been shown [20]. Work needs to be done, however, to further elucidate the roles of these various agents in the progression of precursor lesions to invasive SCC.

Risk factors for progression to cancer

VIN III is the immediate precursor to invasive SCC and portends the highest risk of progression. Several additional risk factors for progression of VIN, usual type or the HPV-associated VIN to vulvar cancer have been identified [42–46], including age over 40, immunocompromised status, previous lower genital tract neoplasia with associated radiotherapy, and proximity to the anal verge and squamous-columnar junction. The association of VIN, usual type and vulvar cancer is seen in patients on steroid therapy and patients who have concomitant HIV infection.

Some risk factors for progression of lichen sclerosus to cancer have been identified, the most significant of which seems to be age. Jones and colleagues [47] reported that women who had cancer had a median age of 75 compared with 63 for a group of women who did not have cancer. Some researchers also have implicated the progression of cancer with women who present with a longer duration of symptoms, such as pruritis, irritation, and soreness [27,48]. Unfortunately, because vulvar cancer that presents with associated lichen sclerosus is without symptoms (up to 93%), this is a less reliable marker. Other researchers have reported an association of leukoplakia and the progression to cancer [49]. With abandonment of this term, however, it no longer serves as a marker. Areas that are thickened and do not respond to topical steroids should be biopsied for further evaluation. Areas that progress to squamous cell hyperplasia or VIN, differentiated type deserve closer attention, although the exact frequency of malignant transformation remains unclear.

Screening and diagnosis of vulvar cancer and vulvar intraepithelial neoplasia

Clinical findings

Vulvar preinvasive and invasive cancer may present with various symptoms, including chronic itching, burning, dyspareunia, erythema, edema, and pain. Bleeding or drainage is often a late symptom of a rather large ulcerative cancer. Most patients, however, are asymptomatic. Lesions may be unifocal or multifocal, frequently appearing white and raised (Fig. 1). They also may have the appearance of gray or red macules or other pigmented lesions. They frequently occur at the posterior vulva or periclitoral regions and extend to adjacent structures such as the clitoris, urethra, vagina, and anus. Self-examination by visualization with a mirror and by palpation can lead to early identification of a suspected abnormality and prompt evaluation.

Vulvar cytology

Analogous to cervical cytology, vulvar cytology attempts to identify cells that seem to be premalignant or malignant. First described by Dennerstein in 1988, scrapings were initially taken with a scalpel [50]. Others have used saline-moistened, cotton-tipped swabs and nylon brushes [51,52]. Features consistent with the precursor lesions are evaluated for overall cellularity,

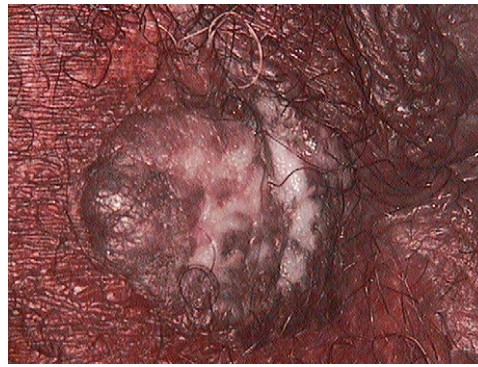

Fig. 1. Colposcopic appearance of a raised, acetowhite vulvar lesion consistent with VIN.

presence of hyperkeratotic, parakeratotic, or parabasal cells, and the presence of dysplastic cells. These methods show an overall correlation with vulvar biopsy of 60% to 91%. Despite their noninvasive nature, their use remains investigational, with vulvar biopsy remaining the gold standard.

Toluidine blue staining

Application of toluidine blue dye was first used by Richart to identify areas of cervical intraepithelial neoplasia [53]. Its application on the vulva has provided mixed results. Dye-stained regions potentially may assist in directing biopsy sites. In 242 patients, Collins and colleagues [54] found a 17% false-positive rate and no false-negative results in ten VIN lesions and nine invasive cancers. Other investigators have shown similar results, which suggests a possible role for toluidine in guiding biopsy sites [55,56]. Unfortunately, hyperkeratotic lesions are only lightly stained, even if neoplastic, whereas benign excoriated regions may stain deeply blue. This difference may lead to higher false-positive and false-negative rates.

Colposcopy and biopsy

Clinical inspection and biopsy are the hallmarks of early diagnosis of high risk skin lesions leading to prompt interventions. Although no pathognomonic finding is agreed upon, visual inspection with colposcopy after application of acetic acid is useful. Because the squamous epithelium of the vulva is keratinized, acetic acid requires several minutes before it is adequately absorbed by the tissue. We generally apply 5% acetic acid–drenched gauze to the vulva for a minimum of 5 minutes before inspection. Extensive evaluation with the colposcope, including in the perianal region, is needed to rule out multicentric lesions (Fig. 2). Because of vulvar cancer's varying appearance, liberal use of biopsy with a punch-type device is recommended to adequately diagnose potential invasive disease.

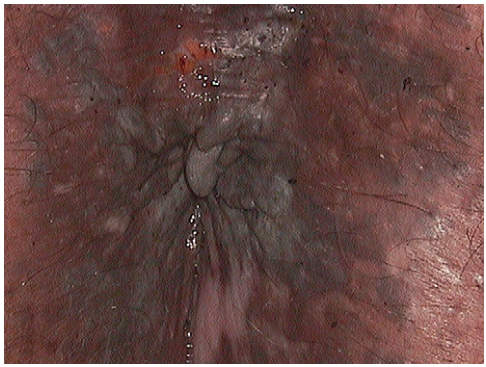

Fig. 2. Colposcopic appearance of perianal VIN lesions.

Management of preinvasive disease

With the increasing incidence of preinvasive vulvar disease in younger women, treatments that are effective but do not distort normal anatomy are becoming more necessary. Because of the controversy of the malignant potential of lichen sclerosus, lichen simplex chronicus, squamous cell hyperplasia, and lichen planus, symptomatic treatment with a moderate strength steroid and close observation is reasonable once malignancy is ruled out. With the recent changes in the terminology for VIN and the abandonment of the classification of mild dysplasia, treatment is warranted once the diagnosis of VIN is made and malignancy is excluded. Treatments include topical agents, laser ablation, wide local excision, skinning vulvectomy, and simple vulvectomy.

Topical agents

Various agents have been investigated for the treatment of premalignant vulvar disease, including 5-fluorouracil (5-FU), dinitriochlorobenzene, bleomycine, and recently, imiquimod. Of the methods, 5-FU is the most popular. 5-FU is a pyrimidine antagonist that inhibits DNA synthesis and prevents cell replication. Treatment generally takes 6 to 10 weeks, with patients experiencing severe inflammation for 2 weeks [57]. Various tissue responses may develop, including local erythema, edema, skin sloughing, and significant pain. Upon completion of therapy, tissue healing is completed in 4 to 6 weeks with little to no scarring. The obvious benefits of 5-FU are avoidance of surgery and minimal scarring. 5-FU also has been studied as an adjuvant therapy. In a randomized, controlled trial, patients treated with 5-FU for HPV-associated vulvar and vaginal lesions after treatment by another modality had a lower recurrence rate than patients not treated [58]. Maintenance therapy was noted to be most effective in women with multiple lesions, multiple organ involvement, or immunosuppression. The inconsistency of success and poor

patient compliance make this a less-than-ideal treatment for most patients; it is generally reserved for individuals who refuse or are unable to undergo other therapies.

Recently, imiquimod was used for the treatment of VIN. Imiquimod is an immune response modulator that is used extensively for the treatment of genital warts [59]. Le and colleagues [60] treated 23 patients with VIN 2 or 3 with imiquimod for 16 weeks. Seventeen patients were available for evaluation. They noted complete regression of the disease in 9 patients and partial response in another 5 patients. Although this treatment is promising, further investigation is needed before any definitive recommendation on its use is made.

Laser ablation

Many consider this therapy the treatment of choice for the treatment of VIN, particularly in multifocal lesions. There is generally good cosmetic healing, and treatment can be accomplished in an outpatient setting. It is also effective, with success rates in excess of 90% [61,62]. The treatment, however, can be painful with prolonged healing times. Greater expertise is generally needed with the laser. We prefer the use of the CO_2 laser at a power density of 600 to 1000 W/cm^2. Based on Benedet's study, we recommend ablating to a depth of 1 mm for nonhairy lesions and 3 mm for hair-bearing regions [63]. Postoperatively, we advise our patients to separate the vulvar folds and take sitz baths three times daily. Pain is managed with nonsteroidal anti-inflammatory drugs and narcotics, as necessary.

Wide local excision, skinning vulvectomy, and simple vulvectomy

Surgical therapy with excision of the lesion is the time-honored treatment of choice. The advantage of this treatment is the ability to obtain complete histologic evaluation of the lesion. It is also less expensive than laser therapy and healing is generally faster. Wide local excision also can be performed in a clinical setting. Although there are no definitive data as to the extent of excision, respected authorities recommend a 5-mm margin of normal epithelium [4]. Paget's disease is thought to commonly extend beyond its clinically apparent lesion, so special care must be taken with the excision. Excising a small portion of the subcutaneous tissue is recommended with intraoperative assessment of margin status. Wide local excision is best used for localized lesions, and infiltration with a local anesthetic agent, such as lidocaine or marcaine, is sufficient. We generally make efforts to preserve the clitoris, anus, and urethra. Upon complete resection of the lesion, primary end-to-end reapproximation of the defect is accomplished with interrupted suture. Although the vulvar skin and mucous membrane are elastic, it may be necessary to undermine the skin to allow for addition mobilization for direct closure.

When multicentric lesions are present, it may become necessary to excise the lesions and substitute the defect with a split-thickness skin graft [64].

This skinning vulvectomy preserves the subcutaneous tissue of the vulva, which maintains good cosmesis and function. DiSaia and Rich [65] later modified the procedure to preserve the clitoris, choosing to scrape off lesions on the glans with a surgical blade. Although the procedure is effective, prolonged bed rest (approximately 7 days) is required to allow for the skin graft to adhere.

When the cosmetics of a skinning vulvectomy are not necessary or the morbidity of prolonged bed rest is of concern, a simple vulvectomy may be preferred. A simple vulvectomy may be necessary for extensive lesions because of the concern for potential invasive cancer. This procedure involves completely excising the skin and a portion of the subcutaneous tissue. After obtaining hemostasis, the underlying skin is undermined and primary closure of the skin edges is performed. Unlike a radical vulvectomy, the perineal fascia is not excised. Plication of the puborectalis, perineal muscles, and anal sphincter may be necessary to provide additional perineal support and minimize tension of the posterior closure. Blood loss is generally more than the aforementioned procedures, and postoperative stool softeners are recommended until the vulva has healed adequately.

Outcomes are similar for laser ablation and excision procedures in various case series. A recent comparative trial that involved various treatments found that vulvectomy was superior to laser therapy, however [66]. With excision procedures, the status of the surgical margins is the best predictor of recurrence [67]. Despite this, patients with positive margins do not always need reoperation unless invasive disease is present. Close observation is often warranted. Counseling on modifiable risk factors, such as smoking cessation, and decreasing high-risk behaviors that place one at risk for HIV or other sexually transmitted infections are wise.

The role of human papillomavirus vaccination

HPV-16 is highly associated with VIN, usual type [12,16]. Recent development of a HPV vaccine shows tremendous promise in the prevention of genital malignancies. Koutsky and colleagues [68] showed 100% efficacy in the reduction of persistent HPV-16 infections. Hampl and colleagues [12] further reviewed the potential effects of the HPV vaccine as it relates to VIN and vulvar cancer. Their findings were consistent with other investigators, noting the presence of HPV type 16 or 18 in 76% of patients who had VIN and 42% of patients who had vulvar cancer. Based on these findings, they concluded that the HPV vaccine could make a significant contribution to the reduction of vulvar cancer in younger women while reducing VIN by two thirds. Based on these and other studies, the Centers for Disease Control and Prevention recommended HPV vaccination in girls aged 11 to 12 years, although immunization can be performed in girls as young as 9 years old [69]. They recommended catch-up vaccines in female patients aged 13 to 26. Full impact on prevention of VIN and invasive cancer is yet to be realized in needed longitudinal trials.

Vagina

Vaginal cancer is extremely rare and accounts for 1% to 2% of cancers of the female reproductive tract [70]. Primary vaginal cancer is the presentation of cancer within the vagina without clinical or histologic evidence of cervical or vulvar cancer or history of these cancers within 5 years of diagnosis. First described by Cruveilhier in 1826, the incidence of vaginal cancer is 0.6 per 100,000 [71,72]. There were 2420 newly reported cases of vaginal cancers (and other genitals) in the United States in 2006, with 820 deaths [1]. The peak incidence is generally in the sixth and seventh decades of life. Secondary vaginal cancer in patients with a history of cervical or vulvar neoplasia is more common and accounts for approximately 80% to 90% of tumors found in this site. Reasons for the development of vaginal cancer after cervical neoplasia can be residual disease after removal of the cervix, field effect of HPV-replicating cervical disease, and radiation treatment given for cervical neoplasia [73].

The cause of vaginal cancer is unknown. Many researchers report similar risk factors as cervical cancer [74]. Risk factors for vaginal cancer are HPV infections, diethylstilbestrol (DES) exposure in utero, immunocompromised state, chronic irritation (pessary use or prolapse), and irradiation for cervical neoplasia [75]. Other possible risk factors are HSV and cigarette smoking [75]. Previous hysterectomy for benign disease was thought to be associated with increased risks; however, recent data show insufficient evidence for such claim.

Vaginal cancers are usually found at a more advanced stage compared with cervical or vulvar cancer. They tend to be more technically difficult to treat and have a lower overall survival rate. Various studies report 40% to 45% 5-year overall survival rates for SCC.

Vaginal cancer types

Most lesions, in situ and invasive, are squamous and account for approximately 85% to 90% of vaginal carcinomas. Adenocarcinomas are responsible for 8% to 10% of vaginal cancers, partly because of exposure to DES. The remaining histologic types include sarcomas, malignant melanomas, lymphomas, and embryonal rhabdomyosarcomas [76].

Precursors to vaginal cancer

Vaginal intraepithelial neoplasia

In 1952, Graham and Meigs [77] were the first to describe vaginal intraepithelial neoplasia (VAIN) occurring several years after total hysterectomy for carcinoma in situ of the cervix. Incidence of disease varies from 0.2 to 2 per 100,000 [72]. Most cases of VAIN are found in patients over age 60 with risk factors similar to those found in patients who have cancer of the vulva and cervix [78]. Approximately 75% of patients who have VAIN currently

or previously had squamous cancer of the vulva or cervix. History of previous hysterectomy for cervical neoplasia is a significant risk factor for VAIN, with its presentation occurring commonly in the upper vagina near the vaginal cuff. HPV infections, history of pelvic irradiation, and immunosuppression are other risk factors.

The terminology of VAIN follows recommendations of the Bethesda system, with VAIN 1 or mild dysplasia called low-grade squamous intraepithelial lesion and VAIN 2 or 3 or moderate or severe dysplasia or carcinoma in situ categorized as high-grade squamous intraepithelial neoplasia. Most cases of VAIN are asymptomatic with no lesion seen. Occasionally, raised white or pink areas may suggest disease. Usually it is discovered on colposcopy performed for an abnormal Pap test result in posthysterectomy cases or in evaluating for cervical disease.

The natural history of VAIN based on a 3-year follow-up study of no treatment suggests a high regression rate of 78% compared with 13% persistence and 9% progression to cancer [79]. Another study reported that 5% of cases of VAIN progressed to invasive cancer; however, only five women in this study had VAIN 3 disease. In treatment for VAIN 3, underlying invasive disease must be considered.

Molecular biology of precursor lesions

Vaginal cancers seem to present a profile somewhere between cervical and vulvar cancer, demonstrating HPV-16 and -18 infectivity and p53 alterations [80]. HPV-16 and -18 have been detected in up to 50% of vaginal cancers. Several investigators have found various mutations or overexpression of the p53 gene in vaginal cancer [80,81]. Using immunohistochemistry, p53 overexpression has been found in 33% to 48% of primary vaginal cancers and mutations of the gene in 22% of invasive cases [81]. The pattern of genomic imbalances using comparative genomic hybridization resembles those of cervical carcinomas [80]. DNA gains were seen in chromosomal arms of 3q (69%), 19 p (50%), and 5 p (50%) [82]. Most tumors also were aneuploidy. DES-exposed patients have shown increased incidence in VAIN, with 30% to 40% of the women having a transformation zone extending into the vagina [83]. Although the mechanism is not clear, DES exposure in utero increases a woman's risk for clear cell adenocarcinoma up to age 40 [84]. Further work needs to be done to elucidate the roles of these various agents in the progression of precursor lesions to invasive cancer, however. In VAIN lesions, HPV seems to be the primary causative agent [85].

Risk factors for progression to cancer

In contrast to cervical intraepithelial neoplasia (CIN), progression in VAIN seems to require a greater period of time. CIN and VAIN share common risk factors, however, such as persistent infection with oncogenic HPV subtypes, sexual activity, early age of first intercourse, smoking, and

immune status. HPV DNA was detected in 60% of patients who had invasive vaginal cancer and in more than 80% of patients who had carcinoma in situ. Antibodies to HPV-16 L1 are also strongly associated with vaginal cancer [74].

Diethylstilbestrol

Young women who are exposed in utero to the synthetic estrogen DES are at risk for clear cell adenocarcinoma of the vagina and squamous dysplasia of the cervix and vagina [83,86,87]. These findings are greater in women whose mothers received DES before the twelfth week of gestation. Typical clinical abnormalities seen are vaginal adenosis, cockscomb cervix, cervical collar, and a transverse vaginal septum [86]. Many of these patients have an extensive cervical and vaginal transformation zone, which increases the potential area for squamous metaplastic changes and HPV susceptibility [83]. The incidence of clear cell adenocarcinoma in women with in utero DES exposure is 1 per 1000 by age 40 [84,88]. Although this patient population is decreasing with time, close follow-up with regular cervical and vaginal cytology and colposcopy and a careful vaginal examination should be done yearly.

Screening and diagnosis of vaginal cancer

Vaginal cytology

Generally, VAIN and vaginal cancers are diagnosed after evaluation of an abnormal Pap test result with colposcopy or biopsy of a gross vaginal lesion. Microscopic features reveal hyperchromasia, nuclear enlargement, and irregular condensation of chromatin. Pap test screening for vaginal cancer is recommended for women with history of hysterectomy for cervical neoplasia. The incidence of vaginal cancer is not increased for women who have a hysterectomy for benign disease, so Pap screening should be limited and adjusted to risk of exposure to HPV or previous exposure to DES [89].

Colposcopy and biopsy

Vaginal cancer may present with painless vaginal bleeding, discharge, and ulcerated lesions in the upper vagina. Advanced disease may present with symptoms that reflect location. Anterior tumors may present with urinary retention, bladder spasm, and hematuria. Posterior tumors may present with rectal symptoms, such as constipation, tenesmus, and bloody stool. Colposcopy after an abnormal Pap test result may reveal unifocal or multifocal acetowhite lesions, which are findings similar to cervical neoplasia. Generally, vaginal cancer is found in the upper one third of the vagina. Careful inspection of the entire vagina is critical because the speculum blades may obscure a lesion, and patients with previous hysterectomies may have malignant lesions buried within the vaginal cuff.

VAIN is usually asymptomatic and probably underestimated, with most cases found after an abnormal Pap test result. Colposcopy with application of 5% acetic acid for 3 to 5 minutes assists in the detection of disease before

inspection (Fig. 3A). Because of atrophy of the vagina from the hypoestrogenic state, Lugol's solution can be helpful in detecting vaginal disease in postmenopausal women (Fig. 3B). Although not as common as in high-grade cervical disease, mosaicism and punctations are a concern for high-grade vaginal disease and should be sampled (Fig. 4A, B). Extensive evaluation of the vaginal with liberal biopsies of acetowhite areas or Lugol's nonstaining areas should be performed (Fig. 4C). Atypical vessels may represent microinvasive disease (Fig. 5). In patients with previous radiation or severe atrophy, however, it may not represent malignant disease. All abnormal areas deserve sampling to rule out invasive disease.

Management of preinvasive disease

Various treatments for VAIN have been described. Few to no definitive prospective trials investigating the various methods are available. Treatment options include topical agents, laser ablation, and surgical resection.

Topical agents

Similar to premalignant vulvar disease, 5-FU and imiquimod have been investigated for the treatment of VAIN. Several investigators have found some success with 5-FU [90,91]. Various treatment strategies have been used with similar efficacies. They generally involve placement of a 5% 5-FU intravaginal suppository for 7 to 10 days with a 1- to 2-week rest period in between the treatments to allow to recovery from the local reactions. Sillman and colleagues [92] investigated the use of 5-FU with surgical resection and found improved success with the combination therapy over chemotherapy alone. It has a 75% success rate as opposed to a 29% rate in patients who receive 5-FU alone.

Preliminary data on the use of imiquimod show some promise in its use with VAIN. Haidopoulos and colleagues [93] found some success in a small

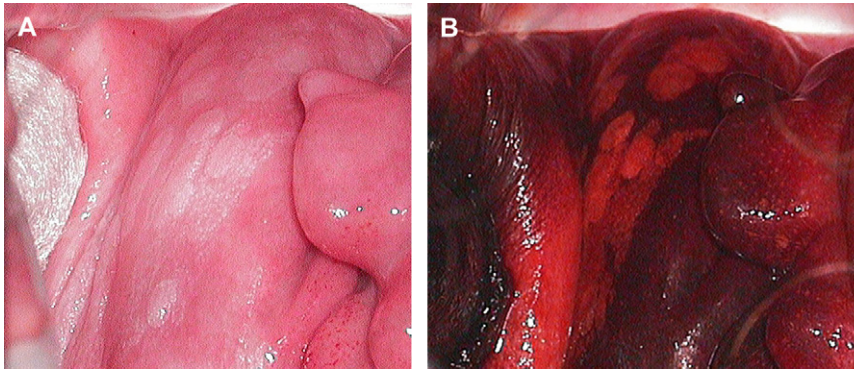

Fig. 3. (*A*) Multifocal VAIN 1 and condyloma after application of 5% acetic acid. (*B*) Multifocal Lugol's nonstaining areas of VAIN 1 and condyloma.

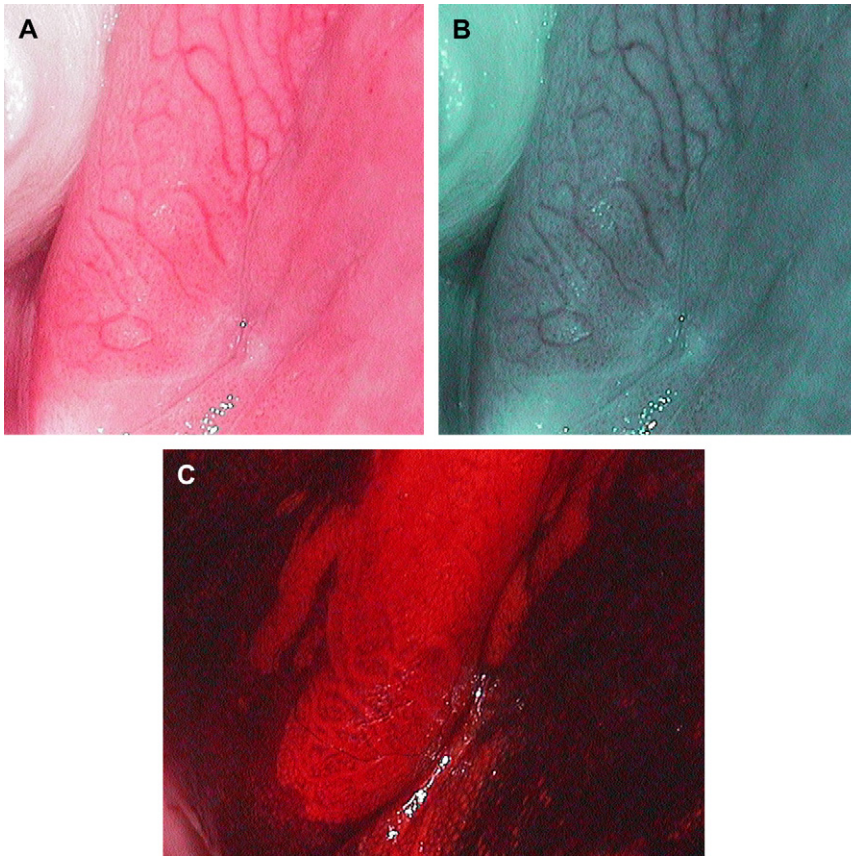

Fig. 4. (*A*) VAIN 3 after 5% application of acetic acid. Dense aceto white epithelium lesions with coarse mosaic tile and punctuations indicative of VAIN 3. (*B*) VAIN 3 visualized under the green filter after application of 5% acetic acid. (*C*) VAIN 3, nonstaining area after application of Lugol's solution.

number of patients treated with imiquimod for VAIN 2/3. Their numbers were limited, with only seven patients treated and five available for follow-up. Further studies are needed to better elucidate the use of this treatment modality.

Laser ablation

Laser therapy of VAIN shows great tolerability with excellent success. Townsend and colleagues [94] were able to successfully treat 92% of patients with VAIN. Eight of the 36 patients treated, however, required two or more treatments. Other studies have shown varying results, with recurrence as high as 57% [90,95]. Benedet and colleagues [96] evaluated the depth of epithelial involvement in women who had VAIN. They found involvement

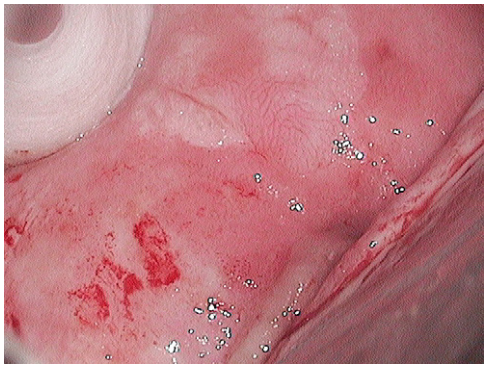

Fig. 5. After 5% acetic acid, colposcopic changes consistent with VAIN 3 and suspicious for microinvasive disease.

between 0.10 and 1.4 mm; therefore, they recommended a depth of ablation of 1.5 mm. Because of the expense of laser equipment and the needed expertise, some experts advocate local excision over the use of laser in most patients who have VAIN.

Radiation

Some investigators have advocated radiation for the treatment of VAIN. In one series of 14 patients who had CIN 3 and 6 women who had VAIN 3 who were treated with radiation, no recurrence was noted [97]. Unfortunately, 12 women had mild to moderate vaginal toxicity, with 2 developing severe symptoms (ie, prominent vaginal atrophy and stenosis). Given the high rate of vaginal symptoms and the difficulty of follow-up assessment and therapy, this treatment has fallen out of favor.

Upper vaginectomy

Surgical resection of diseased vagina is the treatment of choice. Isolated lesions often can be treated in the office under lower anesthesia. Larger lesions, particularly higher up in the vagina, may require a more extensive vaginectomy. The use of colposcopy to aid in delineation of the affected vagina and injection of dilute phenylephrine submucosally to decrease bleeding assist with the vaginectomy immensely. Various studies have reported excellent long-term success rates with low recurrence rates [98,99].

The role of human papillomavirus vaccination

The association of HPV and VAIN has been documented by several investigators [12,100]. Hampl and colleagues [12] found that HPV type 16 or 18 was present in 64% of patients with VAIN 2/3. Koutsky and colleagues [68] found 100% efficacy in the reduction of persistent HPV-16 infections. Based on these and other studies, the Centers for Disease Control

and Prevention recommended HPV vaccination in girls aged 11 to 12, although immunization can be performed in girls as young as 9 years old [69]. They recommended catch-up vaccines for female patients aged 13 to 26 years.

Summary

Vulvar and vaginal cancers are rare and account for approximately 7% of cancers of the female reproductive tract. Vulvar and vaginal neoplasia share similar risk factors: HPV infection, previous CIN or cervical cancer, current smoking, sexual factors, and immunosuppression. Discovery of HPV-related intraepithelial or invasive disease in the vulva or vagina should warrant continued surveillance for subsequent disease in other anogenital sites. In general, low-grade intraepithelial disease at both sites may be followed closely with Pap smears and colposcopy. Several treatment options are available for patients with documented histologic high-grade intraepithelial vulvar or vaginal neoplasia. Common treatment options for both sites are excision, laser vaporization, and 5-FU. After treatment, lifetime follow-up with cytology and colposcopy, as needed, is recommended. With the widespread use of the HPV vaccine, one half to two thirds of vulvar and vaginal cancers may be prevented. Patient education regarding reduction of risk factors for progression and close surveillance of at-risk individuals may prevent the progression to invasive disease.

References

[1] American Cancer Society. Cancer facts and figures 2006. Atlanta (GA): American Cancer Society; 2006.
[2] Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 9 Regs Limited-Use, Nov 2006 Sub (1973-2004), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2007, based on the November 2006 submission. Available at: http://www.seer.cancer.gov.
[3] Homesley HD, Bundy BN, Sedlis A, et al. Assessment of current International Federation of Gynecology and Obstetrics staging of vulvar carcinoma relative to prognostic factors for survival (a gynecologic oncology group study). Am J Obstet Gynecol 1991;164(4):997–1003 [discussion: 1003–4].
[4] DiSaia PJ, Creasman WT. Invasive cancer of the vulva. In: DiSaia PJ, Creasman WT, editors. Clinical gynecologic oncology. St. Louis (MO): Mosby-Year Book; 1997. p. 202–32.
[5] Wilkinson EJ. Premalignant and malignant tumors of the vulva. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract. New York: Springer-Verlag; 1994. p. 87–129.
[6] Bowen JT. Precancerous dermatosis: a study of chronic atypical epithelial proliferation. J Cutan Dis Syph 1912;30:241–55.
[7] Knight RV. Bowen's disease of the vulva. Am J Obstet Gynecol 1943;46:514–24.
[8] Freidrich EG. New nomenclature for vulvar disease: report of the committee on terminology, ISSVD. Obstet Gynecol 1976;47:122–4.

[9] Ridley CM, Frankman O, Jones IS, et al. New nomenclature for vulvar disease: International Society for the Study of Vulvar Disease. Hum Pathol 1989;20:495–6.
[10] Preti M, Mezzetti M, Robertson C, et al. Inter-observer variation in histopathological diagnosis and grading of vulvar intraepithelial neoplasia: results of a European collaborative study. BJOG 2000;107:594–9.
[11] Heller DS. Report of a new ISSVD classification of VIN. J Lower Gen Tract Dis 2007;11: 46–7.
[12] Hampl M, Sarajuuri H, Wentzensen N, et al. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. Obstet Gynecol 2006; 108:1361–8.
[13] Chafe W, Richards A, Morgan L, et al. Unrecognized invasive carcinoma in vulvar intraepithelial neoplasia (VIN). Gynecol Oncol 1988;31:154–62.
[14] Modesitt SC, Waters AV, Walton L, et al. Vulvar intraepithelial neoplasia III: occult cancer and the impact of margin status on recurrence. Obstet Gynecol 1998;92:962–6.
[15] Thuis YN, Campion M, Fox H, et al. Contemporary experience with the management of vulvar intraepithelial neoplasia. Int J Gynecol Cancer 2000;10:223–7.
[16] Scurry J, Flowers LC, Wistuba I, et al. Human papillomavirus, lichen sclerosis and vulvar squamous cell carcinoma. Int J Gynecol Cancer 1998;8:298–306.
[17] Kagie MJ, Kenter GG, Tollenaar RA, et al. p53 protein over-expression, a frequent observation in squamous cell carcinoma of the vulva and in various synchronous vulvar epithelial, has no value as a prognostic parameter. Int J Gynecol Pathol 1997;16:124–30.
[18] Tate JE, Mutter GL, Boynton KA, et al. Monoclonal origin of vulvar intraepithelial neoplasia and some vulvar hyperplasias. Am J Pathol 1997;150:315–22.
[19] Lin MC, Mutter GL, Trivijisilp P, et al. Patterns of allelic loss (LOH) in vulvar squamous carcinomas and adjacent noninvasive epithelia. Am J Pathol 1998;152:1313–8.
[20] Scurry J, Hung J, Flowers L, et al. Ploidy in human papillomavirus positive and negative vulvar squamous cell carcinomas and adjacent skin lesions. Int J Gynecol Cancer 1999;9: 187–93.
[21] Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. Int J Gynecol Pathol 2001;20:16–30.
[22] Friedrich EG, Kalra PS. Serum levels of sex hormones in vulvar lichen sclerosus, and the effect of topical testosterone. N Engl J Med 1984;310:488–91.
[23] Friedrich EG, MacLaren NK. Genetic aspects of vulvar lichen sclerosus. Am J Obstet Gynecol 1984;150:161–6.
[24] Eng AM, Jacobs RA. Lichen sclerosus et atrophicus and squamous cell carcinoma. J Cutan Pathol 1980;7:123–4.
[25] Di Paola GR, Leverone NGR, Belardi MG. Recurrent vulvar malignancies in an 11 year prospectively followed vulvar dystrophy: a gynaecologist's permanent concern. Gynecol Oncol 1983;15:120–1.
[26] Roman LM, Mitchell MF, Burke TW, et al. Unsuspected invasive squamous cell carcinoma of the vulva in young women. Gynecol Oncol 1991;41:182–5.
[27] Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. Int J Gynecol Cancer 1999;9:89–97.
[28] Scurry J, Whitehead J, Healey M. Histology of lichen sclerosus varies according to site and proximity to carcinoma. Am J Dermatopathol 2001;23:413–8.
[29] Kiryu H, Ackerman AB. A critique of the current classification systems of vulvar disease. Am J Dermatopathol 1990;12:377–92.
[30] Edwards L. Vulvar lichen planus. Arch Dermatol 1989;125:1677–80.
[31] Mann MS, Kaufman RH. Erosive lichen planus of the vulva. Clin Obstet Gynecol 1991;34: 605–13.
[32] Dwyer CM, Ker RE, Millan DW. Squamous cell carcinoma following lichen planus of the vulva. Clin Exp Dermatol 1995;20:171–2.

[33] Zaki I, Dalziel KL, Solomonsz FA, et al. The under reporting of skin disease in association with squamous cell carcinoma of the vulva. Clin Exp Dermatol 1996;21:334–7.
[34] Lee YY, Wilczynski SP, Chumakov A, et al. Carcinoma of the vulva: HPV and p53 mutations. Oncogene 1994;9:1655–9.
[35] Milde-Langosch K, Albrecht K, Joram S, et al. Presence and persistence of HPV infection and p53 mutation in cancer of the cervix uteri and the vulva. Int J Cancer 1995;63:639–45.
[36] Pilotti S, D'Amato L, Della Torre G, et al. Papillomavirus, p53 alteration and primary carcinoma of the vulva. Diagn Mol Pathol 1995;4:239–48.
[37] Kim YT, Thomas NF, Kessis TD, et al. p53 Mutations and clonality in vulvar carcinomas and squamous hyperplasia: evidence suggesting that squamous hyperplasias do not serve as direct precursors to human papillomavirus-negative vulvar carcinomas. Hum Pathol 1996; 27:389–95.
[38] Sliutz G, Schmidt W, Tempfer C, et al. Detection of p53 point mutations in primary human vulval cancer by PCR and temperature gradient gel electrophoresis. Gynecol Oncol 1997; 64:93–8.
[39] McConnell DT, Miller ID, Parkin DE, et al. p53 protein expression in a population-based series of primary vulval squamous cell carcinoma and immediate adjacent field charge. Gynecol Oncol 1997;67:248–54.
[40] Rolfe KJ, Crow JC, Benjamin E, et al. Abnormalities in the G1/S cell cycle checkpoint in vulval cancer. BJOG 2000;107:817.
[41] MacLean AB, Reid WM, Rolfe KJ, et al. Role of angiogenesis in benign, premalignant and malignant vulvar lesions. J Reprod Med 2000;45:609–12.
[42] Maclean AB. Precursors of vulvar cancers. Curr Obstet Gynaecol 1993;3:149–56.
[43] Buscema J, Woodruff JD, Parmley TH, et al. Carcinoma in situ of the vulva. Obstet Gynecol 1980;55:225–30.
[44] Al-Ghamdi A, Freedman D, Miller D, et al. Vulvar squamous cell carcinoma in young women: a clinicopathologic study of 21 cases. Gynecol Oncol 2002;84:94–101.
[45] van Seters M, van Beurden M, de Craen AJM. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol 2005;97:645–51.
[46] Brown JE, Sunborg MJ, Kost E, et al. Vulvar cancer in human immunodeficiency virus-seropositive premenopausal women: a case series and review of the literature. J Low Genit Tract Dis 2005;9(1):7–10.
[47] Jones RW, Sadler L, Grant S, et al. Clinically identifying women with vulvar lichen sclerosus at increased risk of squamous cell carcinoma. J Reprod Med 2004;49:808–11.
[48] Hart WR, Norris HJ, Helwig EB. Relation of lichen sclerosus et atrophicus of the vulva to the development of carcinoma. Obstet Gynecol 1975;45:369–77.
[49] Taussig FJ. Cancer of the vulva: an analysis of 155 cases (1911–1940). Am J Obstet Gynecol 1940;40:764–9.
[50] Dennerstein GJ. Cytology of the vulva. J Reprod Med 1988;33:703–4.
[51] Nauth HF, Schilke E. Cytology of the exfoliative layer in normal and diseased vulvar skin: correlation with histology. Acta Cytol 1982;26:269–83.
[52] Levine TS, Rolfe KJ, Crow J, et al. The use of cytospin monolayer technique I: the cytological diagnosis of vulval and anal disease. Cytopathology 2001;12:297–305.
[53] Richart RM. A clinical staining test for the in vivo delineation of dysplasia and carcinoma in situ. Am J Obstet Gynecol 1963;86:703–12.
[54] Collins CG, Hansen LH, Theriot E. A clinical stain for use in selecting biopsy sites in patients with vulvar disease. Obstet Gynecol 1996;28:158–63.
[55] Broen EM, Ostergard DR. Toluidine blue and colposcopy for screening and delineating vulvar neoplasia. Obstet Gynecol 1971;38:775–8.
[56] Joura EA, Zeisler H, Losch A, et al. Differentiating vulvar intraepithelial neoplasia from nonneoplastic epithelial disorders: the toluidine blue test. J Reprod Med 1998; 43:671–4.

[57] Sillman FH, Sedlis A, Boyce JG. A review of lower genital intraepithelial neoplasia and the use of topical 5-fluorouracil. Obstet Gynecol Surv 1985;40:190–220.
[58] Krebs HB. Prophylactic topical 5-fluorouracil following treatment of human papillomavirus-associated lesions of the vulva and vagina. Obstet Gynecol 1986;68(6):837–41.
[59] Edwards L, Ferenczy A, Eron L. Self administered topical 5% imiquimod cream for external anogenital wars. Arch Dermatol 1998;134:25–30.
[60] Le T, Hick W, Menard C, et al. Preliminary results of 5% imiquimod cream in the primary treatment of vulva intraepithelial neoplasia grade 2/3. Am J Obstet Gynecol 2006;184: 377–80.
[61] Townsend DE, Levine RU, Richart RM, et al. Management of vulvar intraepithelial neoplasia by carbon dioxide laser. Obstet Gynecol 1982;60(1):49–52.
[62] Baggish MS, Dorsey HJ. CO_2 laser for treatment of vulvar carcinoma in situ. Obstet Gynecol 1981;57(3):371–5.
[63] Benedet JL, Wilson PS, Matisic J. Epidermal thickness and skin appendage involvement in VIN. J Reprod Med 1991;36(8):608–12.
[64] Rutledge F, Sinclair M. Treatment of intraepithelial carcinoma of the vulva by skin excision and graft. Am J Obstet Gynecol 1968;102:806–18.
[65] DiSaia PJ, Rich WM. Surgical approach to multifocal carcinoma in situ of the vulva. Am J Obstet Gynecol 1981;140:136–45.
[66] Hillemanns P, Wang X, Staehle S, et al. Evaluation of different treatment modalities for vulvar intraepithelial neoplasia (VIN): CO_2 laser vaporization, photodynamic therapy, excision and vulvectomy. Gynecol Oncol 2006;100(2):271–5.
[67] Shafi MI, Luesley DM, Byrne P, et al. Vulval intraepithelial neoplasia: management and outcome. Br J Obstet Gynaecol 1989;96(11):1339–44.
[68] Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002;347(21):1645–51.
[69] Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: recommendations of the advisory committee on immunization practices (ACIP). Morb Mortal Wkly Rep Surveill Summ 2007;56(RR-2):1–24.
[70] Creasman WT. Vaginal cancers. Curr Opin Obstet Gynecol 2005;17:71–6.
[71] Cruveilhier J. Varices des veines du ligament rond, stimulant une hernie inguinale: anomalie remarquable dans la disposition general du peritoine. Cancer ulcere la parol anterieure du vagin et bas-sond de la vessie. Bull Soc Anat Paris 1826;1:199.
[72] Cramer DW, Cutler SJ. Incidence and histology of malignancies of the female genital organ in the US. Am J Obstet Gynecol 1974;118:443–60.
[73] Woodruff JD. Carcinoma in situ of the vagina. Clin Obstet Gynecol 1981;24(2):485–97.
[74] Daling JR, Madeline MM, Schwartz SM, et al. A population-based study of squamous cell vaginal cancer: HPV and cofactors. Gynecol Oncol 2002;84:263–70.
[75] Merino MJ. Vaginal cancer: the role of infectious and environmental factors. Am J Obstet Gynecol 1991;165:1255–62.
[76] Basil J. Vaginal carcinoma. In: Bieber E, Sanfillipo J, Horrowitz I, editors. Clinical gynecology. Amsterdam: Elsevier; 2006. p. 643–9.
[77] Graham JB, Meigs JV. Recurrence of tumour after total hysterectomy for carcinoma in situ. Am J Obstet Gynecol 1952;64:1159.
[78] Hummer WK, Mussey E, Decker DG, et al. Carcinoma in situ of the vagina. Am J Obstet Gynecol 1970;108:1109–16.
[79] Aho M, Vesterinen E, Meyer B, et al. Natural history of vaginal intraepithelial neoplasia. Cancer 1991;68:195–7.
[80] Koyamatsu Y, Yokoyama M, Nakao Y, et al. A comparative analysis of human papillomavirus in types 16 and 18 and expression of p53 gene and Ki-67 in cervical, vaginal, and vulvar carcinomas. Gynecol Oncol 2003;90:547–51.
[81] Skomedal H, Kristensen G, Helland A, et al. TP53 gene mutations and protein accumulation in primary vaginal carcinomas. Br J Cancer 1995;72(1):129–33.

[82] Habermann JK, Hellman K, Freitag S, et al. A recurrent gain of chromosome arm 3q in primary squamous carcinoma of the vagina. Cancer Genet Cytogenet 2004;148:7–13.
[83] Robboy SJ, Noller KL, O'Brien P, et al. Increased incidence of cervical and vaginal dysplasia in 3,980 diethylstilbestrol (DES) exposed young women: experience of the National Collaborative DES-Adenosis (DESAD) Project. J Am Med Assoc 1984;252:2979–83.
[84] Melnick S, Cole P, Anderson D, et al. Rates and risk of diethylstilbestrol-related clear cell adenocarcinoma of the vagina and cervix. N Engl J Med 1987;316:514–6.
[85] Townsend DE. Intraepithelial neoplasia of the vagina. In: Coppleson M, editor. Gynecologic oncology. Edinburgh: Churchill Livingstone; 1992. p. 493–9.
[86] Herbst AL, Ulfelder H, Poskanzer DC, et al. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. N Engl J Med 1971; 284:878–82.
[87] Bornstein J, Kaufman RH, Adam E, et al. HPV associated with vaginal intraepithelial neoplasia in women exposed to DES in utero. Obstet Gynecol 1987;70:75–80.
[88] Vessey MPL. Epidemiological studies of the effects of diethylstilbestrol. IARC Sci Publ 1989;96:335–48.
[89] Fetters MD, Fischer G, Reed BD. Effectiveness of vaginal Papanicolaou smear screening after total hysterectomy for benign disease. J Am Med Assoc 1996;275(12):940–7.
[90] Petrilli ES, Townsend DE, Morrow CP, et al. Vaginal intraepithelial neoplasia: biological aspects and treatment with topical 5-fluorouracil and the carbon dioxide laser. Am J Obstet Gynecol 1980;138:321–8.
[91] Caglar H, Hurtzog RW, Hreschchyshyn MM. Topical 5-FU treatment of vaginal intraepithelial neoplasia. Obstet Gynecol 1981;58:580–3.
[92] Sillman FH, Sedlis A, Boyce JG. 5-FU/chemosurgery for difficult lower genital intraepithelial neoplasia. Contemp Ob Gyn 1985;27:79–101.
[93] Haidopoulos D, Diakomanolis E, Rodolakis A, et al. Can local application of imiquimod cream be an alternative mode of therapy for patients with high-grade intraepithelial lesions of the vagina? Int J Gynecol Cancer 2005;15:898–902.
[94] Townsend DE, Levine RU, Crum CP, et al. Treatment of vaginal carcinoma in situ with carbon dioxide laser. Am J Obstet Gynecol 1982;143:565–8.
[95] Hoffman MS, Roberts WS, LaPolla JP, et al. Laser vaporization of grade 3 vaginal intraepithelial neoplasia. Am J Obstet Gynecol 1991;165:1342–4.
[96] Benedet JL, Wilson PS, Matisic JP. Epidermal thickness measurements I vaginal intraepithelial neoplasia: a basis for optimal CO_2 laser vaporization. J Reprod Med 1992;37: 809–12.
[97] Ogin I, Kitamura T, Okajima H, et al. High-dose-rate intracavitary brachytherapy in the management of cervical and vaginal intraepithelial neoplasia. Int J Radiat Oncol Biol Phys 1998;40:881–7.
[98] Lee RA, Symmonds RE. Recurrent carcinoma in situ of the vagina in patients previously treated for in situ carcinoma of the cervix. Obstet Gynecol 1976;48:61–4.
[99] Curtis P, Shepherd JH, Lowe DG, et al. The role of partial colpectomy in the management of persistent vaginal neoplasia after primary treatment. Br J Obstet Gynaecol 1992;99: 587–9.
[100] Sugase M, Matsukura T. Distinct manifestation of human papillomaviruses in the vagina. Int J Cancer 1997;72:412–5.